https://dx.doi.org/10.4314/dujopas.v10i3a.10
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 3a September 2024*

Evaluation of Some Haemostatic Indices of Selected Women on Hormonal Contraceptives in Benin City, Nigeria

Obazelu P.A*, Emokpae M.A, Iyawe E., Ogbodo F. C

Department of Medical Laboratory Science,
School of Basic Medical Sciences,
University of Benin

Email: progress.obazelu@uniben.edu

Abstract

Thrombotic disorder has been associated with especially older versions of hormonal contraceptives. Even with development of modern version of the medication, the controversies concerning their thrombotic effects still remain. However, screening of women on hormonal contraceptive for the presence of any derangement in the haemostatic profile is not routinely carried out in Nigeria. This study investigated the effects of hormonal contraceptives on haemostatic profile among women. This was a cross-sectional study carried out on 200 apparently healthy female subjects on hormonal contraceptives. They comprised of 50 non- contraceptive users (control subjects) (group A), fifty (50) non-hormonal contraceptive users (group B), 50 subjects on progestin-only contraceptive (POC) (group C) and 50 women on combined oral contraceptive (COC) (group D). Exactly 4.5mL of venous blood was collected aseptically from each participant into a plain container containing 0.5mL of 3.1% tri-sodium citrate anticoagulant, followed by proper mixing. The citrated plasma obtained after centrifugation at 3000 rpm for 15 minutes and separation, was used for the determination of prothrombin time (PT) and activated partial thromboplastin time (APTT). Data analysis was carried out using the statistical package for social sciences (SPSS) version 17.0 software and a p-value <0.05 was considered significant. The Mean±SD prothrombin time {PT (seconds)} was significantly higher (p<0.001) among women on combined hormonal contraceptive than progestin-only contraceptive, non-hormonal contraceptive and controls. Similarly, the activated partial thromboplastin time {APTT (seconds)} was significantly higher (p<0.05) among subjects on combined hormonal contraceptive than progestin-only and non-hormonal contraceptive users but not different when compared with controls. The use of hormonal contraceptives results in altered PT and APPTT and may be an increased risk of thrombogenic among users.

Keywords: Activated partial thromboplastin time, Hormonal contraceptives, Nigerian women, Prothrombin time

INTRODUCTION

Hormonal contraceptives are synthetic biochemical substances used to prevent unintended pregnancy (Adejumo *et al*., 2016). The use of contraceptives have been known to be a reliable means to prevent pregnancy in women in as much as they do not prevent sexually transmitted diseases such as acquired immune deficiency syndrome or viral hepatitis (Akhigbe *et al*., 2008). Hormonal contraceptives come in different forms such as the birth control tablets, the

Author for Correspondence

contraceptive skin patch, the vaginal ring and the hormone-releasing contraceptive coils. The most commonly known methods among married women are injectables (88%), pills (87%) and implants (78%) (NPC, 2019). They influence the levels of hormones in women and prevent the release of mature eggs by the ovary. Some studies indicated that these hormonal contraceptives can cause alterations in some trace elements and vitamins which influence haemopoiesis in humans (Akinloye *et al*., 2011; Palmery *et al*., 2013). Furthermore, other studies indicated that various haematological changes are associated with the use of hormonal contraceptives. These changes include high platelet count which increases the risk of thromboembolism, myocardial infarction, arterial disease and carcinogenicity (Lidegaard *et al*., 2002; Plu-Bureau *et al*., 2013; Martin and Elliot-Sale, 2016). The most extreme but rare side effect has been predisposition to increased risk of thromboembolic occurrences (Hugon-Rodin *et al*., 2014). Moreover, several parameters in the human body (including blood count) has shown physiological variations as part of the normal circadian rhythm, which could be diurnal or seasonal (Thirup, 2003). The older first and second generation tablets such as those containing levornorgestrel or norgestimate appear to cause a lower risk of thrombosis when compared with the third and fourth generation birth control tablets such as those containing dienogest, desogestrel, drospirenone or gestodene (Plu-Bureau *et al*., 2013). Nakada *et al*. (2014) reported that the higher blood count in women in their reproductive years is because oestrogen administration considerably amplified the proliferation of haematopoetic stem cell (HSCs).

Haemostasis is the arrest of bleeding in order to reduce blood loss from an injured blood vessel. It is the process whereby blood kept in a fluid state in the blood vessel. Haemostasis helps to prevent blood loss and stop excessive bleeding through a process called coagulation. Coagulation is a chemical process that leads to formation of fibrin clot (Hall, 2015). The process begins when prothrombin, a coagulation factor is activated e.g. by platelets. Prothrombin is converted to thrombin which acts on fibrinogen, a plasma protein and breaks it down into an insoluble fibrin. The fibrin forms a thread around the platelets, white cells and other cells to form a fibrin clot thereby preventing further blood loss (Hall, 2015).

Blood coagulation is initiated by a succession of finely balanced physical and biochemical changes resulting from damage to the blood vessel called coagulation pathways. These include the extrinsic and intrinsic pathways. The intrinsic is initiated by contact with a foreign surface and the extrinsic is initiated by exposure to tissue factor (Hall, 2015). Prothrombin time (PT) test, activated partial thromboplastin time (APTT) test, used to assay coagulation factors in homeostatic studies (Cheesbrough, 2006). The haemostatic system represents a delicate balance between pro-coagulant, anti-coagulant mechanisms allied to a process of fibrinolysis. Few studies have been carried out regarding the effects of contraceptives on haemostatic parameters in the human body. An increased rate of thrombosis in 1-3 per 100,000 women per year was reported by Abdollahi *et al*. (2003) and Naess *et al*. (2007).

The number of women of child bearing age using hormonal contraceptives is on the increase worldwide. In Nigeria the prevalence of hormonal contraceptive use rose from 3% in 1990 to 10% in 2008. Alterations in haemostatic profile with resultant increase in thrombogenic risk as a potential complication of using these drugs have been documented in Caucasians and in Nigerians. However, screening of women on different hormonal contraceptives for presence of any derangement in the haemostatic profile is not routinely carried out in Nigeria. Hence this study was carried out to explore the haemostatic effects of different types of hormonal contraceptives in women attending family planning clinics around Benin city. The aim of the

study was to determine some haemostatic changes associated with use of different hormonal contraceptives in women of reproductive age.

MATERIALS AND METHODS

Study area

This is was a cross-sectional study of women of reproductive age using different hormonal contraceptives recruited from Faith Mediplex hospital, Central hospital and Planned Parenthood Federation of Nigeria (PPFN) in Benin city, Edo State who are on hormonal contraceptives.

Collection of samples

The total study population consists of two hundred (200) apparently healthy female subjects within reproductive age, between 18years to 45 years were evaluated in the study. They consist of one- hundred (100) female subjects on hormonal contraceptives [50 female subjects on progestin-only contraceptives (group C) and 50 female subjects on combined oral contraceptives (group D)], 50 female subjects on non-hormonal contraceptives (group B) and 50 female subjects' non-contraceptive users (group A) (control).

Female subjects on hormonal contraceptives without chronic illness were recruited for the study. Hospitalized patients, surgical patients and pregnant women or those with chronic illnesses were excluded from the study.

Following the administration of questionnaire and giving of consent to participate in the study, 4.5mL of venous blood was obtained aseptically into a plain container having 0.5mL of 3.1% trisodium citrate anticoagulant. The citrated blood sample was centrifuged at 3000 rpm for 15 minutes and the plasma was separated into another clean tube and used for the evaluation of PT and APTT.

Ethical Consideration

Ethical approval was obtained from Research Ethics Committee on Human Subjects from Edo State Ministry of Health, Benin city (Ref. Number: HA.737/50 issued on 2nd March, 2021). Informed consent for participation of study was sought from each participant prior to inclusion in the study.
Haemostatic parameters {activated partial thromboplastin time (APTT) and prothrombin time (PT)} were analysed manually immediately after sample collection.

Estimation of Haemostatic Parameters

Prothrombin Time

Principle

Citrated plasma sample is added to brain thromboplastin and calcium chloride reagent at 37°C and the time taken for clot formation is measured.

Procedure

Prothrombin time was performed by pipetting 0.1ml of the citrated plasma samples into a clean glass test tube and incubating in a water bath at 37°c for 2 minutes. Then 0.2ml of the pre-warmed thromboplastin/calcium chloride reagent (BIOLABO Diagnostics, Maizy, France) was added to the test tube using an automatic pipette and the stopwatch started

simultaneously while holding the tube in the water and tilting back and forth. The watch was stopped at the first sight of clot formation and the result recorded in seconds (BIOLABO Diagnostics, Maizy, France).

Activated Partial Thromboplastin Time (APTT)

Principle
Kaolin, a surface activator and platelet substitute (phospholipid) are incubated with citrated plasma at 37°C for a definite time. Calcium chloride is added and the time taken for clot development is recorded.

Procedure
A 1:9 of anticoagulant to blood ratio was obtained into a plain container and centrifuged for fifteen (15) minutes at 3000g to get platelet poor plasma using the bucket centrifuge. After which 0.1millilitres (0.1mls) of plasma was distributed into a clean dry test tube. 0.1ml of pre-warmed kaolin/platelet substitute (BIOLABO Diagnostics, Maizy, France) aliquot was added to the test tube and incubated at 37°c for two (2) minutes. The sample was re-calcified with 0.1ml of 0.025M calcium chloride and a stopwatch started instantly while tilting the tube back and forth checking for clot formation. The watch was stopped at the first sight of clot formation and the result recorded in seconds (BIOLABO Diagnostics, Maizy, France).

RESULTS
Table 1 showed the comparison of Mean±SD of haemostatic variables of four groups namely; group A, B, C, and D, representing contraceptive naïve (control), non-hormonal contraceptive, progestin-only, and combined hormonal contraceptive subjects, respectively.
Comparing groups A and B; No significant difference was observed in the prothrombin time (PT) (seconds) between both groups ($p>0.05$). The activated partial thromboplastin time (APTT) (seconds) of group A (38±1.12) was significantly higher compared to that of group B (30.46±0.67) ($p<0.05$)

Comparing groups A and C, the prothrombin time (PT) value of both groups showed no statistical significant difference ($p>0.05$). However, group A shows that APTT (38.00±1.12) was significantly higher ($p<0.05$) compared to group C (32.10±0.63).
The prothrombin time (PT) value of group D (18.32±0.47) was significantly higher ($p<0.05$) compared with that of group A (14.90±0.24), while no statistical significant difference was observed in the APTT values of both groups ($p>0.05$).

Comparing PT and APTT values of groups B and group C, both groups showed no statistical significant difference ($p>0.05$).

Group D showed significantly higher ($p<0.05$) PT (18.32±0.47) and APTT (36.76±0.71), compared to group B, PT (14.88±0.43) and APTT (30.46±0.67).
Also, group D showed significantly higher ($p<0.05$) values of PT (18.32±0.47) and APTT (36.76±0.71), when compared to group C, PT (15.86±0.49) and APTT (32.10±0.63)

Table 1: Mean Comparison of Haemostatic Parameters among the Studied Groups

Parameter	Control subject (A) (n=50)	Non-hormonal contraceptive subject (B) (n=50)	Progestin-only subject (C) (n=50)	Combined hormonal contraceptive subject (D) (n=50)	F Value	p Value
PT (sec)	14.90±0.24^{d}	14.88±0.42^{d}	15.86±0.49^{d}	18.32±0.47^{abc}	12.08	0.001
APTT (sec)	38.00±1.12^{bc}	30.46±0.67^{ad}	32.10±0.63^{ad}	36.76±0.71^{bc}	21.97	0.001

p≤ 0.05- Significant; p ≥ 0.05- Not significant.
PT- Prothrombin Time
APTT- Activated Partial Thromboplastin Time.
a represents significance with control
b represents significance with non-hormonal contraceptive subjects
c represents significance with progestin-only subjects
d represents significance with combined hormonal contraceptive subjects.

DISCUSSION

The activated partial thromboplastin time (APTT) is a performance pointer evaluating the effectiveness of both the intrinsic and the common coagulation pathways. Apart from identifying anomalies in blood clotting, it can be used to monitor the treatment effects in individuals at risk of thrombosis on heparin therapy. It is used in combination with prothrombin time (PT) which measures the extrinsic pathway. The haemostatic results in this study showed that the PT value of the study non- hormonal contraceptive (NHC) subjects was lower than that of the control subjects, though not significant; However the APTT value of the non- hormonal contraceptive users was significantly lower than that of the controls. In a study on the effect of copper ion on blood coagulation, Elias *et al*. (2004) showed that at high concentration (0.01mM), copper ions significantly increased blood coagulation time. The APTT values for progestin-only contraceptive (POC) users were significantly lower in comparison to that seen in the controls; However the PT values for progestin-only contraceptive subjects showed no significant difference when compared to the control subjects. Cheesbrough (2006) stated that a shortened prothrombin time (PT) and activated partial thromboplastin time (APTT) increases the risk of thrombosis in patients.

Meanwhile, there was no significant difference in the APTT of combined hormonal contraceptive (CHC) subjects when compared to controls. This is consistent with the study of Abdalla *et al*. (2008) who reported no significant changes in the values of APTT in combined hormonal contraceptives (CHC) users. An elevated PT value was observed in combined hormonal contraceptives users when compared with the control group, this is in contrasts to the studies of Abdalla *et al*. (2008) who reported a normal PT among combined hormonal contraceptive (CHC) users. Babatunde and Olatunji (2004) in Ilorin reported a normal APTT, but with a shortened PT among CHC users. Aldrighi *et al.* (2006) after conducting combined hormonal contraceptives trials in Sao Paulo, reported significant increase in APTT and PT results. Elsayid *et al*. (2015) in a study conducted among Sudanese women on combined hormonal contraceptives, observed a slight increase in PT values though not statistically significant. Nasir *et al.* (2008) reported a shortened APTT among combined hormonal contraceptive users. The differences reported among these studies may suggest the possible alterations in the normal ranges across different geographical regions and races (Erhabor *et al*., 2014).Comparing the APTT and PT values of progestin- only contraceptive (POC) users and combined hormonal contraceptive (CHC) users with non- hormonal contraceptive (NHC) users, it was observed that the APTT and PT values of POC users were not statistically

different from NHC users, while that of CHC users were both prolonged compared to NHC users. Similarly, combined hormonal contraceptive subjects showed prolonged APTT and PT values when compared with progestin-only contraceptive subjects. Abubakar (2013) stated that haemostatic derangement among hormonal contraceptive users is not streamlined in a particular pattern, and ethnic and/or racial variations may occur.

Oestrogen is associated with several prothrombic variations in coagulation proteins which include increased level of factors II, VII, VIII, X and fibrinogen, decreased levels of antithrombin and protein S and activated protein C resistance (Trenor *et al*., 2011). These changes could manifest in haemostatic parameters as seen in this study and several others. It could also explain why combined oral contraceptives are found to cause a proportionately haemostatic alteration. Progestin-only contraceptives caused significant alterations in haemostatic parameters as observed in POC subjects in this study. This was supported by the findings of Ajayi *et al*. (2007) and Joseph *et al*. (2008) who reported alterations in the haemostatic parameters among Nigerian women on injectable progestin-only contraceptives and implants respectively. However, it has been postulated that the thrombogenic risk associated with progestin-only contraceptives depends on the type, mechanism of delivery and length of therapy (Trenor *et al*., 2011).

CONCLUSION

The use of hormonal contraceptives results in altered haemostatic profile with an increased procoagulant activity conferring a higher thrombogenic risk on the users compared to non-users. This thrombogenic risk also varies with the hormonal content with the combined oestrogen and progestogen preparations having a higher risk than the progestin-only contraceptives.

ACKNOWLEDGMENTS

We want to specially thank the staff and management of Faith Mediplex Hospital, Central hospital and Planned Parenthood Federation of Nigeria (PPFN) in Benin City, Edo State for allowing us use their facilities for this research

REFERENCES

Abdalla, T. M., Kordofani, A. A. Y. and Nimir, A. A. H. (2008). Haemostatic studies in Sudanese women on oral contraceptive pills. *Khartoum Medical Journal,* **1**(3): 116-118.

Abdollahi, M., Cushman, M. and Rosendaal, F. R. (2003). Obesity: risk of venous thrombosis and the interaction with coagulation factor levels and oral contraceptive use. *Journal of Thrombosis and Haemostasis*, **89**: 493-498.

Abubakar, S. B. (2013). Some haemostatic changes in women on hormonal contraceptives attending family planning clinic at Ahmadu Bello University Teaching Hospital Shika-Zaria, Nigeria. *Faculty of Internal Medicine,* **19**: 49-55.

Adejumo, E. N., Adedeji, I. O. and Akinmulero, A. O. (2016). Effect of hormonal contraceptives on the total antioxidant status of women from Isolo, Lagos state. Nigeria. *Journal of Biosciences and Medicine,* **4**: 107-111.

Ajayi, O., Ajayi, O. and Obarhua, E. (2007). Haemostatic Evidence of Thrombotic Signaling in Nigerian Women on Injectable Contraceptives. *Haematologica*, **92**(2): 434-434.

Akhigbe, R. E., Azeez, M. O., Ige, S. F., Oyeyipo, L. P., Ajao, F. O. and Alade, A. O. (2008). Haematological effects of long-term administration of combined oral contraceptive in rats. *International Journal of Pharmacology*, **4**(5): 403-406.

Akinloye, O., Adebayo, T. O., Oguntibeju, O. O., Oparinde, D. P. and Ogunyemi, E. O. (2011). Effects of contaceptives on serum trace elements, calcium and phosphorus levels. *West Indian Medical Journal*, **60**(3): 308-315.

Aldrighi, J. M., De Campos, L. S., Eluf Gebara, O. C., Petta, C. A. and Bahamondes, L. (2006). Effect of a combined oral contraceptive containing 20 microg ethinyl estradiol and 75 microg gestodene on hemostatic parameters. *Gynecological Endocrinology,* **22**(1): 1–4.

Babatunde, A. S. and Olatunji, P. O. (2004). Short-term effect of oral contraceptive pills on some haemostatic parameters in healthy Nigerian women. *The Nigerian Postgraduate Medical Journal,* **11**(4): 246–250.

Cheesbough, M. (2006). Investigation of bleeding disorders. District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press Publication. Pp. 340-347.

Elsayid, M., Elbasheer, M. A. M., Elgari, M. M. and Elfaki, T. E. M. (2015). The Effect of Contraceptive Pills on Coagulation Tests among Sudanese Women in Khartoum State-Sudan. *International Journal of Science and Research,* **5**(10): 773-777.

Erhabor, O. I., Isaac, I. Z., Kaoje, A. U., John, R. T. and Suleiman, S. A. (2014). Assessment of Some Coagulation Parameters among Clients on Hormonal Contraceptive in a Tertiary Health Facility in Sokoto, North Western, Nigeria. *Journal of Hematology and Thromboembolic Diseases,* **2**: 139.

Hall, J. E. (2015). Hemostasis and Blood Coagulation. Guyton and Hall textbook of Medical Physiology. 12th edition. Chapter 36. Saunders Elsevier Inc. Philadelphia, PA, USA. Pp. 451-460

Hugon-Rodin, J., Gompel, A. and Plu-Bureau, G. (2014). Epidemiology of hormonal contraceptives-related venous thromboembolism. *European Journal of Endocrinology*, **171**: 221–230.

Joseph, J. T., Abdulazeez, A. A. and Obisesan, O. A. (2008). Effect of hormonal contraceptives on some haemostatic parameters in women attending family planning clinics in Jos, Nigeria. *Nigeria Journal of Health and Biomedical Science*, **7**(1): 15-18.

Lidegaard, O., Edstrom, B. and Kreiner, S. (2002). Oral contraceptives and venous thromboembolism: A five-year national case-control study. *Contraception*, **65**(3): 187-196.

Martin, D. and Elliott-Sale, K. (2016). A perspective on current research investigating the effects of hormonal contraceptives on determinants of female athlete performance. *Revista Brasileira de Educacao Fisica e Esporte*, **30**(4): 1087-1096.

Naess, I. A., Christiansen, S. C., Romundstad, P., Cannegieter, S. C., Rosendaal, F. R. and Hammerstrom, J. (2007). Incidence and mortality of venous thrombosis: A population-based study. *Journal of Thrombosis and Haemostasis*, **5**: 692–699.

Nakada, D., Oguro, H., Levi, B. P., Ryan, N., Kitano, A. and Saitoh, Y. (2014). Oestrogen increases haematopoietic stem cell self-renewal in females and during pregnancy. *Nature*, **505**: 556-564.

Nasir, A., Qamaruddin, B. and Salman, N. A. (2008). Effect of low dose oral pill on haemostatic parameters in a set of Pakistani population. *The Journal of the Pakistan Medical Association*, **58**(5): 229–233.

National Population Commission (NPC). (2019). Nigeria Demographic and Health Survey 2018. Abuja. Pp. 1- 469.

Palmery, M., Saraceno, A., Vaiarelli, A. and Carlomagno, G. (2013). Oral contraceptives and changes in nutritional requirements. *European Review for Medical and Pharmacological Sciences*, **17**: 1804-1813.

Plu-Bureau, G., Hugon-Rodin, J., Maitrot-Mantelet, L. and Canonico, M. (2013). Hormonal contraceptives and venous thromboembolism: An epidemiological update. *Best Practice and Research Clinical Endocrinology and Metabolism*, **27**(1): 25-34.

Thirup, P. (2003). Haematocrit: Within subject and seasonal variation. *Sports Medical Journal,* **33**: 231-243.

Trenor, C. C., 3rd, Chung, R. J., Michelson, A. D., Neufeld, E. J., Gordon, C. M., Laufer, M. R. and Emans, S. J. (2011). Hormonal contraception and thrombotic risk: a multidisciplinary approach. *Pediatrics*, **127**(2): 347–357.