Original Article

Effect of Low Dose Oral Pill on Haemostatic Parameters in a Set of Pakistani Population

Nasir Ali Afsar[1], Qamaruddin Barakzai[2], Salman Naseem Adil[3]

Department of Pharmacology[1,2], Ziauddin Medical College, Ziauddin University, Karachi, Department of Pathology[3], The Aga Khan University, Karachi, Pakistan.

Abstract

Objective: To observe the adverse effects of low dose combination contraceptive pills on blood coagulation in a set of local population.

Methods: Between December 2002 to December 2003 a comparative cross-sectional study was conducted at the Department of Pharmacology, Ziauddin Medical University, Karachi and Aga Khan University Hospital, Karachi. Fifty women of reproductive age were divided in two equal groups; one being the users of combination oral contraceptive pills (ethinyl estradiol and levonorgestrel) and the other being matching controls not taking any hormonal contraceptives. We studied, CBC, PT and INR, APTT, BT and platelet aggregation against ADP, collagen, epinephrine and ristocetin.

Results: PT, INR, and platelet aggregation response to ADP, collagen, epinephrine, and ristocetin were not significantly different among the groups. However, APTT was shortened in users of contraceptives ($p = 0.003$).

Conclusion: The referred oral contraceptive is associated with enhanced activity of intrinsic pathway of secondary haemostasis (JPMA 58:229;2008).

Introduction

The estrogens and progestins are widely used contraceptives; however their safe use has remained under debate. Thromboses had been associated with both the components, i.e. estrogens[1] and progestins[2] of such pills. As the use of these contraceptives gained momentum, much larger and diverse population got involved with reports of epidemiological differences regarding the adverse outcomes of the hormonal contraceptives.[3-4] In order to decrease the risk of adverse effects, modern combination oral contraceptive (COC) pills have low estrogen content, but even at lower doses, such hormonal therapy users run a greater risk of circulatory adverse effects as compared to the nonusers[5-7], warranting careful monitoring. In Pakistan, it is observed that, the rational prescription of hormonal contraceptives is not uniformly practiced. To complicate the problem, there are certain factors prevailing locally that might pose a potentially higher thrombotic risk among users of hormonal contraceptives, such as chronic rheumatic heart disease, which is often not detected timely.[8] In addition there is a concern over population explosion and an overzealous drive to control it, led by both public and private sector. To achieve the target, the hormonal contraceptives are used indiscriminately.

As described earlier, it is reported that there is a difference in incidence of adverse outcome(s) attributable to different populations.[3,4,9,10] Combination oral contraceptives increase ADP (Adenosine diphosphate) - induced aggregation and fibrinogen binding.[11] They also induce marked and permanent increase in cell membrane lipid biosynthesis.[12] Since platelet membrane lipids play an important pro-coagulant role in haemostasis, an enhanced synthesis can have a profound impact on haemostatic physiology, when considered along with other changes. The role of genetic factors as a major determinant of platelet response is also reported.[13] It is also reported[14] that vWF (von Willebrand Factor) antigen expression as well as the amount of functional vWF activity was reproducibly and significantly higher in estradiol-treated endothelial cells when compared with control values, largely through an enhanced endothelial production of vWF as well as a slight increase in endothelial cell replication. This may further explain the increased adherence of platelets to vascular endothelium and propensity of thrombosis. Another study[15] showed that hormonal contraceptive treatment shortened the APTT and euglobulin lysis time.

In addition, levonorgestrel, a progestin, has been associated with increased serum factors V and X levels, shortened PT and APTT[2], thus favouring hypercoagulability. The same drug is part of the COC pill most widely available in the local market.

Taking into account all these above mentioned factors it can be inferred that the use of hormonal contraceptives is likely to cause adverse prothrombotic effects, especially when the picture is still not clear about the risks of taking such hormonal preparations in different

populations.[16,17] Therefore, we designed this study to observe the effects of hormonal contraceptives on haemostatic parameters in the local setting.

Subjects and Methods

The current study was designed as a comparative cross sectional study from December 2002 to December 2003 at Department of Pharmacology, Ziauddin Medical College, Ziauddin University, Karachi, in collaboration with Department of Haematology, The Aga Khan University, Karachi. The study was approved by Ethical Review Committee of Ziauddin University, Karachi. The study population comprised of fifty females of reproductive age divided into two equal groups. One group (n = 25) comprised of females exposed to low dose combination oral contraceptives (ethinyl estradiol 30 µg and levonorgestrel 150µg taken for 21 days in a cycle) and the other group (n = 25) was of matching control subjects, not using any hormonal contraceptives. All the subjects were inducted from various clinics/health centers in Karachi, offering family planning services. All the subjects gave informed consent at the time of induction. Only healthy and non-pregnant women of age group 20-45 years, who had at least one normal cycle after delivery/abortion and showed their willingness for participation were inducted. The only difference between the groups was the use/no use of combination oral contraceptive pills for at least preceding four months. At the time of induction, it was ensured that all women who were nulliparous and with a history of smoking/addiction, bleeding or thrombotic illness, menstrual disorders, any condition characterized by physiological stress, such as surgery and trauma or those using any antiplatelet agent, any procoagulant or anticoagulant were excluded. Bleeding Time, complete blood cell count, absolute indices, platelet aggregation studies against ADP, collagen, epinephrine and ristocetin, prothrombin time/ INR, activated partial thromboplastin time (APTT) were the parameters investigated.

Platelet aggregation was measured in platelet rich plasma with Chrono-Log Lumi-aggregometer 400® according to the manufacturer's specifications. The principle of detection was "turbidimetry". When platelets are free they impart the turbidity to the plasma thus hindering the light while it passes through it. With increasing platelet aggregation, the turbidity and thereby the resistance offered to light beam passing through it is reduced. This change in light intensity is detected by the instrument.

PT, INR and APTT were done using Sysmex® CA-1500 Automated Blood Coagulation Analyzer according to the manufacturer's specifications. The principle of detection was "scattered light detection". When blood is not clotted, it scatters little light and lets pass more through it. However, a clot hinders and thereby scatters more light. This interruption in light path is detected by the instrument.

The data analysis was done on computer package EPI Info® ver. 6.0 software of CDC (Center for Disease Control, Atlanta, USA). The results were given in the text as mean + standard deviation (S.D.) for quantitative variables and as number and percentage for qualitative variables. Student's t-test was applied to compare mean and standard deviation of quantitative variables between groups. Chi-square test was applied to compare the proportion/ percentages of qualitative variables between groups. In all statistical analyses, only a p-value < 0.05 was considered significant.

Results

All the study population was compared for the baseline characteristics (Table 1), showing no significant difference. The median duration of use for COC was 78 (Range: 13 - 470) weeks and 64% were using the pill for a year or above.

A comparison of the haemostatic parameters (Table 2) showed a statistically significant difference regarding mean bleeding time values among groups. The mean value was shorter in the oral contraceptive group as compared to controls. However, the difference regarding the platelet count as well aggregation to ADP, collagen, epinephrine, and ristocetin was not significant. Among the groups a comparison of prothrombin time (PT) as INR, and activated

Table 1. Base-Line Characteristics of Study Population.

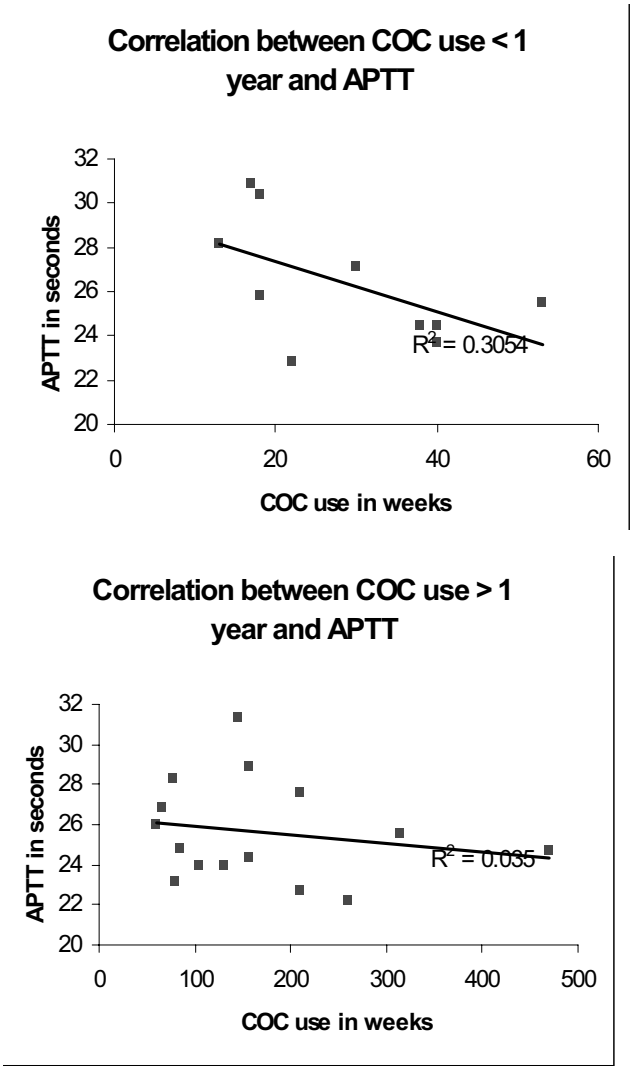
Parameter (units)	COC (n=25)	Controls (n=25)	P-value
	Mean S.D	Mean S.D	
Age (Years)	32 ± 5.9	31 ± 5.5	0.594
Body Mass Index	24.2 ± 6.1	23.5 ± 4.7	0.667
Pulse (per min.)	83.8 ± 6.3	82.8 ± 6.0	0.567
Systolic Blood Pressure (mmHg)	114 ± 9.2	112 ± 7.1	0.641
Diastolic Blood Pressure (mmHg)	74 ± 8.2	75 ± 7.5	0.721
Respiration (per minute)	17.1 ± 1.1	16.6 ± 1.3	0.213
Number of Pregnancies	4.1 ± 2.1	3.2 ± 2.0	0.132
Parity	3.8 ± 1.9	2.8 ± 1.7	0.063
RBC count (106/µl)	4.4 ± 0.51	4.6 ± 0.39	0.111
Haemoglobin (g/dl)	11.9 ± 1.14	12.1 ± 1.24	0.226
Haematocrit (%)	35.8 ± 3.27	37.3 ± 3.18	0.102
MCV (fl)	81.4 ± 8.67	81.0 ± 7.73	0.872
MCH (pg)	27.2 ± 3.32	26.7 ± 2.96	0.629
MCHC (g/dl)	33.3 ± 0.93	32.9 ± 1.13	0.186
TLC (103/µl)	9.1 ± 3.30	8.5 ± 1.89	0.568

All values given as mean ± standard deviation
COC = Combination Oral Contraceptives

Table 2. A comparison of hemostatic parameterseheh of study population.

Parameter (units)	COC (n=25)	Controls (n=25)	P-Value
	Mean ± S.D	Mean ± S.D	
Platelet Count (103/µl)	258 ± 100	294 ± 69	0.145
Bleeding Time (min)	1.9 ± 0.48	2.6 ± 0.78	0.001*
Aggregation to ADP (%)	48.9 ± 15.4	48.3 ± 20.5	0.901
Aggregation to Epinephrine (%)	47.6 ± 14.8	49.3 ± 22.4	0.745
Aggregation to Collagen (%)	49.1 ± 14.2	47.1 ± 20.6	0.700
Aggregation to Ristocetin (%)	46.8 ± 14.9	46.9 ± 19.5	0.974
Prothrombin Time (seconds)	11.8 ± 2.27	12.4 ± 1.08	0.207
INR	1.02 ± 0.08	1.02 ± 0.09	0.814
APTT (seconds)	25.9 ± 2.61	28.3 ± 2.76	0.003*

All values given as mean ± standard deviation
* Significant P-value



COC - Combination oral contraceptive

partial thromboplastin time (APTT) was done (Table - 2). PT as well INR was not significantly different. APTT was shorter among the users of oral contraceptive pills as compared to the nonusers and showed a negative correlation, albeit weakly (R = 0.55, R2 = 0.305; Figure - 1) during first year of use, but not with prolonged use (R = 0.18, R2 = 0.035; Figure 1) despite being maintained at a value less than the controls.

Discussion

Despite the limitation launch of level blinding, this study gives an insight into an important health related issue. Our study suggests that at the therapeutic levels of the particular combination oral contraceptive, which is most the widely available pill in Pakistan, there is no altered platelet aggregation response to ADP, collagen, epinephrine and ristocetin. Previous studies[17-19] reported enhanced platelet response among the users of hormonal contraceptives. Genetic differences in the platelet surface glycoprotein structures, such as GPIIb/IIIa receptors are in part involved in the population-to-population variation regarding the platelet aggregation response.[13,20] Though the sample size is small, it hints that the specific hormonal contraceptive studied, is less likely to disturb the platelet homeostasis to the point where it can manifest. The reason for this observation may be attributed to multiple factors such as genetic makeup, dietary habits or other mechanisms thus paving the way for further studies.

Though bleeding time was lower in users of combination oral contraceptives as compared to nonusers, no inference can be made on the basis of the bleeding time alone. The reason for this includes inter-individual variation in forearm epidermal thickness, age, skin temperature, venous pressure, direction of incision, experience of the personnel, and lack of blinding in the study.

Literature suggests[2,6,16,21-23] that combination oral contraceptives predispose to thromboses. In some of these studies, APTT (representing intrinsic and common pathway) and PT as INR (representing extrinsic and common pathway) were reported shorter among users of combined oral contraceptive pills. Besides this many factors favouring coagulation were reported elevated. Recently, Denes et al[24] have reported in a large cohort that postmenopausal women taking hormone replacement therapy are more vulnerable to adverse cardiovascular outcomes. A similar notion had been suggested elsewhere.[25] It is important to understand that postmenopausal hormone replacement therapy employs much lower doses of estrogen and progestins than required for contraception. Our study shows significantly shorter APTT rather than PT (INR) in users thus hinting towards the accelerated activity in intrinsic pathway of haemostasis. A negative correlation was noted between APTT values and

duration of use of the COC, with statistical significance only during the first year of use. In the subsequent years, though the trend continued, it did not show significance suggesting a gradual decline in APTT. However the sample size is small and needs confirmation through a larger study. Therefore it is difficult to figure out the precise importance of this particular finding.

Since this is not a randomized, double-blind, placebo-controlled trial, we cannot ascertain the cause-effect relationship of the observations. Nonetheless, the findings are important in the perspective of the social, cultural and personnel-related circumstances prevailing locally. Recruitment to such a study is not easy partly because the said pills are in access even without the prescription of a qualified physician. Media tends to pour them into masses without projection of pros and cons. Moreover, the population is not well aware of the potential benefits of enrolment in such studies. Obtaining consent is also difficult because most of the users are not willing to interact regarding their contraceptive practices. Lastly, the funding opportunities are minimal especially to the private sector. This study was realized after the hard work of the health care providers at the family planning facilities, consistency of the investigators and support from the grant-providing institution.

As a result of this study, further studies, preferably double-blinded, placebo-controlled, are warranted in this regard addressing the questions such as the importance of the genetic polymorphism in different genes affecting the platelet function, and the quantitative assay of the various clotting factors, especially those involved in intrinsic and common pathways. Till that time the correct approach, at least in our set up could be to discourage oral pill use unless suggested by a physician after proper screening and then following up the user with periodic history, physical examination and laboratory analysis. In a developing country like Pakistan, where only a small fraction of the GDP is reserved for health care, literacy rate is low, many people do not have access to health care facilities, community-oriented care is not the practice and quackery is not yet abolished, this becomes even more imperative. Due to a countrywide scarcity of emergency and advanced life support facilities, if a hormonal contraceptive user develops a serious cardiovascular adverse effect, the mortality is expected to be high.

Financial Support

This study was supported by a grant from Ziauddin University, Karachi.

Acknowledgement

We are thankful to the colleagues at Ziauddin Medical University who extended their cooperation and to all the staff at Family Health Hospital, Britto Road, Karachi for their help. The study would not have been possible without the support and guidance of Dr. Shamim, Dr. Huma and Ms. Shahnaz. We are also grateful to all the staff at Clinical Laboratory, The Aga Khan University Hospital, Karachi for their cooperation.

References

1. Inman WH, Vessey MP, Westerholm B, Engelund A. Thromboembolic disease and the steroidal content of oral contraceptives. BMJ. 1970; 2:203-09.
2. Viegas OA, Singh K, Koh S, Singh P, Ratnam SS. The effects of Norplant on clinical chemistry in Singaporean acceptors after 1 year of use: I. Haemostatic changes. 1: Contraception 1988; 38:313-23.
3. Leck I, Thomson JM, Bocaz JA, Barja P, Bonnar J, Daly L, et al. A multicentre study of coagulation and haemostatic variables during oral contraception: variations with geographical location and ethnicity. Task Force on Oral Contraceptives - WHO Special Programme of Research, Development and Research Training in Human Reproduction. Int J Epidemiol. 1991; 20:913-20.
4. Bocaz JA, Barja P, Bonnar J, Daly L, Carrol A, Coutinho E, et al. Differences in coagulation and hemostatic parameters in normal women of childbearing age from different ethnic groups and geographical locations. Task Force on Oral Contraceptives- WHO Special Programme of research, Development and Research Training in Human Reproduction. Thromb Haemost 1986; 55:390-95.
5. Cushman M, Kuller LH, Prentice R, Rodabough RJ, Psaty BM, Stafford RS et al. Estrogen Plus Progestin and Risk of Venous Thrombosis. JAMA. 2004; 292: 1573-80.
6. Lidegaard O, Edstrom B, Kreiner S. Oral contraceptives and venous thromboembolism: a five-year national case-control study. Contraception. 2002; 65:187-96.
7. Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case-control study. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet. 1995; 346:1575-82.
8. Population Reports. May-June 1982; Series A, No. 6 [Serial online]. Available at http://www.jhuccp.org/pr.
9. Chan P, Yeh CJ, Pan WH. Influence of Contraceptive Pill Use, Premenopause and Postmenopausal State on Hemostatic Parameters in Ethnic Chinese Women. J Thromb Thrombolysis 1996; 3:09-12.
10. Tsakok FH, Koh S, Yuen R, Ratnam SS. Coagulation Studies in Asian women using injectable progestogen for contraception. Int J Gynaecol Obstet. 1980; 18:105-08.
11. McGregor L, Toor B, McGregor JL, Renaud S, Clemetson KJ. Effects of oral contraceptives, or lanosterol, on ADP-induced aggregation and binding of 125I-fibrinogen to rat platelets. Thromb Res. 1984; 33:517-22.
12. Ciavatti M, Davenas E, Blache D, Monnier MA, Renaud S. Biosynthesis of platelet lipids in relation to aggregation in women using oral contraceptives. Contraception. 1982; 25: 629-38.
13. O'Donnell CJ, Larson MG, Feng D, Sutherland PA, Lindpaintner K, Myers RH, et al. Genetic and environmental contributions to platelet aggregation: the Framingham heart study. Circulation. 2001; 103:3051-56.
14. Harrison RL, McKee PA. Estrogen stimulates von Willebrand factor production by cultured endothelial cells. Blood. 1984; 63:657-64.
15. David JL, Gaspard UJ, Gillain D, Raskinet R, Lepot MR. Hemostasis profile in women taking low-dose oral contraceptives. Am J Obstet Gynecol. 1990; 163 (1 Pt 2):420-23.
16. Grady D, Wenger NK, Herrington D, Khan S, Furberg C, Hunninghake D, et al. Postmenopausal hormone therapy increases the risk for venous thromboembolism. The Heart and Estrogen/progestin Replacement Study (HERS). Ann. Intern. Med. 2000; 132: 689-96.
17. Spitzer WO, Lewis MA, Heinemann LA, Thorogood M, MacRae KD. Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case-control study. Transnational Research Group on Oral Contraceptives and health of Young Women. BMJ. 1996; 312:83-88.
18. Norris LA, Bonnar J. Haemostatic changes and the oral contraceptive pill. Baillieres Clin Obstet Gynaecol. 1997; 11:545-64.

19. Setiabudy RD, Affandi B, Witjaksono B, Wirawan R, Hendratna M, Hidayat E. The effect of Norplant on some hemostatic parameters in Indonesian women. Southeast Asian J Trop Med Public Health. 1993; 24 S1:234-36.

20. Feng D, Lindpaintner K, Larson MG, Rao VS, O'Donnell CJ, Lipinska I, et al. Increased platelet aggregability associated with platelet GPIIIa PlA2 polymorphism: the Framingham Offspring Study. Arterioscler Thromb Vasc Biol. 1999; 19:1142-47.

21. Archer DF, Mammen EF, Grubb GS. The effects of a low-dose monophasic preparation of levonorgestrel and ethinyl estradiol on coagulation and other hemostatic factors. Am J Obstet Gynecol. 1999; 181(5 Pt 2):63-66.

22. Creatsas G, Kontopoulou-Griva I, Deligeoroglou E, Digenopoulou L, Tsangaris A, Kallipolitis G, et al. Effects of two combined monophasic and triphasic ethinylestradiol/gestodene oral contraceptives on natural inhibitors and other hemostatic variables. Eur J Contracept Reprod Health Care. 1997; 2:31-38.

23. Kunz F, Pechlaner C, Tabarelli M, Solder E, Zwierzina WD. Influence of oral contraceptives on coagulation tests in native blood and plasma. Am J Obstet Gynecol. 1990; 163 (1 Pt 2):417-20.

24. Denes P, Larson JC, Lloyd-Jones DM, Prineas RJ, Greenland P. Major and minor ECG abnormalities in asymptomatic women and risk of cardiovascular events and mortality. JAMA. 2007; 297:978-85.

25. Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, et al. Estrogen plus progestin and the risk of coronary heart disease. N Engl J Med. 2003; 349:523-34.