EFFECT OF A 'PROGESTIN ONLY' CONTRACEPTIVE ON PLATELET AGGREGATION IN A PAKISTANI SET OF POPULATION

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Background: Safe use of progestin-only hormonal preparations has remained quite controversial though they are being employed in various disorders very commonly. These hormones result in a number of adverse effects in the body, the most drastic being predisposition to higher risk of thromboembolism with reports of involvement of platelets. Despite a large number of studies, no definite inference had been derived regarding the effects of these agents on blood coagulation. It has been reported that the response in various ethnic groups may vary. Therefore this study is designed to observe the effects of progestin-only hormonal contraceptives regarding primary hemostasis in our population. **Methods**: We carried out this study on 50 women divided into two equal groups of 25 each, namely, a group of users of Progestin-only injectable hormonal contraceptive Norethisterone oenanthate 200 mg used as a 2-monthly injection and a control group and studied platelet aggregation against ADP, Collagen, Epinephrine and Ristocetin. **Results**: There was no statistical difference regarding the platelet aggregation studies against ADP, collagen, epinephrine and ristocetin between users and nonusers of progestin-only injectable contraceptive. The menstrual irregularities were found to be more frequent in users of injectable hormonal contraceptives than in non-users, especially amenorrhea and irregularities of menstrual flow. In users of injectable hormonal contraceptives, hemoglobin and hematocrit were slightly better maintained as compared to non-users. **Conclusion**: This suggests that Norethisterone oenanthate at currently used doses does not increase the platelet aggregation against ADP, collagen, epinephrine and ristocetin in our population.

Key Words: Progestin, Norethisterone, Contraceptive, Coagulation, Thrombosis, Thrombophilia, Platelet Aggregation.

INTRODUCTION

Progestins are widely used agents for multiple indications including contraception. Progestin-only hormonal contraceptives act by preventing ovulation through multiple mechanisms. They diminish frequency of Gonadotropin Releasing Hormone pulses,[1] produce thick, viscous cervical mucus to reduce sperm penetration and turn the endometrium non-receptive for implantation.[2]

Safe use of these hormonal preparations has remained quite controversial though they are being commonly employed in various disorders. These hormones result in a number of adverse effects in the body, however, the most drastic adverse effect is predisposition to higher risk of thromboembolic phenomena. Studies suggest that using progestin increase platelet count and aggregability, thus predisposing to hypercoagulability.[3-5] These agents also have antiplasmin and antithrombin activity.[6]

It is possible that hormonal contraceptives do not pose equal risk of thrombogenesis in different populations.[7,8] There are differences in the coagulation and haemostatic tests[9] between women using hormonal contraceptives from widely diverse geographical areas, the cause of which is not certain, however it could be related to the apparent varying incidence of thrombosis.[10]

The picture is still not clear about the risks of taking such hormonal preparations[11-16] in different populations.

Although there is a tremendous amount of research being carried out in the world today regarding the effects of these hormonal preparations on hemostatic mechanism, it is lacking in Pakistan despite the wide spread use of hormonal contraceptives.

We know that there are genetic and epidemiological differences between the response to female hormonal preparations.[17] WHO has also recommended that such studies should be conducted in different settings to bring about a clearer picture.[7,8,18,19]

Therefore we have designed this study to observe the effects of progestin-only hormonal contraceptives regarding primary hemostasis in our population.

MATERIAL AND METHODS

We conducted this study from October 2002 to September 2003 at Department of Pharmacology, Ziauddin Medical University, Karachi, in collaboration with Department of Hematology, The Aga Khan University, Karachi.

A total of 50 healthy females of reproductive age were inducted in the study from

various clinics providing family planning services and divided into two groups, namely, users and non-users, based on the use of norethisterone enanthate 200 mg 2-monthly injection for preceding 3 months at least. All subjects gave written informed consent. Subjects were evaluated by taking detailed history, performing physical examination as well as certain laboratory investigations, like complete blood count, bleeding time, serum glucose, urine examination. All had normal menstrual history and had at least one normal cycle after delivery/ abortion.

All those potential participants were excluded who had not given birth to a healthy live baby, had a history of menstrual irregularity before start of the contraception or had taken hormonal preparation due to any cause, were smoking or had any addiction, bleeding or thrombotic illness, any condition that is characterized by physiological stress, such as surgery, trauma etc. or were using any procoagulant or anticoagulant.

The study parameters included bleeding time, platelet count and morphology and platelet aggregation study against ADP, collagen, epinephrine, ristocetin. For platelet aggregation studies, 9 ml blood sample, collected through venipuncture was added to plastic tubes containing 1 ml sodium citrate solution. We used Chrono-Log Lumi-aggregometer 400® assembly according to the manufacturer's specifications, using platelet rich plasma. Each reading was taken for 5-minutes. The percent aggregation was calculated from the final chart reading (CR) as follows: Percent Platelet Aggregation = 90-CR / 90-10 × 100

The data was fed in the computer package MicrosoftExcel® and analysis was done on computer package EPI Info® ver. 6.0 software of CDC (Center for Disease Control, Atlanta, USA). 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. a p-value <0.05 was considered significant.

RESULTS

All the study population was compared for the baseline characteristics *(Table 1)* and demographic data *(Table 2)* to ensure the satisfactory similarity between the groups. The median duration of use for progestin-only contraceptives was 83 (Range: 13–470) weeks. A comparison of complete blood picture revealed that the women on progestin-only injectable contraceptives had greater mean hemoglobin, hematocrit, erythrocyte counts and absolute indices. However, only hemoglobin percentage differences were statistically significant. A statistically significant difference regarding mean bleeding time values among groups was seen. The mean value was shorter in the injectable contraceptive group as compared to control group. Otherwise, there was no statistically significant difference among the participants regarding various parameters.

Table 1: Base-Line Characteristics of Study Population

Parameter	*Users (n=25)*	*Non-Users (n=25)*	*P-Value*
	Mean ± S.D	*Mean ± S.D*	
Age (Yrs)	29 ± 6.7	31 ± 5.5	0.611
Height	1.55 ± 0.06	1.56 ± 0.07	0.566
Weight	59.3 ± 12.1	56.8 ± 9.6	0.568
BMI	24.8 ± 5.2	23.5 ± 4.7	0.656
Pulse /min.	85.8 ± 7.5	82.8 ± 6.0	0.128
Systolic BP	115 ± 7.7	112 ± 7.1	0.226
Diastolic BP	76 ± 5.6	75 ± 7.5	0.546
Respiration /min	17.2 ± 1.4	16.6 ± 1.3	0.190
RBC	4.7 ± 0.38	4.6 ± 0.39	0.628
Hemoglobin %	13.1 ± 1.04	12.1 ± 1.24	0.029
Hematocrit	38.8 ± 3.46	37.3 ± 3.18	0.093
MCV	84.2 ± 4.81	81.0 ± 7.73	0.086
MCH	28.1 ± 1.92	26.7 ± 2.96	0.054
MCHC	33.4 ± 0.78	32.9 ± 1.13	0.082
Total Leukocyte Count	9.2 ± 2.32	8.5 ± 1.89	0.229
Platelet Count	304 ± 97	294 ± 69	0.682
Bleeding Time	2.0 ± 0.60	2.6 ± 0.78	0.005

All values given as mean ± standard deviation

Table 2: Demographic Characteristics of Study Population

Parameters	*Users (n=25)*	*Non-Users (n=25)*
Ethnicity		
Baluch	2 (8)	2(8)
Punjabi	2 (8)	2 (8)
Pakhtoon	5 (20)	5 (20)
Sindhi	9 (36)	10 (40)
Urdu speaking	7 (28)	6 (24)
Socio-economic status		
< Rs. 5000/-	11 (44)	13 (52)
Rs. 5000-10,000/-	8 (32)	8 (32)
> Rs 10,000/-	6 (24)	4 (16)

All values given as number (percentage)

A comparison of the platelet aggregation studies *(Table 3)* showed that platelet aggregation to ADP, collagen, epinephrine, and ristocetin was not significantly different between the groups.

The obstetrical characteristics showed significant difference between groups regarding menstrual regularity and flow *(Table 4)*. As compared to 96% of subjects with regular menstrual cycles in the control group, only 48% in injectable

hormonal contraceptives group had regular menstrual cycle. All the women reported that prior to drug use their menstrual cycle was regular with a difference of not more than ± 3 days. The menstrual flow was estimated on history by taking into account the average number of the sanitary pads consumed during menstruation. As compared to 92% of subjects with moderate menstrual flow (defined as a bleeding of 25-80 ml) in the control group, only 2 % in injectable hormonal contraceptives group had moderate menstrual flow, rest falling in heavy (defined as a bleeding of > 80 ml), scanty (defined as a bleeding of <25 ml), or amenorrheic group.

Table 3: A Comparison of Platelet Aggregation* Response of the Study Population

Parameter	Users (n=25)	Non-users (n=25)	P-Value
Percent Platelet Aggregation to ADP	49.9 ± 16.7	48.3 ± 20.5	0.759
Percent Platelet Aggregation to Epinephrine	46.2 ± 16.2	49.3 ± 22.4	0.579
Percent Platelet Aggregation to Collagen	46.4 ± 16.1	47.1 ± 20.6	0.889
Percent Platelet Aggregation to Ristocetin	46.4 ± 15.3	46.9 ± 19.5	0.899

All values given as mean ± standard deviation
**Calculated as per the formula: Percent platelet aggregation = 90-CR / 90-10 × 100*

Table 4: Menstrual History of the Study Population

Parameters	***Users (n=25)***	***Non-Users (n=25)***	***P-Value***
Menstrual Regularity			
Regular	12 (48)	24 (96)	0.001
Irregular	10 (40)	1 (4)	
Amenorrhea	3 (12)	0	
Menstrual Flow			
None	3 (12)	0	0.009
Scanty	4 (16)	2 (8)	
Moderate	13 (52)	23 (92)	
Heavy	5 (20)	0	

All values given as number (percentage)

DISCUSSION

Safety has been a serious concern regarding hormonal contraceptive use. Initially they were developed as combination oral pills, containing both estrogen and progestin. Early pills had higher estrogen. Soon after the start of their use, studies demonstrated an increased incidence of thromboses among women taking the pill[20-22] related to the amount of estrogen in the various versions of the pill. Therefore, progestin-only pills were developed [23]. However, progestins are also held responsible for the similar outcomes.[4,5,8] Progestins may enhance the platelet phase of hemostasis, through multiple mechanisms, such as by increasing the platelet count and aggregability.[3-5] One study[4] reported increased platelet aggregation response to ADP among Indonesian users of progestin-only contraceptives. There are studies, which showed that the genetic differences in the platelet surface glycoproteins including GPIIb/IIIa receptors are in part involved in population to population variation regarding the platelet aggregation response.[24-27] As we know these glycoproteins act as receptors for various pro-aggregation agonists. Some studies suggest that among the users of hormonal contraceptives oxidative stress is observed, which also leads to free radical generation and enhanced platelet aggregation response.[28]

Our study suggests that norethisterone enanthate 200 mg 2-monthly injection does not affect the platelet aggregation versus ADP, collagen, epinephrine and ristocetin in our setting. On this particular aspect, the contemporary literature has little to offer. Our findings do not come in accord with other studies showing altered platelet response after using progestins for few years.[3-5] In our study, there were some participants who did not show any change in platelet aggregation even after using them continuously for five years or more. This could be attributed at least in part to possible difference in various platelet surface glycoproteins. Further studies to study the polymorphism in platelet surface glycoproteins are warranted.

In the present study, platelet aggregation was done only against more commonly studied agonists. There is a long list of other pro-aggregation agonists against which further studies could be done. Moreover, we studied platelet aggregation only among users of most commonly used progestin-only contraceptive. Further studies for platelet aggregation response among users of other progestins should be done to bring about a more comprehensive picture.

Although bleeding time is shortened among users, it may not be a significant finding due to two reasons: one, it is still in the normal range; two, there are multiple variables, including, defining the endpoint of bleeding, epidermal thickness in the forearm between races, age, skin temperature, venous pressure, direction of incision, and experience of the operator.

Though it was not the primary aim of the present study, however, it was observed that frequency of menstrual irregularities was higher in users of injectable hormonal contraceptives than in non-users. Among users of injectable hormonal

contraceptives, hemoglobin and hematocrit were slightly better as compared to non-users but erythrocyte count was not significantly different, suggesting that the change is due to less menstrual loss rather than erythroplasia. This could be expected as the users of progestin-only contraceptives had higher chances of developing amenorrhea or scanty menstruation. This is in accordance with the findings of earlier studies.[29,30]

CONCLUSION

Norethisterone oenanthate 200 mg 2-monthly injection did not increase the platelet aggregation against ADP, collagen, epinephrine and ristocetin in our set of population, suggesting that the specific hormonal contraceptives studied, at least do not disturb the platelet homeostasis to the point where it can manifest. Difference in the genetic makeup is a reasonably possible factor underlying the observed results, at least in part.

Declaration

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