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A three-year evaluation of hemostatic function in Singaporean Norplant acceptors

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Abstract

In a longitudinal study involving 100 Singaporean women, results indicate that the acceptors persist in having a possibly increased predisposition to thrombosis, as evidenced by a significant increase in platelet count and aggregability. Furthermore, the shortened prothrombin time and activated partial thromboplastin time, together with changes in vitamin K factors, still indicate the possible enhanced potential for hypercoagulation.

Introduction

Epidemiologic studies have indicated that the slight but significant increased risk of venous thrombo-embolism associated with the use of the combined contraceptive pill is related to the dose of estrogen in the pill [1]. These observations were supported by laboratory findings of changes in different coagulation and fibrinolytic parameters in users of these contraceptives [2,3]. The effects of progestogens on hemostatic function seem to be minimal but cannot be ruled out completely [4–6]. Moreover, there is very little detailed information on the use of levonorgestrel as a single agent and its effect on the changes induced in hemostatic function.

Given the long-acting nature of the progestogen-only Norplant^R system, this study was designed to study the effects of Norplant implants on hemostatic function in a representative Southeast Asian population.

Materials and methods

One-hundred women were recruited for this study. These volunteers were healthy, non-smoking, non-alcohol drinking and non-lactating Singaporean women who had chosen the Norplant system for contraception. Clinical assessment and blood sampling for the study were done prior to insertion of the implants and after 6, 12, 24 and 36 months of use. The subjects were used as their own controls. Blood samples

were obtained from fasting subjects, from the antecubital vein contralateral to the arm in which the implants were inserted, at the same time of the day (0900–1100 hours) on each occasion with the subjects having rested for at least half an hour prior to venipuncture.

Using plastic syringes, nine parts of blood was added to one part of 0.21 M Hepes (Sigma) in 0.129 M tri-sodium citrate (Merck) in cold plastic tubes. Mixing was done by inverting the tubes several times and keeping them cold in crushed ice. The blood was spun in a refrigerated centrifuge for 10–15 minutes at 2000 *g*. For platelet aggregation studies, the blood was kept at room temperature and platelet rich plasma (PRP) was obtained by centrifuging the blood at 180 *g* for 5 minutes. Immunoelectrophoresis of a α_2-macroglobulin (α_2-M), α_2-antitrypsin (α_2-AT) and α_2-antiplasmin (α_2-AP) was performed using plain sera. For plasminogen, ε-caproic acid (EACA) plasma was used. Serum with EACA-thrombin was used for immunological test for fibrinogen degradation products (FDP).

Laboratory tests using different methods were then performed to study the following hemostatic parameters: prothrombin time (PT) was estimated by the one stage method using home-made saline brain [7]. For activated partial thromboplastin time (APTT), Thomson's method was used [8]; for hematocrit and platelet count, Coulter Counter ZBI was used. Fibrinogen was estimated in plasma [9]. Coagulation Factor II [10], one stage assay for Factor V and VII using human deficient plasma [7], Factor VII [11] and Factor X [12] and antithrombin III (ATIII) [13] were also measured. Plasminogen activator activity on fibrin plate [14,15] and FDP [16] were also estimated. Platelet aggregation using ADP (Sigma) final concentration 10 μM and 3 μg equine collagen (Horm-Chemie) was estimated [17]. Laurell's rocket method was used to determine factor VIIIR: Ag, α_2-M, α_1-AT, α_1-AP, Protein C, plasma plasminogen and antithrombin III using antibodies purchased from Boehringer, Germany [18]. However for α_2-AP, the antibody was from Mochida, Japan and for Protein C, the antibody was from Diagnostic Stago, France.

Freeze-dried pooled HEPES-citrate plasma from at least 20 normal volunteers not on any medication was used as secondary standards. The volunteers were also used as normal controls for PT, APTT and ATIII clotting assays. Factor VIIIC and VIIIR:Ag were calibrated against the 1st and 2nd International Standard from the National Institute of Biological Standards and Controls (NIBSC), Holly Hill, England while Protein C was calibrated against standards from Diagnostic Stago, France. The locally prepared freeze-dried human brain thromboplastin used throughout this study has an International Sensitivity Index (ISI) of 1:16. Locally prepared International Reference Control was used for most assays in our internal quality control program, where test variations were monitored using Cusums plot. Commercial Platelet Control (Sarstedt, W. Germany) was used for control. As no standard or controls were available for platelet aggregation studies, the test variation was kept to a minimum by performing the test at about 90 minutes after blood collection. Streptokinase 100 iu/ml (Boehringer) was used as the internal reference control for plasminogen activator activity in fibrin plate.

The coefficient of variation of the various tests using different batches of reference control plasma or sera during the study period are summarized: PT (1.9–3.0%),

APTT (3.2–3.9%), fibrinogen (4.5–4.9%), Factor II (4.1–6.6%), V (6.6–8.9%), VII (3.3–4.8%), VIIIC (6.0–9.0%), X (7.7–9.7%), VIIIR:Ag (6.8–8.9%), plasminogen activator (11.0–14.0%), plasminogen (6.3–7.9%), ATII (functional) (2.3–2.5%), ATIII (antigen) (4.8–6.2%), Protein C (5%), α_2-AP (4.2–7.9%), α_2-M (5.4–5.6%) and α-AT (6.1–7.0%).

To assess the significance of differences from mean values, the paired 't' test was used. Further comparisons were also made by observing percentage changes in mean postinsertion levels compared to preinsertion levels which were designated as 100%. In the case where the results were found to be skewed, Wilcoxon signed rank test of statistical significance using median distribution was used to assess the statistical significance of the changes observed.

Results

Table 1 Hemostatic parameters in Singaporean Norplant acceptors: screening tests

Parameters studied	*Preinsertion*	*After 6 months of use*	*After 1 year of use*	*After 2 years of use*	*After 3 years of use*
Hemoglobin					
n	96	96	96	76	65
mean ± SD (g/dl)	12.1 ± 1.2	13.1 ± 1.4	13.4 ± 1.2	12.6 ± 1.3	12.1 ± 1.5
p value		< 0.001	< 0.001	< 0.001	NS
% difference from preinsertion mean	100	108.0	110.7	104.1	101.7
Hematocrit					
n	96	96	96	76	65
mean ± SD (L/L)	0.38 ± 0.03	0.40 ± 0.03	0.39 ± 0.03	0.38 ± 0.03	0.38 ± 0.03
p value		< 0.001	< 0.001	NS	NS
% difference from preinsertion mean	100	105.2	102.6	100.0	100.0
PT					
n	97	97	97	76	65
mean ± SD (s)	13.5 ± 0.7	13.2 ± 0.8	12.9 ± 0.8	12.9 ± 0.7	12.5 ± 0.7
p value		< 0.001	< 0.001	< 0.001	< 0.001
% difference from preinsertion mean	100	97.6	96.3	96.3	94.8
APTT					
n	97	97	97	76	65
mean ± SD (s)	42.2 ± 3.9	38.6 ± 2.8	38.6 ± 2.8	38.8 ± 2.7	41.0 ± 2.6
p value		< 0.001	< 0.001	< 0.001	< 0.05
% difference from preinsertion mean	100	91.6	91.9	93.0	97.4

Table 2 Hemostatic parameters in Singaporean Norplant acceptors: coagulation factors

Parameters studied	*Preinsertion*	*After 6 months of use*	*After 1 year of use*	*After 2 years of use*	*After 3 years of use*
Fibrinogen					
n	97	97	92	76	65
mean ± SD (g/L)	3.00 ± 0.52	3.23 ± 0.57	3.09 ± 0.54	2.99 ± 0.43	3.11 ± 0.46
p value		< 0.01	< 0.05	NS	NS
% difference from preinsertion mean	100	107.7	103.0	100.0	102.3
Factor II					
n	97	97	92	76	65
mean ± SD (%)	103.9 ± 13.9	99.1 ± 9.3	94.6 ± 9.3	99.5 ± 7.1	99.6 ± 10.8
p value		< 0.001	< 0.001	< 0.001	< 0.05
% difference from preinsertion value	100	95.3	90.3	95.4	96.3
Factor V					
n	97	97	92	76	65
mean ± SD (%)	101.5 ± 18.0	115.7 ± 18.1	116.7 ± 15.6	99.5 ± 12.5	95.7 ± 10.5
p value		< 0.001	< 0.001	NS	NS
% difference from preinsertion mean	100	114.0	114.4	98.6	97.3
Factor VII					
n	97	95	92	76	65
mean ± SD (%)	118.3 ± 21.8	99.5 ± 16.9	102.7 ± 17.6	98.6 ± 18.0	96.3 ± 18.5
p value		< 0.001	< 0.001	< 0.001	< 0.001
% difference from preinsertion mean	100	84.1	85.7	82.3	81.3
Factor VIII					
n	97	97	92	76	65
mean ± SD (units/ml)	1.06 ± 0.98	1.04 ± 0.92	1.10 ± 0.92	0.91 ± 0.25	0.89 ± 0.27
p value		NS	NS	NS	NS
% difference from preinsertion mean	100	98.0	102.8	87.5	92.7
Factor VIIIR:Ag					
n	96	96	92	72	65
mean ± SD (units/ml)	0.84 ± 0.28	0.80 ± 0.28	0.83 ± 0.31	0.89 ± 0.31	0.98 ± 0.29
p value		NS	NS	NS	NS
% difference from preinsertion mean	100	95.0	97.6	104.7	110.1
Factor X					
n	97	97	92	76	65
mean ± SD (%)	79.4 ± 15.1	88.1 ± 14.1	87.1 ± 14.1	92.9 ± 13.5	87.2 ± 13.5
p value		< 0.001	< 0.001	< 0.001	< 0.001
% difference from preinsertion mean	100	110.9	109.1	118.2	114.7

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Table 3 Hemostatic parameters in Singaporean Norplant acceptors: fibrinolytic activity

Parameters studied	*Preinsertion*	*After 6 months of use*	*After 1 year of use*	*After 2 years of use*	*After 3 years of use*
Plasminogen activator					
n	97	97	92	74	65
mean ± SD (mm^2)	152.3 ± 50.3	155.0 ± 57.2	142.9 ± 55.6	121.9 ± 38.9	159.6 ± 53.3
p value		NS	NS	< 0.001	NS
% difference from preinsertion mean	100	102.0	93.7	82.1	107.5
Fibrinogen degradation products					
n	97	97	92	76	65
mean ± SD (μg/ml)	1.53 ± 1.09	2.28 ± 1.38	1.23 ± 0.64	1.61 ± 0.78	1.38 ± 0.72
p value		< 0.001	NS	NS	NS
% difference from preinsertion mean	100	155.9	90.5	110.3	89.6
Plasminogen antigen					
n	97	97	92	76	65
mean ± SD (mg/dl)	12.8 ± 2.0	13.1 ± 1.9	12.9 ± 2.1	12.8 ± 1.5	11.9 ± 1.5
p value		NS	NS	NS	NS
% difference from preinsertion mean	100	101.5	100	100.8	96.0

(1) Hemostatic changes

Tables 1–5 give the values of the variety of hemostatic factors studied. These include the screening tests and also the individual coagulation factors, fibrinolytic activity, coagulation inhibitors and platelet function. In view of the large number of subjects involved in the study, many of the differences over time achieved statistical significance using the paired 't' test but the majority of these were of little or no clinical significance.

The hemoglobin concentration increased from 12.1 g/dl to 13.4 g/dl during the first year. The hemoglobin concentration decreased subsequently during the next two years to reach its preinsertion concentration at the end of three years. Both the PT and APTT showed a significant shortening during the first year. The PT progressively shortened further in the subsequent two years, whereas the APTT increased progressively during the same period. However, despite these differences, both PT and APTT were significantly shorter than their preinsertion mean at the end of three years (Table 1).

Table 4 Hemostatic parameters in Singaporean Norplant acceptors: inhibitors of coagulation

Parameters studied	*Preinsertion*	*After 6 months of use*	*After 1 year of use*	*After 2 years of use*	*After 3 years of use*
Antithrombin III (functional)					
n	97	97	92	76	65
mean ± SD (%)	104.3 ± 8.2	105.8 ± 8.3	106.2 ± 9.4	101.1 ± 7.9	99.8 ± 7.1
p value		NS	NS	< 0.05	< 0.01
% difference from preinsertion mean	100	101.4	102.3	98.1	95.9
Antithrombin III (antigen)					
n	96	96	92	76	65
mean ± SD (%)	0.226 ± 0.02	0.239 ± 0.02	0.243 ± 0.02	0.232 ± 0.02	0.241 ± 0.02
p value		< 0.001	< 0.001	< 0.05	< 0.001
% difference from preinsertion mean	100	105.7	107.5	103.6	108.1
Protein C					
n	96	96	92	76	65
mean ± SD (%)	91.7 ± 14.8	87.8 ± 13.1	92.9 ± 12.1	88.7 ± 11.1	89.0 ± 12.7
p value		NS	NS	NS	NS
% difference from preinsertion mean	100	95.6	101.2	97.0	98.2
α1-Antiplasmin					
n	96	96	91	76	65
mean ± SD (mg/dl)	5.22 ± 0.72	5.33 ± 0.60	5.26 ± 0.60	5.20 ± 0.61	5.05 ± 0.57
p value		NS	NS	NS	NS
% difference from preinsertion mean	100	102.1	100.4	100.8	100.8
α2-Macroglobulin					
n	96	96	92	75	65
mean ± SD (g/L)	2.37 ± 0.54	2.89 ± 0.70	2.83 ± 0.75	2.72 ± 0.69	2.60 ± 0.70
p value		< 0.001	< 0.001	< 0.001	< 0.001
% difference from preinsertion mean	100	122.4	120.4	117.7	113.6
α1-Antitrypsin					
n	96	96	92	76	65
mean ± SD (g/L)	2.03 ± 0.38	2.02 ± 0.36	1.94 ± 0.31	1.79 ± 0.35	1.95 ± 0.26
p value		NS	< 0.05	< 0.001	NS
% difference from preinsertion mean	100	99.9	95.6	86.9	96.5

Table 5 Hemostatic parameters in Singaporean Norplant acceptors: platelet function

Parameters studied	*Preinsertion*	*After 6 months of use*	*After 1 year of use*	*After 2 years of use*	*After 3 years of use*
Platelet count					
n	95	95	94	75	65
mean ± SD (× 10^9/L)	206.9 ± 52.1	268.3 ± 72.6	278.5 ± 69.1	268.7 ± 68.3	256.5 ± 69.2
p value		< 0.001	< 0.001	< 0.001	< 0.001
% difference from preinsertion mean	100	129.6	134.9	129.4	125.6
Platelet aggregation (+ ADP)					
n	97	97	85	69	65
mean ± SD (max T %)	50.4 ± 13.8	55.1 ± 24.7	60.7 ± 20.2	60.2 ± 18.3	58.5 ± 11.0
p value		NS	< 0.001	< 0.001	< 0.001
% difference from preinsertion mean	100	109.3	122.1	121.4	125.8
Platelet aggregation (+ collagen)					
n	94	94	85	65	65
mean ± SD (max T %)	61.2 ± 10.3	57.7 ± 16.2	71.3 ± 8.9	81.3 ± 11.1	80.3 ± 10.3
p value		NS	< 0.001	< 0.001	< 0.001
% difference from preinsertion mean	100	94.3	118.0	135.0	136.1

Regarding the coagulation factors, the vitamin K dependent Factors II and VII showed a significant decrease, while Factors V and X showed a significant increase during the first year of use. Over the subsequent two years, the concentration of factor II increased minimally, while the concentration of Factor VII decreased further. However, despite these changes, the concentrations of both Factor II and VII were still significantly lower at the end of three years, as compared to the preinsertion concentrations. Factor V, which had shown a 14.4% increase during the first year, decreased significantly to a value slightly below its preinsertion concentration at the end of the second and third year. On the other hand, the concentration of Factor X continued to increase significantly and was 14.7% above its preinsertion concentration at the end of three years (Table 2).

Besides a short but marked increase in FDP in the first six months, no other appreciable changes in parameters relating to fibrinolytic activity were observed in the three years of Norplant use (Table 3).

In terms of coagulation inhibitors, the only consistent changes seen over the three years were the significant elevation of antithrombin III (antigen) and α_2-macroglobulin concentrations (Table 4). The other parameters did not show any definite trend of change.

Major changes were observed in platelet activity. Mean platelet count rose significantly from 207 × 10^9/L to 279 × 10^9/L at the end of one year. Despite a

progressive decrease in the subsequent two years, the mean platelet count of 257 × 10^9/L at the end of three years was still significantly higher than the preinsertion platelet count (Table 5). Platelet aggregation using ADP or equine collagen showed a significant rise during the three years and both parameters were significantly increased as compared to their preinsertion mean at the end of three years (Table 5).

Discussion

At the end of three years of use, certain changes seen in the hemostatic system in the first year [19,20] are found to be still continuing. The increased platelet count and enhanced platelet aggregation suggest that our Norplant acceptors may have a possibly increased predisposition to thrombosis. Furthermore, the shortened PT and APTT, together with changes in the vitamin K dependent factors indicate the possible enhanced potential for hypercoagulation. While it is true that there is no single laboratory test or combination of tests that will reliably predict a patient who will develop a thrombo-embolic event [21], the results of this on-going study merit serious consideration.

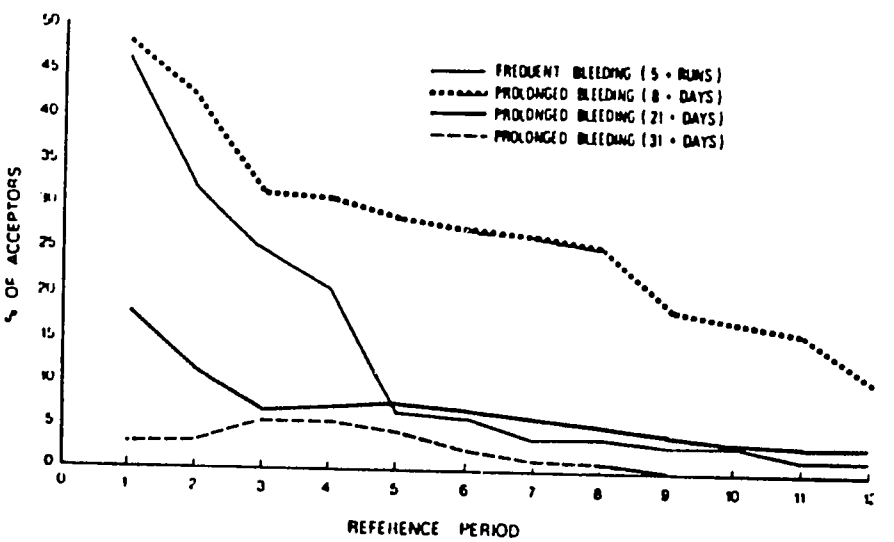

Figure 1 Percentage of Norplant acceptors with increased frequency of menstruation and prolonged bleeding/spotting by reference period

The findings of this study on the changes in hemostatic function are at variance with those of Shaaban *et al.* [22]. We observed a decrease rather than an increase in Factor VII and an increase rather than a decrease in antithrombin III antigen levels. The Egyptian study observed changes over a six-month period only and the differences may well also reflect the different populations studied.

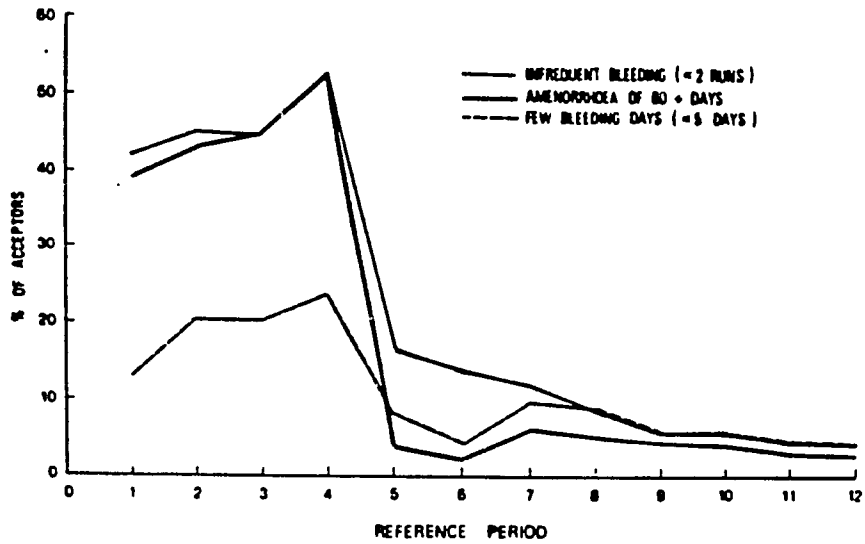

Figure 2 Percentage of Norplant acceptors with decreased frequency of menstruation and amenorrhea by reference period

Conclusion

The results of the effect of Norplant implants on hemostatic function in Singaporean acceptors emphasize the compelling need for long-term close surveillance of the Norplant system in different population subsets and for the entire five-year period of implant use among acceptors.

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Resumé

Dans l'étude longitudinale effectuée sur 100 femmes singapouriennes, les résultats indiquent que celles-ci ont continué à présenter ce qui est peut-être une prédisposition accrue à la thrombose, ainsi que l'a fait ressortir l'augmentation significative du nombre de plaquettes et de leur possibilité d'agrégation. En outre, le raccourcissement du temps de prothrombine et l'accélération du temps de thromboplastine partielle associés aux changements des facteurs de la vitamine K indiquent encore la possibilité d'une élévation potentielle de l'hypercoagulation.

Resumen

En el estudio longitudinal efectuado con 100 mujeres de Singapur, los resultados indican que continúan presentando lo que puede ser una predisposición acrecentada a la trombosis, según lo señalado por un aumento significativo del número de plaquetas y su posible agregación. Por otra parte, el acortamiento del tiempo de protrombina y la aceleración del tiempo de tromboplastina parcial asociados con los cambios de los factores de la vitamina K continúan indicando la posibilidad de un aumento potencial de la hipercoagulación.

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