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Oral Contraceptive Types in Relation to ABO Blood Groups Among Saudi Women of Different Reproductive Age Groups and Impact on Venous Thromboembolism

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Abstract

The risk varies of venous thromboembolism (VTE) is related to the type of Oral Contraceptive (OC) generations used. This study evaluated the effect of OC types in relation to ABO blood group on the risk of VTE among Saudi women. Thrombin generation (TG) was measured in the plasma of these women in the presence and absence of platelet rich plasma, platelet poor plasma and thrombomodulin or activated protein C. OC usage increased thrombin generation parameters ETP and Peak height by 9.81% and 16.04%, respectively. An increased risk of VTE was seen among women on third generation OCs as compared to those using second generation products. Within OC generation, we found that for those on fourth generation OCs, their ETP increased by 36.18% as compared to those using second generation and by 6.07% in those using third generation compared to those using second generation. There was significant difference with respect to ABO blood groups and OC generation types but larger sample size is required. Women who are 40 years and older and using third generation OC had a higher risk of having thrombosis (11.84%), as compared to those on second generation OC (8.79%), and for those using fourth generation OC (5.03%). An association between different OC groups and non-O blood group in thrombosis generation was noted. TG parameters were significantly increased in relation to BMI when comparing to OC users versus non-users. In addition, inhibition of TG parameters in the presence of recombinant human thrombomodulin (TM) and activates protein C (APC) were significantly increased.

Introduction

Deep Vein Thrombosis (DVT) is the presence of thrombus deeply into the pelvis or legs. It is associated with many burdensome and serious complications including pulmonary embolism (PE) and development of symptoms and signs of chronic venous insufficiency, known as Post-thrombotic Syndrome (PTS).[1,2] The incidence rate of Venous Thromboembolism (VTE) among adults is 1-2 per 1000 people, and age is considered to be an important risk factor. Aging contributes to an increased VTE incidence rate by around 1% annually.[3-5] VTE amplifies its recurrence at an annual rate of 5-10% after the first event. Moreover, such an occurrence is often associated with PTS. As a result of DVT, up to 40% of patients will develop an association between ABO blood type and the risk for VTE,[6] coronary heart disease[1] and atherosclerosis.[7] An increased bleeding tendency

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has been reported for blood group O.[8,9] In contrast, non-O blood group increased the risk of thrombosis caused by increased levels of FVIII-Von Willebrand.[8,10]

There are many other risk factors[3,11] for DVT including inherited blood clotting disorders, family history of DVT/PE, obesity, surgical procedures or injuries, pregnancy and especially the use of oral contraceptive (OC) pills[12] or hormone replacement therapy. A typical observation from several studies was a distinct relation between elevated body fat or body mass index (BMI) [13,14] and an increased incidence of thrombotic events and associated complications.[1,15,16] Different mechanisms were suggested to explain the role of obesity in increasing the risk of thrombotic events and included decreased fibrinolysis, increasing platelet aggregation and hyperfibrinogenemia.[17] Previously due to religious considerations, the use of OCs was not popular in the Saudi community. However, more recently, other factors have emerged including those who are working and changes in the habits of the Saudis in general.[18]

OC pills are a combination of 2 main components, estrogen and progestin. They are divided into 4 generations based on the type of progestin they contain. First generation progestin includes norethindrone, lynestrenol, ethynodiol diacetate, and norethisterone; second generation includes levonorgestrel and nongestural; third generation includes desogestrel, gestodene, and norgestodene; and fourth generation includes drospirenone and dienogest. The fourth generation is not recommended for those with predisposition to develop breast cancer.[19,20] The most commonly used types of OCs by Saudi women are second and third generation types.

The combined estrogen and progestogen OCs are known to increase the thromboembolic risk by unbalancing coagulation homeostasis and inducing a procoagulant state.[21]

The use of OCs has been associated with excessive thrombin generation (TG), as assessed by comparative studies of endogenous thrombin potential (ETP) between users and non-users, as well as between different progestogens.[22] This excess in TG is explained by increased levels of procoagulant factors such as factors VII, VIII and II with concomitant decrease in anticoagulant factors such as antithrombin and protein S.[23,24]

The procoagulant effect of OC use is manifested by lower protein S and antithrombin III levels, elevated levels of plasminogen, fibrinogen and specific clotting factors, and increased activated protein C (APC) resistance.[25] The extent of the hemostatic effect is related to the amount of estrogen and the type of progestogen.[26,27]

There are not obvious reasons to explain prescribing second or third generation OCs in practice. Second generation OCs are prescribed based on over age 40, duration of use, and family history of thromboembolism to reduce risk of VTE. Both BMI and lifestyles factors played a role in OC selection.

Methods

Study Population

This study was approved by the ethical committee at King Abdulaziz University. Our subjects were approached by our university obstetrics-gynecology department at the patient clinic. Eligible women for inclusion were Saudis and between 18 and 45 years of age who were non-smoker and not overly obese. Women taking oral anticoagulants or antiplatelet drugs during the prior month, over age 50 years, smokers or overly obese were excluded. The study objective was clearly explained to all participants and they each signed an informed written consent according to the Helsinki Declaration. In total, 115 women were included in this study, of which 47 women were using OCs for a period ranging from 3 months to 17 years and 68 women were not using OCs and served as the control group.

Blood Sample Collection and Preparation

Vacutainer® tubes containing 0.105 mol/L trisodium citrate (ratio 9:1; Becton Dickinson, Pont de Claix, France) were used for blood collection. Platelet poor plasma (PPP) was prepared by centrifuging the citrated blood twice at $4000 \times g$ for 10 min at $25°C$. After centrifugation, the supernatant was collected and frozen at $-80°C$ until needed.

Normal pooled plasma (NPP) was prepared in-house by collecting blood from 10 healthy control donors. The blood was centrifuged at $2,500 \times g$ for 5 min and the plasma from each donor was collected and pooled together. Hereafter, the plasma pool was subjected to an ultra-centrifugation at $10,000 \times g$ for 10 min and aliquoted. The aliquots were stored in liquid nitrogen until needed.

Reagents

Recombinant tissue factor (TF) was obtained from Innovin (Dade-Behring, Marburg, Germany). The source of Z-Gly-Gly-Arg-aminomethylcoumarine (ZGGR-AMC) was Bachem (Basel, Switzerland). The synthetic phospholipids were received from Avanti Polar Lipids Inc. (Alabaster, AL, USA). The preparation of the calibrator, α_2-macroglobulin-thrombin complex ($\alpha 2M$-FIIa), was done as described elsewhere.[28] Hepes buffers containing either 5 mg/ml or 60 mg/ml bovine serum albumin (BSA5 and BSA60, respectively) were prepared as reported elsewhere.[29] Recombinant human thrombomodulin (TM)[12] was obtained from Asahi Kasei Pharma (Tokyo, Japan) and APC was a kind gift from Veronique Regnault.[9]

Calibrated Automated Thrombinography

TF was premixed with phospholipids and 10 µl of this mixture was placed into the wells of a flat bottom 96-well polystyrene plate with 10 µl of either BSA5 buffer or TM or APC to achieve a final concentration of 1 pM TF, 4 µM phospholipids, with or without 2.5 nM TM or APC. In the calibration wells, 20 µl of $\alpha 2M$-FIIa was added for correcting inner filter effect, plasma color and substrate consumption. Next, 80 µl of PPP was dispensed into each well. Thrombin generation was started by addition of 20 µl of the fluorogenic substrate and calcium to the wells according to the method described by Al Dieri

et al.[29,30] NPP was also measured to normalize the data of each measurement.

Fluorescent signals were measured at excitation/emission wavelengths of 390/460 nm and analyzed using Thrombin scope software (Maastricht, Netherlands). Thrombin generation results were expressed as ETP (nmol/L min), lag time (min) and peak height (nmol/L). ETP and peak height data were normalized to the data obtained by measuring NPP at 5 pM TF. In the presence of TM or APC, TG parameters were expressed relative to the parameters in the absence of TM or APC (lag time and TTP as ratio to corresponding parameter without TM or APC; peak height and ETP and lag time as % inhibition by TM or APC).

Statistical Methods

Statistical analysis was performed using the Statistical Package for Social Science version 24.0 for windows (SPSS Inc., Armonk, NY, USA) for testing the difference among thrombin generation parameters and subject characteristics. MedCalc Statistical software version 19.0.3 for windows (MedCalc, Acacialaan 22, Ostend, Belgium) was used for graphing and charts. The data was expressed as mean $\pm$ SD (standard deviation). The Student's *t*-test was used to compare the differences between OC user and non-user. In addition, 1-way ANOVA, 2-way ANOVA, and 3-way ANOVA were carried out to investigate whether significant difference existed among thrombin generation parameters in relation to subject characteristics (age group, BMI levels, blood groups, and OC generation). Statistical significance was set at *p < 0.05*.

Results

Study Population

Our study population included 115 Saudi women of whom 47 (40.8%) were on OCs and 68 (59.1%) were non-users and served as the control group (Table 1)[AQ2].

Based on blood group, we classified the participants and controls as blood group O and non-O blood group. For participants they were 38.3% group O and 61.7% non-O and for the control they were 47.6% group O and 52.4% non-O. As for OC generation usage, 21.3% were using second generation, 55.3% third generation and 23.4% fourth generation (Table 2).

Referring to Table 3, it is noted that there was a significant difference between OC users and non-users with respect to thrombin generation parameters. OC usage increased thrombin generation parameters ETP and Peak height by 9.81% and 16.04%, respectively. There was significant difference in the lag time (P value = 0.011) between OC users and non-users.

Table 4 shows the relationship of subject characteristics to thrombin generation parameters including lag time, ETP and Peak height. There was a significant difference with respect to BMI levels, blood groups, and OC generation types except for the age category. There was no significant difference in all age groups but for those 40 years and above, they were more likely to develop thrombosis than those less than 40 years for the rise of thrombin generation parameters ETP and Peak height by 10.12% and 2.69%, respectively. BMI level had a significant impact on thrombin generation parameters ETP and Peak height. The same was true for blood group and OC generation. Subjects with B blood group had the lowest thrombin parameter levels, and the highest thrombin generation parameters were observed in those with AB blood group. Within OC

Table 1. Characteristics of OC Users and Non-Users in the Study.

Parameter	OC users Number	OC users %	Non-user Number	Non-user %	p-value
Age (years)					
Under 40	34	72.3	41	60.3	0.182
Over 40	13	27.7	27	39.7	
BMI					
Underweight	1	2.2	4	10.0	0.094
Normal	19	42.2	9	22.5	
Overweight	9	20.0	14	35.0	
Obese	16	35.6	13	32.5	
Unknown	2	4.1	28	41.1	
Blood group					
O-Individuals	18	38.3	10	47.6	0.831
A-Individuals	10	21.3	4	19.0	
B-Individuals	12	25.5	5	23.8	
AB-Individuals	7	14.9	2	9.5	
O group	18	38.3	10	47.6	0.428
Another group	29	61.7	11	52.4	
OC generation					
Second	10	21.3	-	-	-
Third	26	55.3	-	-	
Fourth	11	23.4	-	-	

Abbreviations: OC, Oral Contraceptive; BMI, Body Mass Index.

Table 2. Distribution of OC Generation Use in the Study Among Different Age Groups and Blood Types.

OC generation	Age group Under 40 N (%)	Age group Over 40 N (%)	Blood Class O Individual N (%)	Blood Class Non – O N (%)	Blood groups O N (%)	Blood groups A N (%)	Blood groups B N (%)	Blood groups AB N (%)
Second	6 (12.8)	4 (8.5)	2 (4.3)	8 (17.0)	2 (4.3)	2 (4.3)	5 (10.6)	1 (2.1)
Third	20 (42.6)	6 (12.8)	12 (25.5)	14 (29.8)	12 (25.5)	5 (10.6)	4 (8.5)	5 (10.6)
Fourth	8 (17.0)	3 (6.4)	4 (8.5)	7 (14.9)	4 (8.5)	5 (10.6)	1 (2.1)	1 (2.1)

Abbreviations: OC, Oral Contraceptive.

generation, we found that for those on fourth generation OCs, their ETP increased by 36.18% as compared to those using second generation and by 6.07% in those using third generation compared to those using second generation.

Table 5 shows the relationship of subject characteristics on thrombin generation parameters' inhibition ETP and Peak for TM and APC. There was a significant difference regarding BMI levels. There was no significant difference for blood groups, OC generation types, and age category.

Two way ANOVA showed the effect of combined factors of blood group and OC generation on thrombin parameters lag

time, ETP, and Peak height (Table 6). A significant effect was noted between the combination factors of blood group and OC generation on ETP and Peak height, which means that subjects using fourth generation OC with blood group O had the highest ETP and were more likely to develop thrombosis as compared to second generation OC users with non-O blood group who had the lowest ETP and were more prone to bleeding tendency. Moreover, those using third generation OC with non-O blood group were more likely to develop thrombosis (1.89%).

Two-way ANOVA showed the effect of combined factors of age group and OC generation on thrombin parameters lag time, ETP and Peak height (Table 7). There was no significant effect of combined age group and OC generation on either thrombin generation parameters ETP or Peak height. Those using fourth generation OC and who were 40 years old and above had the highest thrombin generation parameters in comparison to those who were under 40 years. The implication is that women using fourth generation OC and are over 40 years are more likely to have thrombosis than others. Women using second generation OC and less than 40 years are more likely to have bleeding as compared to others.

In addition, those who are 40 years and older and using third generation OC had a higher risk of having thrombosis (11.84%), as compared to those on second generation OC (8.79%), and for those using fourth generation OC (5.03%).

Discussion

The use of OCs has been a controversial subject in Saudi Arabia due to religious beliefs and was banned for a long time in the past. However, Saudi society has witnessed many changes lately and women have become more educated as professionals and are a major resource in the work force. As a result, they

Table 3. Effect of OC Use on Thrombin Generation.

	OC users		Non − user		
Parameter	Mean	± SD	Mean	± SD	p-value
Lag time (min)	3.56	1.11	4.05	0.82	0.011*
ETP (nmol/L)					
TG	1525.59	361.12	1389.27	298.68	0.033*
TM	890.09	563.43	623.29	296.38	0.005*
TM (%)	-43.27	26.14	-55.85	17.29	0.007*
APC	923.62	554.15	606.64	353.45	0.001*
APC (%)	-42.02	25.05	-58.46	20.03	0.001*
Peak height (nmol/L)					
TG	258.81	104.73	223.03	81.39	0.043*
TM	177.79	120.24	119.04	81.63	0.005*
TM (%)	-36.82	21.61	-50.83	16.32	0.000*
APC	161.57	124.88	93.79	81.01	0.002*
APC (%)	-45.53	23.51	-63.22	18.17	0.000*

* Statistically significant results (p < 0.05).
Abbreviations: OC, Oral Contraceptive; SD, Standard Deviation; ETP, Endogenous Thrombin Potential; TG, Thrombin Generation; TM, Thrombomodulin; APC, Activated Protein C.

Table 4. Relationship of Subject Characteristics to Thrombin Generation for OC Used.

	Lag time (min)			ETP (nmol/L)			Peak height (nmol/L)		
Parameter	Mean	± SD	p-value	Mean	± SD	p-value	Mean	± SD	p-value
Age (years)									
Under 40	3.66	1.31	0.361	1487.96	340.63	0.235	256.85	105.34	0.838
Over 40	3.29	0.88		1638.49	413.21		263.95	107.20	
BMI									
Underweight	8.67	-	0.000*	1036.00	-	0.017*	155.99	-	0.039*
Normal	3.59	0.64		1376.34	257.51		216.11	68.75	
Overweight	3.06	1.09		1614.21	319.33		314.49	101.83	
Obese	3.33	1.11		1727.44	426.77		292.14	126.04	
Blood group									
O − Individuals	3.72	1.64	0.101	1622.19	479.52	0.025*	264.38	128.69	0.025*
A − Individuals	3.66	0.72		1540.75	167.58		250.68	71.45	
B − Individuals	3.86	0.62		1218.46	228.57		195.65	73.91	
AB − Individuals	2.53	0.71		1673.67	361.12		348.69	57.55	
OC generation									
Second	3.67	0.91	0.173	1366.15	244.41	0.002*	229.75	84.29	0.010*
Third	3.77	1.33		1449.06	280.68		235.62	84.29	
Fourth	2.96	1.00		1860.42	440.02		340.05	124.44	

*Statistically significant results (p < 0.05).
Abbreviations: OC, Oral Contraceptive; BMI, Body Mass Index.

Table 5. Relationship of Subject Characteristics on Thrombin Generation for OC Used.

Parameter	Lag time (nmol/L)				ETP (nmol/L)				Peak height (nmol/L)			
	TM % Mean ± SD	P	APC % Mean ± SD	P	TM % Mean ± SD	P	APC % Mean ± SD	P	TM % Mean ± SD	P	APC % Mean ± SD	P
Age												
Less than 40	-2.68 ± 7.79	0.384	18.03 ± 10.16	0.136	-45.18 ± 27.16	0.369	-42.93 ± 25.86	0.658	-38.37 ± 22.89	0.401	-46.65 ± 24.09	0.568
Over 40	-0.39 ± 6.19		10.15 ± 24.46		-36.24 ± 21.88		-38.68 ± 22.89		-32.00 ± 17.04		-41.74 ± 22.15	
BMI												
Underweight	-	0.034*	-	0.610	-86.97	0.005*	-88.51	0.002*	-80.01	0.002*	-93.51	0.003*
Normal	-4.19 ± 7.01		19.22 ± 11.19		-52.03 ± 22.37		-52.19 ± 19.36		-42.05 ± 17.60		-54.11 ± 16.12	
Overweight	3.62 ± 5.12		13.14 ± 28.70		-18.98 ± 14.89		-19.32 ± 16.88		-16.59 ± 15.87		-25.03 ± 20.12	
Obese	-2.47 ± 7.95		15.63 ± 7.04		-40.34 ± 26.89		-37.83 ± 26.18		-37.37 ± 21.14		-42.13 ± 24.80	
Blood group												
O – Individuals	-1.36 ± 7.87	0.018*	13.50 ± 21.35	0.722	-43.95 ± 30.57	0.058	-43.06 ± 29.84	0.067	-37.71 ± 22.53	0.070	-47.37 ± 28.78	0.069
A – Individuals	-4.57 ± 6.67		16.19 ± 9.70		-50.31 ± 17.57		-46.75 ± 14.39		-40.59 ± 20.01		-47.36 ± 13.22	
B – Individuals	-5.68 ± 5.90		16.51 ± 11.82		-52.15 ± 23.81		-51.75 ± 22.89		-44.55 ± 20.49		-54.76 ± 17.52	
AB – Individuals	4.68 ± 4.92		21.45 ± 3.75		-20.20 ± 17.02		-20.38 ± 17.95		-18.08 ± 14.79		-25.26 ± 19.78	
OC generation												
Second	-4.31 ± 7.65	0.574	11.71 ± 10.70	0.592	-41.14 ± 24.35	0.138	-42.97 ± 25.99	0.067	-37.37 ± 20.83	0.249	-50.38 ± 20.86	0.050*
Third	-1.35 ± 7.38		17.17 ± 18.76		-49.34 ± 27.16		-47.90 ± 25.18		-40.53 ± 21.47		-50.10 ± 22.91	
Fourth	-1.74 ± 7.67		17.74 ± 6.91		-29.19 ± 21.24		-25.38 ± 17.31		-26.98 ± 21.75		-29.71 ± 22.43	

* Statistically significant results (p < 0.05).
Abbreviations: OC, Oral Contraceptive; BMI, Body Mass Index; ETP, Endogenous Thrombin Potential; TM, Thrombomodulin; APC, Activated Protein C.

Table 6. Effect of OC Generation and Blood Groups on Thrombin Generation Parameters for OC Users.

OC generation	Lag time (nmol/L)			ETP (nmol/L)			Peak height (nmol/L)		
	O group Mean ± SD	Other groups Mean ± SD	P value	O group Mean ± SD	Other groups Mean ± SD	P value	O group Mean ± SD	Other groups Mean ± SD	P value
Second	4.33 ± 1.89	3.50 ± 0.64	0.486	1499.84 ± 377.36	1327.95 ± 219.67	0.004*	177.63 ± 15.51	242.78 ± 100.02	0.026*
Third	3.97 ± 1.73	3.59 ± 0.9		1433.89 ± 271.98	1460.98 ± 296.97		221.96 ± 75.53	247.33 ± 92.28	
Fourth	2.64 ± 1.06	3.15 ± 0.99		2394.22 ± 423.62	1631.64 ± 164.54		435.01 ± 147.72	285.79 ± 73.83	

*Statistically significant results (p < 0.05).
Abbreviations: OC, Oral Contraceptive; ETP, Endogenous Thrombin Potential.

Table 7. Effect of OC Generation and Age Groups on Thrombin Generation Parameters for OC Used.

OC generation	Lag time (nmol/L)			ETP (nmol/L)			Peak height (nmol/L)		
	Less than 40 Mean ± SD	40 & above Mean ± SD	P value	Less than 40 Mean ± SD	40 & above Mean ± SD	P value	Less than 40 Mean ± SD	40 & above Mean ± SD	P value
Second	3.68 ± 1.09	3.64 ± 0.72	0.845	1327.28 ± 278.68	1443.89 ± 176.60	0.958	198.42 ± 76.26	276.76 ± 105.28	0.415
Third	3.85 ± 1.46	3.49 ± 0.83		1415.75 ± 297.36	1583.39 ± 156.45		241.57 ± 86.65	215.79 ± 79.86	
Fourth	3.16 ± 1.03	2.45 ± 0.85		1832.76 ± 303.46	1924.94 ± 765.57		338.88 ± 128.41	343.17 ± 140.32	

*Statistically significant results (p < 0.05).
Abbreviations: OC, Oral Contraceptive; ETP, Endogenous Thrombin Potential.

started to use OCs.[31] OCs consist of estrogen and progestogen. Such a combination carries variable risk for thrombosis depending on the generation type used. OCs have been used in obstetric practice in Saudi Arabia. The main problem associated with their use, especially when used at late reproductive age, is related to the considerable risk for developing thrombosis. We used second, third and fourth generation OCs to evaluate their effect on TG parameters and hence their tendency to cause thrombosis. We also included blood group type of the participants to investigate the effect on predisposition to thrombosis. Moreover, we took into consideration the age groups of the study population and their relation to liability to develop thrombosis. There are many previous reports[32,33] on the effect of OC use on thrombin generation in other parts of the world, but our current study is the first to be conducted in Saudi Arabia.

Regarding age, women who were less than 40 years old mostly used the third generation OCs and those who were older than 40 used second generation OCs. It was also noted that OCs led to increased thrombin generation parameters either ETP and/or Peak in all participants. Increased generation of thrombosis may induce VTE risk factors due to resistance of prothrombin and deficiency of antithrombin as reported by Al Dieri et al.[30] Our results are in agreement with those reported by Rosendaal et al.[34] who stated that a prolonged use of OCs enhanced thrombosis generation. Bloemen et al.[35] also reported that there was a significant incremental increase in ETP and peak levels accompanied with shortened lag time in women who used OCs.

In this study, higher BMI were associated with a higher baseline TG profile, including lag time, higher peak height and ETP levels, compared to women with a normal weight. Moreover, overweight, but not obese women, are more exposed to OC-related prothrombotic effect, as demonstrated by the significant reduction in both TM and APC inhibiting effects, compared to women with normal weight. This considerably highlights that TG regulation by TM and APC is impaired in overweight women, while it is relatively conserved in obese ones. Moreover, it is well recognized that having a high BMI is associated with VTE, and the risk of developing thromboembolic events is 3-fold greater for a BMI >25 kg/m^2 and more than 5-fold greater for a BMI >30 kg/m^2.[11] A study by Beijers et al.[36] investigated the effect of both BMI and body fat percentage on thrombin generation and found a positive relation with TG parameters peak and ETP. However, this relation was only observed in female participants and not in males.[36] The same findings were observed in a study by García-Raso and Sillero 2014[11] who found a significant association of body fat percentage with the tendency to thrombosis in females but not in males. Both obesity and OC use are known predisposing factors for VTE.[26] Nevertheless, due to the presence of several confounders and the variability of the risk estimates, the absolute risk of OC use-related thromboembolic events among obese women is not accurately evaluated to date, and the same clinical recommendations for the screening and prevention of VTE applies for all weight categories.[37] In a report by Sonnevi

et al.[38] they measured TG in plasma of women with a history of VTE and concluded that obesity was correlated with higher ETP; this only occurs when using a high concentration of TF 10 pmol/L. This was in line with the analysis of a previous study that showed a linear correlation between lag time and ETP with BMI. In contrast, after adding APC, no difference in TG was observed between obese and non-obese patients.[14] On the other hand, another study that compared OCs users with non-users indicated that women on OCs had a weaker APC-induced inhibition of TG.[39] Our results are in harmony with those of others who reported that the increase of prothrombosis was associated with obesity that in turn was linked to higher TG profile, peak, and ETP.[18] Consequently, obesity might lead to damaging TG regulation through APC and TM and finally end up with thrombosis.[40,41]

When evaluating thrombin generation parameters ETP and Peak separately and combined effect of blood group and OC generation, there was a significant effect of blood group and OC generation on both thrombin parameters ETP and Peak, which means that those who are using fourth generation OCs and with blood group O have the highest ETP and are more likely to develop thrombosis as compared to second generation OC users with non-O blood group who have the lowest ETP and are more prone to bleeding tendency, moreover, those using third generation OC with non-O blood group are more likely to develop thrombosis by 1.89%. In a different study, the effect of blood group and OC generation used was a larger decline in antithrombin III for women with blood type O using the highest estrogen dose preparation and for women with non-type O using the lowest progestin dose preparation; plasma levels of OCs were associated with changes in the most extreme levels of antithrombin III.[42] The Al Dieri et al.[30] report is in line with our study because the demonstrated increased formation of thrombin in plasma always induces a risk of VTE, whether it is due to deficiency of antithrombin or protein C, an excess of prothrombin resistance, and non-O blood group. These results were in agreement with the result of Mohamed et al.[18] who didn't detect any significant difference in any parameters of TG when using the 3 generations of OCs (second, third, and fourth). Moreover, Tchaikovski et al.[43] didn't record any difference in ETP between users of the 3 different OCs generations, but they found the third generation caused an inhibition to APC more than the second generation. It has also been reported that the third generation of OCs has a more resistant APC profile than the second generation of OCs.[44]

Conclusion

This study found an increased risk for VTE among Saudi women on third generation OCs compared to those using second generation. There is a significant difference with respect to ABO-blood groups and OC generation types. Age was associated with risk for thrombosis. In contrast to the increased risk of thrombosis seen in non-O blood group individuals, the O blood group was associated with bleeding tendency. Moreover, Higher BMI were associated with significant increase in TG

parameters lag time, ETP and Peak. Regarding the inhibition of TG parameters ETP and peak in the presence of TM and APC, there is a significant difference in relation to BMI, while there is an increase although not significant in relation to ABO blood group and OC types. Special attention was devoted to the fact that women develop a resistance to APC because of OC use. Women who used third generation OC showed higher resistance to APC than women who used second generation OC.

A limitation of this study is the wide variation in the period of the OC usage which ranged from 3 months up to 17 years. It would be prudent to investigate the effect of duration of OC usage on thrombosis.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Approval

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