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Original article

The role of factor V Leiden and prothrombin G20210A mutations for clotting in Sudanese women under oral contraceptive use

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ABSTRACT

Background & objectives: The risk of venous thromboembolism (VTE) in women using combination oral contraceptives (COC) is attributed to changes in hemostasis. These changes are greatly affected in women with a hereditary thrombotic predisposition. The present study investigated the relationship between genetic mutations associated with deep venous thrombosis and oral contraceptive use in pregnant women.

Materials and methods: This was a case-control study to detect factor V Leiden (FVL) and prothrombin G20210A mutations in Sudanese pregnant women. Three hundred individuals were included in this study (200-study group; 100- control group). Both these groups were diagnosed as having deep vein thrombosis (DVT). Genetic mutations were determined using RT-PCR. Proteins C, protein S, antithrombin III (ATIII), fibrinogen, prothrombin time (PT), international normalize ratio (INR), thrombin time (TT) and activated partial thromboplastin time (APTT) were measured.

Results: The results of genetic analysis for factor V Leiden mutation showed that two patients had the mutant allele (A A), twenty-two had mixed type (GA), and the remaining one hundred seventy-six had wild type (G G). The entire control group had only one the mixed type (GA), remaining had wild type (G G). Regarding prothrombin, G20210A mutation showed only the wild type (G G) in both the study group and the control group. Protein C, protein S and plasma fibrinogen was significantly lower in the study group compared to the control group. No statistically significant difference was observed in ATIII, PT, TT and APTT between the study group and the control group.

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Interpretation & conclusion: The oral contraceptives were associated with increased risk of developing deep vein thrombosis (DVT) among Sudanese pregnant women. The prevalence of factor V Leiden mutations was more than prothrombin mutations among pregnant women.

1. Introduction

Oral contraceptives use has increased throughout the world including developing countries (Svoboda, 2020; Naz et al., 2012). The increased risk of venous thrombosis due to the use of oral contraceptives (OC) was reported in 1961. Since this first report, many studies have identified the risk of venous thrombosis with the use of oral contraceptives, and some have reported about 2–6 times more risk of developing deep vein thrombosis (DVT) (Khialani et al., 2020; Dragoman et al., 2018; McDaid et al., 2017).

Furthermore, changes in hemostasis have been reported to contribute for the increased risk of venous thromboembolism (VTE) associated with use of combined oral contraceptives (COCs). The use of COC has also been linked to an increased risk of ischemic stroke and/or venous thromboembolism, especially in women with inherited thrombophilia (i.e., thrombogenic mutations) such as the factor V Leiden or prothrombin gene mutations, or protein C, protein S, or antithrombin deficiency (Duliceket al., 2018).

The World Health Organization (WHO) classifies "a known thrombogenic mutation" as "*a condition which represents an unacceptable health risk if [combined hormonal contraception] is used* (Hiedemann et al., 2019)." In contrast, the WHO defines routine thrombophilia screening before starting COCs as unnecessary due to the low prevalence of thrombogenic mutations and high screening costs in a clarification for this recommendation. There is no comprehensive review of the reasons for and against genetic screening from the patient's viewpoint in the current literature. This viewpoint is particularly pertinent in light of a growing focus on patient-centered treatment, shifting attitudes toward genetic testing, and shifting policy landscapes (Hiedemann et al., 2019). Women have more autonomy in this phase when policies provide for direct patient access to thrombophilia testing, as well as policies that allow women to get oral contraceptives from a pharmacist without a prescription (Hiedemann et al., 2019).

Overall, this point seems straightforward; however, there are two essential considerations from the patient's perspective. First, long-term use of COCs can increase the risk of thrombosis. Thus, among carriers of thrombogenic mutations who use COCs for a long time, the lifetime risk of thrombosis is likely even at decreasing annual level (Vernon et al., 2017). Secondly, number of published articles demonstrate that a few women who have tested positive for thrombophilia are likely to be denied effective contraception even if they have never experienced VTE. This reasoning is definitely paternal in order to learn about potential health risks and eliminate the opportunity to compare and review them. Some patients may have an acquired thrombophilia, which carries similar risks for VTE and subsequent sequalae (Zöller et al., 2020). In some women, especially those with lupus, an autoimmune disease, pregnancy or a major illness may cause the following thrombotic predisposition and the likelihood of thrombotic events to cause antiphospholipidantibody syndrome (Sammaritano, 2020). Women receiving COCs with or without a prescription have little or no knowledge of thrombotic predisposition and the risks associated with COCs (Gialerakiet al., 2018). Therefore, this study was designed to detect the thrombotic risk of oral contraceptive use, factor V Leiden and prothrombin mutation in Sudanese pregnant women.

2. Material & method

2.1. Study population

Sudanese pregnant women were recruited to assess the risk of thrombosis with oral contraceptive use, Factor V Leiden (FVL) and prothrombin G20210A mutations in this cross-sectional study. This study was conducted in Khartoum state (Sudan) from January 2016 to August 2019. Pregnant women who used oral contraceptive were recruited in the study group while pregnant women who did not used oral contraceptives were included in the control group and both were diagnosed as having DVT. The inclusion criteria included pregnant women who used oral contraceptive and pregnant women who did not use oral contraceptive, both having diagnosed with DVT. The exclusion criteria were non-pregnant women. The patients recruited in the study were already diagnosed as having DVT by duplex ultrasonography and contrast venography. The duration of pregnancy varied from 6 months to 8 months. Most of the participants became pregnant between 3 and 6 months after stopping the OCs while about few participants took more than 6 months to become pregnant with three of them becoming pregnant at 8 months.

2.2. Sample collection

Five milliliters of blood were collected from all participants after consideration of all safety procedures according to WHO guidelines (WHO guidelines on drawing blood: best practices in phlebotomy (2010)). The collected blood samples were immediately divided into two containers as follows; Four and half milliliters of blood was transferred to a container having 3.2% w/v trisodium citrate (9:1), plasma was separated immediately by centrifugation at 3000*g* for 15 min at room temperature and stored in 0.5 ml aliquots at −80 ^{0}C, for coagulation assays. The remaining blood sample blood sample in EDTA was used for genomic deoxyribonucleic acid (DNA) extraction using Jena Bioscience, Blood DNA preparation kit, according to the manufacturer leaflet and stored at −20 °C till use.

2.3. Data collection methods

Data were collected from cases and controls, using a designed questionnaire while the results were obtained from laboratory experiment. The questionnaire was validated for content validity and construct validity by two experts each from clinical laboratory sciences and medicine.

2.4. Sample size and techniques

The total sample size was 200 pregnant women of varying age, who used oral contraceptive and admitted to Soba Teaching Hospital (Khartoum). Sample size was estimated by applying the classical equation (Statistics, Libretexts, 2021)

n= (Zσ2/d) 2

Z = is the value of specified level of significance

σ = is the standard deviation of the cases.

d = is the difference between the case mean and control mean.

In the present study, the values were n= (1.5 × 2.52 / 0.5)2 = 225 women, approximately = 200.

Case finding method was used to enroll all pregnant women with thrombosis using contraceptive pill (patients' group), while simple random technique was used to select the control group. A whole blood sample was collected from 200 women of patients group and from 100 women of control group.

2.5. Ethical consideration

Research purpose and objectives were explained to each participant, and their consent for participation in the study was obtained. The research work was approved by the Research Committee of College of Pharmacy, AlMaarefa University, Saudi Arabia (MCST (AU)-COP 1525/RC)

2.6. Tests performed

Prothrombin Time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT), thrombin clotting time (TT), fibrinogen Level, protein C, protein S and antithrombin III were determined. All these were tested using kits following manufacturer's instructions. Prothrombin gene mutation and factor V Leiden gene mutation were determined through PCR analysis. The kits were procured from Spinreact (Spain), ThermoScientific, SolisBioDyne (Estonia), INtRON Biotechnology Inc., TechnoChrom (Germany).

2.7. Statistical methods

The statistical analysis of laboratory results and data were done by using the statistical package for social sciences (SPSS). Computer program version 25 was used for data processing. Pearson correlation coefficient and Welch's *t* test were used.

3. Results

3.1. Distribution of the study participants

There was no significant difference in the weight/kg, height/m, age/year, age groups /year, recurrent DVT, family history, ICU, abortion, and recurrent abortion between patients and controls (P > 0.05). An extremely significant difference (p < 0.000) was seen between patients and controls in the duration of oral contraceptive uses /month, and oral contraceptive (OC) (Table 1).

3.2. Comparison of coagulation profile

The specific values obtained in each experiment are summarized in Table 2. There was a significant difference (p < 0.05) between patients and controls in PT, INR, Fib, PS, and PC, and no significant difference was observed in the APTT, TT, and ATIII between the study populationand the controls (P > 0.05).

3.3. Correlation of study participants' general characteristics with coagulation profile

There was a positive medium correlation between weight/kg (0.534), height/m (0.312) and PT\sec in the patient's group (P < 0.05), and there was no correlation between duration of oral contraceptive uses /month, age/year, and PT\sec in the patient's group. There was a positive medium correlation between weight/kg (0.534), height/m (0.312) and INR in the patients group (P < 0.05) and a weak positive correlation between the duration of oral contraceptive uses /month (0.172) and APTT\sec in the patients group (Table 3).An analysis of patients' characteristics with different parameters revealed no significant difference except in PC% with respect to age, wherein a significant decrease in PC% was observed in patients who were above 35 years (76.8 ± 21.0) when compared to those aged less than 25–30 years (88.0 ± 23.6) and those less than 20 years (89.7 ± 25.4). The data for other values are not given as they were not significantly different.

3.4. Factor V Leiden genotype and alleles in DVT patients

As shown in Table 4, there was a significant difference between patients and controls in FV Leiden genotypes (P < 0.05), of the 200 participants in the study group, 176 were 88.0% had normal homozygous (GG), 22 (11.0%) had heterozygous (GA), and 2 (1.0%) had homozygous (AA). Of the 100 controls, 99 (99.0%) had normal homozygous (GG), one (1.0%) had heterozygous (GA), and none of the control had homozygous (AA). Also, there was a significant difference between patients and controls in FV Leiden alleles (P < 0.05), of 200 patients, 187.0 (93.5%) alleles were (G), and 13 (6.5%) alleles were (A). And of the100 controls, 99.0 (99.0%) alleles were (G), and there was 1.0 (1.0%) allele was (A) indicating that the oral contraceptive was more effective in FV Leiden genotypes than non-users of oral contraceptives.

3.5. Comparison of coagulation profile between FV Leiden genotype in DVT patients

An overall summary of the results is given in Table 5. There was no significant difference between FV Leiden genotypes and coagulation profile in patients (P > 0.05).

3.6. Comparison of general characteristics with FV Leiden genotypes

As shown in Table 6, there was no significant difference between FV Leiden genotypes and general characteristics in patients (P > 0.05).

3.7. Comparison of duration of oral contraceptive use with FV Leiden genotypes

The specific values used for each experiment are summarized in Table 7. There was significant difference (p < 0.05) between types of oral contraceptive, and duration in FV Leiden genotypes. The Familiar (OC) at duration of 6.5 months use had 96 (GG)genotype (48.0%), 11(GA) genotype (5.5%), and 2 (AA) genotype (1.0%), and the Vominor (OC) at duration 8.7 months use had 20 (GG) genotype (10.0%), 5 (GA) genotype(2.5%), and none had (AA) genotype (0.0%), and the Brevicon (OC) at 6.6 months had 22 (GG) genotype (11.0%), 3 (GA) genotype (1.5%), and nonehad (AA) genotype while the Listerine (OC) at3.4 months use had 19 (GG) genotype (9.5%) and none had (GA) genotype or the (AA) genotype. Micronor (OC) at duration of 8.6 months use had 14 (GG) genotype (7.0%), 1 (GA) genotype (0.5%), and none(AA) genotype finally Yasmin (OC) at duration of3.9 months use had 5 (GG) genotype(2.5%),1 (GA) genotype (0.5%), and no(AA) genotype.

3.8. Comparison of prothrombin G20210A genotypes between patients and controls

There was no difference between patients and controls in Prothrombin G20210A genotypes, all the 200 patients (100.0%), and all the 100 controls (100.0%) had normal homozygous (GG) (Table 8).

Table 1
The distribution of the study participants according to their characteristics within each study groups.

General characteristics		Study groups			
		Patients (n = 200)		Controls (n = 100)	
Weight/kg (mean ± SD)		65.2 ± 8.3		66.9 ± 7.6	
Height(m) (mean ± SD)		1.7 ± 0.11		1.7 ± 0.09	
Duration of oral contraceptive (month)		6.5 ± 6.2		0.0 ± 0.0***	
Age/year (mean ± SD)		27.7 ± 7.4		28.7 ± 7.8	
Characteristics		Freq.	Percent	Freq.	Percent
Age groups* /year (frequency)	<25	85	42.5	39	39.0
	25–35	70	35.0	34	34
	Above 35	45	22.5	27	27.0
Recurrent DVT (frequency)	Yes	19	9.5	7	7.0
	No	181	90.5	93	93.0
Family history (frequency)	Yes	10	5.0	4	4.0
	No	190	95.0	96	96.0
ICU (frequency)	Yes	35	17.5	15	15.0
	No	165	82.5	85	85.0
Abortion (frequency)	Yes	24	12.0	14	14.0
	No	176	88.0	86	86.0
Recurrent – abortion (frequency)	Yes	5	2.5	4	4.0
	No	195	97.5	96	96.0
Oral contraceptive (frequency)	Yes	200	100.0	0	0.0
	No	0	0.0	100	100.0***
Type of oral contraceptive (frequency)	Familiar	109	54.5	0	0.0
	Vominor	25	12.5	0	0.0
	Brevicon	25	12.5	0	0.0
	Listerine	19	9.5	0	0.0
	Micronor	15	7.5	0	0.0
	Yasmin	7	3.5	0	0.0

*** *P < 0.001.*

Table 2
Comparison of differences in means for coagulation profile between patients and controls.

Parameters	Study groups		*P* value
	Patients (n = 200)	Controls(n = 100)	
PT /sec (mean ± SD)	25.0 3 ± 3.16	25.89 ± 2.79*	0.023
INR (mean ± SD)	2.10 ± 0.26	2.17 ± 0.23*	0.021
APTT /sec (mean ± SD)	31.42 ± 1.73	31.72 ± 1.72	0.158
Fib mg/dl (mean ± SD)	251.86 ± 71.19	281.75 ± 69.12***	0.001
TT /sec (mean ± SD)	9.07 ± 1.37	9.27 ± 1.98	0.369
P.S% (mean ± SD)	80.18 ± 28.87	93.19 ± 28.20***	0.000
P.C% (mean ± SD)	86.21 ± 24.26	94.48 ± 26.69***	0.008
ATIII% (mean ± SD)	72.58 ± 29.14	74.65 ± 26.49	0.551

* *P < 0.05.*
*** *P < 0.001.*

4. Discussion

This study determined the thrombotic risk associated with OC use, FV Leiden, and prothrombin G20210A mutation in Sudanese pregnant women. Both the groups used in the study had pregnant women who were diagnosed as having deep vein thrombosis (DVT) and it is known that pregnancy is a risk for venous thromboembolism (VTE) in young women (Nichols et al., 2020, Ali et al., 2020) with highest risk around delivery days (Nicholset al., 2020). An earlier study reported that the risk of venous thrombosis is five-fold during pregnancy and 60-fold in the first three months after delivery compared to non-pregnant women (Reid, et al., 2010, Alsheef et al., 2020, Allais et al., 2004).

All combined oral contraceptives are reported to increase the risk of venous thrombosis by two-fold to six-fold. The incidence of venous thrombosis in female is low; about three per 10,000 woman years. Nevertheless, venous thrombosis among women of reproductive age is large, owing to the fact that many women use oral contraceptives (Stegeman et al., 2013, Jonard et al., 2016).

The mean age of studied subjects in the study was 27.7 years in the study group and 28.7 years in the control group. As mentioned earlier, age is an important risk factor for VTE (Lidegaard et al., 2011; Oger, 2000). The use of COCs in women of 50 years or older to control menopausal symptoms showed higher risk of venous thrombosis (Roach et al., 2013). The risk of venous thrombosis was more in women with one or more thrombophilic defects. However, in the present study, there was no correlation between COCs use and age that showed that influence of COCs on VTE risk was not dependent on age in the Sudanese women.

In current study, there was a significant difference between patients and controls in PT, INR, Fib, PS, and PC, whereas no significant difference was observed in the APTT, TT, and ATIII between

Table 3
The correlation of study participants' (patients) general characteristics with different parameters.

General characteristics	**Correlation**							
	PT/Sec	**INR**	**APTT/Sec**	**TT/sec**	**Fib mg/dl**	**PS%**	**PC%**	**ATIII%**
Weight/kg	0.534***	0.534***	−0.059	0.310***	−0.236***	0.133	0.073	0.155*
Height/m	0.312***	0.312***	−0.063	0.180*	−0.117	0.096	0.123*	0.133
Duration of oral contraceptive /month	−0.036-	−0.035-	0.172*	−0.048-	−0.001-	−0.021-	−0.047-	−0.001-
Age/year	0.028	0.028	0.040	−0.021	0.018	0.005	−0.168*	−0.058

* *P < 0.05,* ***P < 0.001.*

Table 4
Frequency of Factor V Leiden genotype and alleles in DVT patients.

FV Genotype		Study groups		P value
		Patients n = 200 (frequency/percent)	Control n = 100 (frequency/percent)	
Normal HomozygousGG		176 (88%)	99(99.0%)	0.005
Heterozygous GA		22 (11.0%)	1(1.0%)	
Homozygous AA		2(1.0%)	0(0.0%)	
Total		200 (100%)	100(100.0%)	
FV Allele	G	187(93.5%)	99 (99.0%)	0.033
	A	13(6.5%)	1 (1.0%)	
Total		200(100%)	100(100%)	

Table 5
Comparison of differences in mean, for coagulation profile between FV Leiden genotype in DVT patients.

Parameters	Study groups (n = 200 patients) (mean ± SD) FV genotype			P. value
	GG 176	GA 22	AA 2	
PT /(mean sec)	25.1 ± 3.2	24.6 ± 3.1	24.2 ± 1.0	0.711
INR (mean)	2.1 ± 0.3	2.1 ± 0.3	2.0 ± 0.1	0.703
APTT / (mean sec)	31.4 ± 1.7	31.3 ± 1.6	31.4 ± 3.4	0.897
TT /(mean sec)	9.1 ± 1.3	8.7 ± 1.5	8.8 ± 2.6	0.384
Fib /(mean sec)	263.0 ± 69.3	239.3 ± 87.7	288.2 ± 24.6	0.536
P.S(mean %)	80.0 ± 27.4	82.5 ± 39.9	69.5 ± 19.1	0.814
P.C(mean %)	86.3 ± 25.0	86.6 ± 18.7	78.5 ± 20.5	0.902
ATIII (mean %)	72.4 ± 28.6	75.1 ± 34.2	62.5 ± 27.6	0.815

Table 6
The distribution for DVT patients according to their characteristics with FV Leiden genotypes.

General characteristics		Study groups (patients n = 200)						P. value
		FV genotype						
		GG 176		GA 22		AA 2		
Weight/(mean kg)		65.3 ± 8.5		64.8 ± 6.7		59.5 ± 3.5		0.599
Height/ (mean m)		1.7 ± 0.1		1.7 ± 0.1		1.6 ± 0.1		0.599
Duration of oral contraceptive /(mean month)		6.6 ± 6.3		6.3 ± 5.3		4.0 ± 5.7		0.821
Age/(mean year)		28.0 ± 7.6		26.0 ± 6.0		23.5 ± 0.7		0.342
Age groups* /year(frequency)		Freq.	Percent	Freq.	Percent	Freq.	Percent	P. value
	<25	74	37.0%	9	4.5%	2	1.0%	0.178
	25–35	59	29.5%	11	5.5 %	0	0.0%	
	Above 35	43	21.5%	2	1.0%	0	0.0%	
Recurrent DVT (frequency) Recurrent DVT	Yes	17	8.5%	2	1.0%	0	0.0%	0.896
	No	159	79.5%	20	10.0%	2	1.0%	
Family history (frequency)	Yes	10	5.0%	0	0.0%	0	0.0%	0.488
	No	166	83.0%	22	11.0%	2	1.0%	
ICU (frequency)	Yes	29	14.5%	5	2.5%	1	0.5%	0.367
	No	147	73.5%	17	8.5%	1	0.5%	
Abortion (frequency)	Yes	20	10.0%	3	1.5%	1	0.5%	0.240
	No	156	78.0%	19	9.5%	1	0.5%	
Recurrent - abortion (frequency)	Yes	4	2.0%	1	0.5%	0	0.0%	0.792
	No	172	86.0%	21	10.5%	2	1.0%	
Oral contraceptive (oc) (frequency)	Yes	176	88.0%	22	11.0%	2	1.0%	–
	No	0	0.0%	0	0.0%	0	0.0%	
Type of oral contraceptive (frequency)	Familiar	96	48.0%	11	5.5%	2	1.5%	0.562
	Vominor	20	10.0%	5	2.5%	0	0.0%	
	Brevicon	22	11.0%	3	1.5%	0	0.0%	
	Listerine	19	9.5%	0	0.0%	0	0.0%	
	Micronor	14	7.5%	1	0.5%	0	0.0%	
	Yasmin	5	2.5%	2	1.0%	0	0.0%	

patients and controls. This indicates that contraception associated risk of VTE will increase the risk of thrombosis subsequently. The results of the present also indicate that family history of DVT is an independent risk factor for DVT both in the COC users and the controls. The observations also agree with the results reported earlier in a Swedish nationwide case-control study (Zöller et al., 2013) that showed prevalence of family history of VTE to be lower among COC users compared to non-COC users.

Table 7
Comparison of differences in means for Duration between types of oral contraceptive in FV Leiden genotypes.

Study groups (patients n = 200)				(patients n = 200)					
Type of oral contraceptive	N	Duration	P. Value	FV					
		Mean ± SD		GG		GA		AA	
				176		22		2	
Familiar	109	6.5 ± 5.6	0.050	96	48.0%	11	5.5%	2	1.0%
Vominor	25	8.7 ± 7.2		20	10.0%	5	2.5%	0	0.0%
Brevicon	25	6.6 ± 7.1		22	11.0%	3	1.5%	0	0.0%
Listerine	19	3.4 ± 6.6		19	9.5%	0	0.0%	0	0.0%
Micronor	15	8.6 ± 5.9		14	7.0%	1	0.5%	0	0.0%
Yasmin	7	3.9 ± 5.4		5	2.5%	2	1.0%	0	0.0%
Total	200	6.5 ± 6.2		176		22		2	

Table 8
Frequency of Prothrombin G20210A genotypes in the patients and controls.

Prothrombin genotypes		Groups	
		Patients	Control
Normal HomozygousGG (frequency)	Count	200	100
	% of Total	100%	100%
Heterozygous GA (frequency)	Count	0	0
	% of Total	00.0%	00.0%
Homozygous AA (frequency)	Count	0	0
	% of Total	00.0%	00.0%

Another finding of this study in pregnant women with deep vein thrombosis is that the common genetic thrombophilic defects, i.e., factor V: A1691, was strongly associated with an increased risk for the disease and this risk was augmented with oral contraceptive use. The 12% prevalence of the factor V mutation observed in the study group indicates a risk for thrombosis. There was a significant difference between patients and controls in FV Leiden genotypes ($P < 0.05$), of the 200 patients, 88.0% had normal homozygous (GG), 11.0% had heterozygous (GA), and 1.0% had homozygous (AA). Out of the 100 controls, there were 99.0% had normal homozygous (GG), one case (1.0%) had heterozygous (GA), and none of the control had homozygous (AA). Also, there was a significant difference between patients and controls in FV Leiden alleles ($P < 0.05$), of the 200 patients, 93.5% alleles were (G), and 6.5 % alleles was (A)while in the controls, 99.0% alleles were (G) 1.0%. carried (A) allele. That means the oral contraceptive was more effective in FV Leiden mutation genotypes than non-user oral contraceptives. There are many reports indicating an association between use of OCs and development of VTE in women with known hereditary thrombophilia (Andersen et al, 1998). However, in this study when comparing family history of patients, with FV Leiden genotype, we found only 10/200 (5.0%) had a family history of genotype GG with no genotype GA, and genotype AA, and the remaining patients had no family history (190/200). We found 166 (83%) had genotype GG, 22(11.0%) had GA, and 2(1.0%) had AA (p-value 0.488). Earlier studies show that VTE risk in women using OCs and carriers of FV Leiden is higher (about 35- to 99-fold) compared to non-users of OCs who do not have this prothrombotic mutation (Bloemenkamp et al., 2000, Wierbowski et al., 2018). Regarding the result of prothrombin gene mutation, there was no significant difference between patients and controls, all of the 200 patients, and the 100 controls were detected with normal homozygous (GG), and eventually absence of heterozygous (GA), and homozygous (AA) polymorphisms.

The third generation oral contraceptives containing 30 mg or less of ethinyl-estradiol and gestodene or desogestrel as progestin are reported to increase risk of venous thromboembolism when compared to older preparations that haslevonorgestrel as progestin (Kluft and Lansink, 1997). We found no association between thrombosis and the types of oral contraceptives, those of second generation being the most frequently used among patients (109/54.5%-Familiar, 19/9.5%-Listerine, and 15/7.5%-Micronor) than the third generation (25/12.5%-Vominor, 25/12.5%-Brevicon, and 7/3.5%-Yasmin). When the frequency of types of oral contraceptives was compared with FV Leiden mutation genotypes among generations, we found that among the second generation, out of 143 cases, 129 had GG genotype, 12 had GA genotype, and 2 had AA genotype. Also, we found the distribution of this genetic polymorphism (Alharbi et al., 2021) among the third generation; out of 57 cases 47 were GG genotype, whereas GA genotype represented 10 cases, and no AA genotype were detected. This shows, that the second generation was associated with an increased risk of thrombosis compared to the third generation. The consumption of third generation pills in Sudanese general population is similar to that observed in other parts of the world. Since, a strong association was observed between oral contraceptive use in women with genetic and prothrombin and factor V mutations, it is suggested that determination of genetic mutation before the prescription of COCs should be carried out. The results of the current study support earlier report that showed an increased VTE with use of COCs containing levonorgestrel (Jick, and Hernandez, 2011). This study also confirmed a significant interaction between thrombosis and duration of COC use (Lidegaard et al., 2009).

5. Conclusions

The study concluded that the oral contraceptives is associated with increased risk of developing venous thromboembolism among pregnant women than non-users. The prevalence of factor V Leiden mutations is more than prothrombin mutations among the pregnant women. The oral contraceptives are more effective in combination with factor V Leiden mutation (heterozygous or homozygous) in development of venous thromboembolism among pregnant women than non-users. The non-significant relations indicated that the prothrombin mutations are not genuine risk factors for deep vein thrombosis in Sudanese population. The naturally occurring anticoagulants: protein C, and protein S are deficient in pregnant women, who used oral contraceptive.

6. Recommendations

Following the ever-underlying effect of OC, the need for refining the estrogen and progestin components into forms that are less harmful to human health is impeccable. In connection to such threats on health connected to the use of OC, the study made the following list of recommendations.

1- It is mandatory to validate these finding and design new ones specific for COC users.
2- Prescription of OC must be accompanied with investigations of family history and coagulation regulatory proteins.
3- Prescription of OC must be accompanied with investigations of factor V Leiden polymorphisms.
4- Effectiveness of these finding should be assessed and compared with current practice in management studies.
5- Their cost-effectiveness in contraceptive counseling should also be evaluated before implementation in clinical practice.
6- Reducing the estrogen element in the component of OC medicines, in regard to its significant contribution to thrombosis.
7- It is possible to use alternative forms of family planning, which includes condoms or observation of safe days, to avoid the unavoidable effects of oral contraceptive.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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