Original Articles

IMPACT OF FACTOR V LEIDEN POLYMORPHISM IN PATIENTS WITH PCOS

Veselin P. Penkov,
Katya S. Kovacheva[1],
Georgi M. Golemanov[2],
Galia A. Georgieva[2],
Peter D. Ivanov2,
Regina S. Komsa-Penkova[2],
Olamide Ajewole[3]

Repromed Medical Center – Pleven, Bulgaria
[1]Medical Genetics Section, Faculty of Pharmacy, Medical University – Pleven, Bulgaria
[2]Biochemistry Section, Faculty of Pharmacy, Medical University – Pleven, Bulgaria
[3]Student III course, Faculty of Medicine, Medical University – Pleven, Bulgaria

Corresponding Author:
Georgi M. Golemanov,
Biochemistry Section, Faculty of Pharmacy, Medical University – Pleven
1, St. Kl. Ohridski Str., 5800 Pleven, Bulgaria
e-mail: g.golemanov@abv.bg

Received: September 30, 2019
Revision received: December 01, 2019
Accepted: December 02, 2019

Summary

The present study aimed to evaluate the impact of factor V Leiden (FVL) polymorphism within the reproductive problems encountered by patients with polycystic ovary syndrome (PCOS). A total of 92 female patients with PCOS and 101 healthy controls were included in the study. Clinical and laboratory parameters were examined. The full history of each patient was taken. Single nucleotide polymorphism rs6025 in F5 was genotyped in PCOS patients and compared to the genotype frequency of the healthy controls. The data were analysed for correlation with infertility and pregnancy loss in PCOS patients. The prevalence of FVL polymorphism was higher, however not significantly, in PCOS patients compared to that of the control group (respectively OR=2.238, 95 % CI 0.777±6.449, p=0.104). The carriers of FVL polymorphism showed a higher rate of primary infertility (30.0% versus 12.5%, OR=3.143, 9 % CI 0.686±14.388, p=0.047) and their total reproductive failure rate was higher (60.5% versus 47.2%, OR=1.819, 95% CI 0.632±9.259, p=0.117). Carriage of FVL polymorphism in PCOS patients is associated with primary infertility and a presumed cause of the further investigations needed to understand the impact of FVL on PCOS. Carriage of FVL polymorphism in PCOS patients is associated with a higher rate of primary infertility, which draws attention to the role of this factor in the aetiology of the PCOS-related subfertility. Further investigations are needed to understand the impact of FVL on PCOS.
Key words: factor V Leiden, polycystic ovary syndrome, primary infertility

Introduction

Polycystic ovary syndrome (PCOS) is considered as one of the most common endocrine diseases with unidentified aetiology, in addition to being one of the causes of reproductive problems in women [1]. First described in 1935 by Stein and Leventhal as a reproductive disorder, it is a heterogeneous, metabolic, and chronic disorder that arises from interactions between inherited, endocrine, and environmental factors [2].

Women with the syndrome of polycystic ovaries have difficulty conceiving and are at an increased risk of spontaneous abortion after spontaneous or assisted reproduction. The prevalence of the disorder ranges

from 6 to 21% in women of reproductive age [3]. Most women with PCOS suffer from obesity and insulin resistance [4], and the components of metabolic syndrome (MS) are characterized by chronic inflammation [5].

Although there is no consensus on the PCOS pathogenesis, there is strong evidence that PCOS could be classified as a genetic disease with a heritability of endocrine, metabolic, thrombophilic, and other features [6-8]. Some previous studies have investigated the impact of thrombophilia on the development of PCOS mainly in the carriers of plasminogen activator inhibitor-1 (PAI-1) and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism. The analysis of the results was related to the pro-inflammatory effects of PAI-1 and high homocysteine in MTHFR [9-10]. Glueck et al. first published the results on the impact of FVL in PCOS patients, complicated with recurrent pregnancy losses (RPL). They stated that PCOS patients with RPL had a higher proportion of activated protein C and a higher prevalence of factor with V Leiden mutations if compared to PCOS patients without RPL" [11]. Factor V Leiden is an autosomal dominant genetic condition, expressed with the change from arginine to glutamine at position 506 due to a substitution of base G to base A. The resulting FVL variant cannot be easily degraded by activated protein C (APC). Several other studies presented the effect of FVL on the clinical parameters in PCOS patients with conflicting results [8, 9]. Tsanadis et al. reported no evidence that factor V Leiden differed between PCOS patients and healthy individuals [12]. Kazerooni et al. presented results that demonstrated an association of FVL mutation with pregnancy losses in patients with PCOS [13]. Regardless of the progress in investigating the role of FVL as a single risk factor and in combination with multiple factors, its impact on the progression of PCOS and related reproductive complications remains controversial [14].

Materials and Methods

Ninety-two women with polycystic ovaries were selected from a total of 2230 patients of the Clinic of Gynaecology and Obstetrics, Medical University-Pleven for the period of 2014-2018. The patients included in the study were selected based on clinical examinations and full clinical histories.

PCOS diagnosis was made according to the revised Rotterdam 2003 criteria and the adopted consensus by the European Society of Human Reproduction and Embryology, and the American Society for Reproductive Medicine [15]. The presence of any two of the following criteria was essential for diagnosing PCOS: 1) chronic oligomenorrhea for more than 35 days, or less than six menstrual periods in the previous year or amenorrhea; 2) clinical and/or biochemical signs of hyperandrogenism, 3) polycystic ovary morphology diagnosed by transvaginal sonography with the findings of 12 or more follicles (d=2-9 mm), and/or increased size of the ovaries (>10 mL).

Women with congenital adrenal hyperplasia, bilateral tubal block, organic uterine or ovarian pathology, thyroid dysfunction, Cushing's syndrome, diabetes, chronic hypertension, and recent infections or inflammation were excluded from the study.

The blood samples were taken in the early follicular phase of the menstrual cycle, after at least three months of amenorrhea. Oral contraceptives or other drugs influencing hormone levels (including statins or aspirin) were stopped at least three months before investigation.

The control group included 101 healthy unrelated women without a history of PCOS pregnancy loss, hirsutism, and hyperandrogenism, without a family history in terms of PCOS and similar BMI distribution.

Informed consent was obtained from all the patients and controls. The study protocol was approved by the Ethics Committee of Medical University-Pleven, Bulgaria.

DNA Analysis

Venous blood was collected in vacutainers with 0.084 ml 15% EDTA (Becton, Dickinson, and Company). DNA was isolated following the procedure of the GFTTM Genomic Blood DNA Purification Kit (Amersham Pharmacy Biotech Inc) and quantified using the agarose gel procedure.

Protocol for testing of FVL

Replacement of G with A at the 1691 position in the Factor V gene results in the loss of one

restriction site of Mnl I restrictase. After the incubation of the amplification products from the Mnl I DNA region, samples with FVL do not undergo the restriction by Mnl in the second specific site, thus providing less electrophoretic bands (DNA fragments), as compared to controls after the separation in the agarose gel (2.5%). The primers used have the following nucleotide sequence:5 'primer 5 ' CCC AGT GCT TAA CAA GAC CA 3'; 3' primer 5 ' TGT TAT CAC ACT GGT GCT AA 3' The reaction conditions for the investigated genetic defect were described previously [16]. The amplification was carried out by thermocycler (Techne, Ver. 11.04, Techne, Laboratory Equipment/keison.co.uk).

Hormonal assay

Hormonally related assays included serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, dehydroepiandrosterone sulfate (DHEAS), estradiol, sex hormone-binding globulin SHBG. Serum levels were measured with Chemiluminescent Immunoassay (CLIA) IMMULITE, Siemens, Roche. All the hormonal assays were performed at the Hospital Clinical Laboratory.

Statistical Analysis

The Statistical Package SPSS Windows, version 23.0 (SPSS, Chicago, IL, USA) was used for data analysis. The differences among group means were compared using a one-way analysis of variance (ANOVA), and the Bonferroni test was used as a post hoc test with ANOVA. Categorical variables were analyzed using the Chi-squared and Fisher's exact tests where applicable. Data are reported as mean ± standard deviation. A two-sided p-value <0.05 was considered statistically significant.

Results

Patient Main characteristics

A total of 92 women with PCOS (mean age 30.3 years) and 101 healthy control individuals (mean age 32.0 years) were included in the study. The patients were with a body mass index (BMI) 25.15 kg/m2. The clinical data, according to the criteria of the Rotterdam consensus (PCOS, n=92) were analysed. The patients had complaints of irregular periods and heavy menstrual bleeding, reproductive problems, obesity, and acne. The main demographic and anthropometric characteristics of the patients with PCOS and the controls are presented in Table 1.

There was no significant difference between the investigated groups (exact Fisher test> 0.05) by age, weight, and BMI. However, the two groups significantly differ (p <0.05) in oligomenorrhea, hirsutism, hyperandrogenism, and clinical parameters. The clinical parameters are presented in Table 2.

The ratio between LH and FSH was >3 in 17% and the total testosterone level was elevated in 34% of PCOS patients (Table 2).

Results from DNA Analysis

The patients and controls were investigated for prevalence of FVL polymorphism. The results of DNA analysis showed a higher but not

Table 1. Demographic, anthropometric and life style factors in PCOS patients and controls.

Parameters	PCOS (n=92)	Controls (n=101)
Age (mean, yrs.)	30.264±0.65	32.01±0.72
Age range (yrs.)	22-45	19-49
Height (m)	164.49±6.36	1.66±0.01
Weight (kg)	69.22±2.02	64.7±2.4
BMI (kg/m^2)	25.15 0.70	24.39 ± 0.55
Overweight	38.63	
Obesity (%)	22.72	
Waist circumference (cm)	82.46±5.9	74.35±3.7
Hip circumference (cm)	100.37±4.76	94.6±1.8
Waist-to-hip ratio (WHR)	0.80.60±0.03	0.70±0.03
Ferriman Gallwey score	6.5±0.7	5.1±0.6
Average ovarian volume (ml)	13.1±1.3	6.8±0.7^b

significantly higher carriage of FVL in PCOS patients (12.5%) as compared to controls (6.3%), OR=2.238, 95%, CI 0.777±6.449, p=0.104 (Figure 1, Table 3).

Incidence of Reproductive Failure in PCOS patients (carriers and non-carriers of FVL polymorphism)

The carriers of FVL polymorphism had a higher rate of reproductive problems. The total reproductive failure rate found in carriers was higher than that in non-carriers: 60.5% versus 47.2%, respectively (OR=1.819, 95% CI 0.632±9.259, p=0.117).

The primary infertility rate was very high in the carriers of FVL, as compared to non-carriers (30.0% versus 12.5%, OR=3.143, 95% CI 0.686±14.388, p=0.047). The secondary infertility was low (5.6%) in carriers of FVL as compared to non-carriers: 13.3% (OR 0.821, p>0.05), as well total infertility rate, which was 35.6% versus 25.8% in non-carriers (OR 1.263, p>0.05).

The proportion of unsuccessful pregnancies (missed abortions) was higher (not significantly)

Table 2. Levels of hormone and factors in the serum of PCOS patients and controls.

	PCOS (n=92)	Controls (n=101)	Reference range
Testosterone (nmol/l)	1.56±0.111	0.98±0.083	0.52-2.4 nmol/l
SHBG nmol/l	58.9750±9.46203	56.3806±8.29971	27.1-128 nmol/l
DHEAS (nmol/l)[a]	7.56±0.835	4.12±0.682	1.9-7.7 nmol/l
Estradiol (pg/ml)	55.23±6.092	21.43±8.1	15-350 pg/ml
LH (IU/litre)	10.13±2.537	12.1±1.3	1.9-12.5 IU/L
FSH (IU/liter)	8.99±1.068	5.52±1.033	4.7-21.5mIU/mL

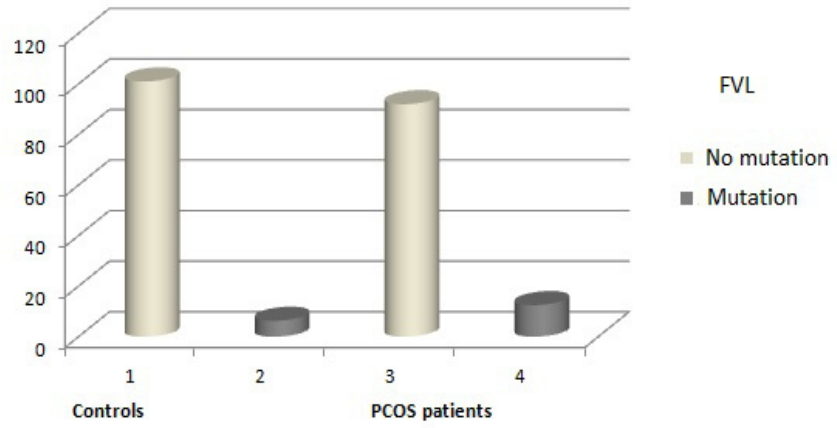

Figure 1. Prevalence of carriage of Leiden mutation in the FV gene in PCOS patients as compared to controls

Table 3. Prevalence, Odds Ratio, Pearson Chi-Square, Likelihood Ratio, Fisher's Exact Test, and 95% CI of FVL in the FV gene in PCOS patients, as compared to controls

Statistical values FVL	Prevalence in patients %	Prevalence in controls %	Odds Ratio	95% Confidence Interval	Pearson Chi-Squared	Likelihood Ratio	Fisher's Exact Test
Total Patients	12.5	6.3	2.238	0.777±6.449	2.319	2.309	0.104

Table 4. Occurrence/incidence Odds Ratio and 95% CI, Pearson Chi-Square, Fisher's Exact Test, of Spontaneous abortions, Missed abortions, Infertility, Total Reproductive Failure in PCOS patients carriers and non-carriers of FVL polymorphism.

Statistical values	Carriers of FVL (%)	Non Carriers (%)	Total	Odds Ratio	95% Confidence Interval	Pearson Chi-Squared	Fisher's Exact Test
Spontaneous Abortions	22.2	33.3	32.3	0.571	0.111±2.940	0.458	0. 445
Missed abortions	10.3	6.2	6.6	1.689	0.177±16.112	0.289	0.283
Pregnancy loss	30.3	34.6	34.1	0.811	0.195±3.383	0.137	0.581
Primary Infertility	30.0	12.5	15.5	3.143	0.686±14.388	2.358	0.047
Secondary Infertility	5.6	13.3	11.7	0.821	0.632±22.860	0.397	0.507
Infertility	35.6	25.8	26.9	1.263	0.297±5.336	0.896	0.481
Reproductive failure	60.5	47.2	53.6	1.819	0.632±9.259	1.102	0.117

in the carriers of FVL polymorphism (10.3% versus 6.2%, OR=1.689, p>0.05), but not in the patients with early pregnancy losses (Table 4).

Discussion

Polycystic ovary syndrome is characterized by a diversity of reproductive and metabolic abnormalities. PCOS is the primary cause of anovulatory infertility and poor pregnancy outcome. Although it is expected that thrombophilia could contribute to reproductive problems in PCOS patients, the data in the literature is still controversial.

Aiming to investigate the role of inherited thrombophilic factor – FVL polymorphism in the pathogenesis of the PCOS related reduced fertility, we analysed the patients for the prevalence of this polymorphism and an association between the carriage of FVL polymorphism and impaired fertility.

The main finding of our study was a higher rate of primary infertility in PCOS patients, who were carriers of FVL polymorphism, unlike non-carriers.

FVL polymorphism leads to a higher activity of the prothrombin complex and, thus, to higher thrombin activity. As a consequence, decidua cells could produce the antiangiogenic factor FMS, which inhibits enzymes of the proliferation of extravillous cytotrophoblasts [17-18]. Possibly, in women with a higher level of activated thrombin, the hemostasis could be easily misbalanced, which could compromise a blastocyst invasion and the implantation process, with a consequent effect on fertility.

FVL is known as a risk factor that predisposes to thrombotic complications, including pregnancy losses. It is known as a factor contributing to pronounced activation of thrombin, and related coagulation and proinflammatory complications.

The data obtained in a previous study we carried out demonstrated that FVL was a risk factor mainly for early pregnancy losses (from 5 wg to 14 wg) [19-20]. In this study, the rate of unsuccessful pregnancies was comparable in both carriers and non-carriers of FVL. In the subgroup of missed abortions, the rate was a little higher in the carriers of FVL polymorphism, but the difference was not significant. The results of Glueck (2003) showed that PCOS patients with RPL had a higher proportion of activated protein C and a higher prevalence of FVL, as compared to PCOS patients without RPL. We did not find any associations between RPL and FVL carriage, maybe due to the small number of PCOS patients with RPL (data not presented).

The present study provided evidence that there was no strong association between PCOS and FVL genotype. Although the OR was 2.338 and Pearson Chi-Squared was also high, the result was not significant. The results of our

study are in agreement with the investigation of Tsanadis et al. [12] and Athiomo [21]. They reported that there was no evidence that factor V Leiden differed between the PCOS and controls. The role of FVL was higher in carriers than in non-carriers, and though the OR was high, it was not significantly higher. The PCOS patients are characterized by a high primary infertility rate, and in our study FVL demonstrated a plausible role in its development. FVL polymorphism leads to a higher activity of prothrombin complex and, thus, to higher thrombin activity.

The identification of candidate genes with a specific contribution to the pathogenesis of PCOS seems to be effective for the establishment of the specific molecular basis for PCOS in the future. More studies with larger sample sizes on various populations should be conducted to define whether this particular polymorphism plays any role in the aetiology of PCOS and reduced infertility.

Conclusions

Carriage of FVL polymorphism in PCOS patients is associated with a higher rate of primary infertility, which draws attention to the role of the factor in the aetiology of the PCOS-related subfertility. Further investigations are needed to understand the impact of FVL on PCOS.

Acknowledgement

The study was conducted with financial support from Medical University – Pleven (Project 20/2018).

References

1. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. Endocr Rev. 2016;37(5):467-520. doi:10.1210/er.2015-1104.
2. Melo AS, Dias SV, Cavalli RC, Cardoso VC, Bettiol H, Barbieri MA, et al. Pathogenesis of polycystic ovary syndrome: multifactorial assessment from the foetal stage to menopause. Reproduction. 2015;150(1):R11-24.
3. Zueff LFN, Martins WP, Vieira CS, Ferriani RA. Ultrasonographic and laboratory markers of metabolic and cardiovascular disease risk in obese women with polycystic ovary syndrome. Ultrasound Obstet Gynecol. 2012;39(3):341-7.
4. Sir-Petermann T, Angel B, Maliqueo M, Carvajal F, Santos JL, Pérez-Bravo F. Prevalence of type II diabetes mellitus and insulin resistance in parents of women with polycystic ovary syndrome. Diabetologia. 2002;45:959-64.
5. Kiddy DS, Sharp PS, White DM, Scanlon MF, Mason HD, Bray CS, et al. Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases. Clin Endocrinol (Oxf). 1990;32(2):213-20.
6. Legro RS, Strauss JF. Molecular progress in infertility: polycystic ovary syndrome". Fertility and Sterility. 2002;78 (3):569-76.
7. Crosignani PG, Nicolosi AE. Polycystic ovarian disease: heritability and heterogeneity. Hum Reprod. 2001;7(1):3-7.
8. Sampson M, Kong C, Patel A, Unwin R & Jacobs HS. Ambulatory blood pressure profiles and plasminogen activator inhibitor (PAI-1) activity in lean women with and without the polycystic ovary syndrome. Clin Endocrinol. 1996;45(5):623-9.
9. Lee YH, Song GG. Plasminogen activator inhibitor-1 4G/5G and the MTHFR 677C/T polymorphisms and susceptibility to polycystic ovary syndrome: a meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2014;175:8-14.
10. Orio F Jr, Palomba S, Cascella T, Tauchmanova L, Nardo LG, Labella D, et al. Is plasminogen activator inhibitor-1 a cardiovascular risk factor in young women with polycystic ovary syndrome? Reprod Biomed Online. 2004;9(5):505-10.
11. Glueck CJ, Wanga P, Bornovali S, Goldenberg N, Sieve L. Polycystic ovary syndrome, the G1691A factor V Leiden mutation, and plasminogen activator inhibitor activity: associations with recurrent pregnancy loss. Metabolism. 2003;52(12):1627-32.
12. Tsanadis G, Vartholomatos G, Korkontzelos I, Avgoustatos F, Kakosimos G, Sotiriadis A, et al. Polycystic ovarian syndrome and thrombophilia. Hum Reprod. 2002;17(2):314-9.
13. T. Kazerooni, F. Ghaffarpasand, N. Asadi, Z. Dehkhoda,M. Dehghankhalili, Y. Kazerooni. Correlation between thrombophilia and recurrent pregnancy loss in patients with polycystic ovary syndrome: A comparative study. J Chin Med Assoc. 2013;76(5):282-8.
14. Moini A, Tadayon S, Tehranian A, Yeganeh LM, Akhoond MR, Yazdi RS. Association of thrombophilia and polycystic ovarian syndrome in women with history of recurrent pregnancy loss. Gynecol Endocrinol. 2012;28(8):590-593.

15. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1):41-7.
16. Komsa-Penkova R, Kovacheva-Kotseva K, Angelova S, Savov A, Semionova M. [Selected methods of DNA analysis and clinical applications]. Pleven: Medical University - Pleven; 2004. Bulgarian
17. Lash GE, Naruse K, Innes BA, Robson SC, Searle RF, Bulmer JN. Secretion of angiogenic growth factors by villous cytotrophoblast and extravillous trophoblast in early human pregnancy. Placenta. 2010;31(6):545-8.
18. Schatz F, Guzeloglu-Kayisli O, Arlier S1, Kayisli UA, Lockwood CJ. The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding. Hum Reprod . 2016;22(4):497-515.
19. Ivanov P, Komsa-Penkova R, Kovacheva K, Konova E, Simeonova M. Influence of factor V LEIDEN carrier on the development of reproductive disorders in different periods of pregnancy. Medical review. 2008;1:68-71.
20. Ivanov P, Konova E, Gecheva S, Cviatkovska C, Izmajlov A, Komsa-Penkova R. et al. Factor V Leiden in assisted reproductive technology. Obstetrics and Gynecology.2013; 6:11-14.
21. Atiomo WU, Condon J, Adekanmi O, Friend J, Wilkin TJ, Prentice AG. Are women with polycystic ovary syndrome resistant to activated protein C? Fertil Steril. 2000;74:1229–1232.