RJNMSR
Review Journal of Neurological & Medical Sciences Review

Volume 3, Issue 8, 2025

ASSOCIATION OF FACTOR 5 LEIDEN MUTATION IN HYPERTENSIVE DISORDERS OF PREGNANCY: A CASE-CONTROL STUDY OF PAKISTANI FEMALES' GENETIC SUSCEPTIBILITY

Amna Noor[1], Saima Rubab Khan[2], Mushayyada Rathore[3], Muhammad Ghufranullah[4], Syed Imran Ali Shah[*5], Zia ur Rehman[6]

[1]*M. Phil Scholar (Biochemistry/Basic Medical Sciences), Superior University, Lahore.*
[2]*Professor*
[3]*Assistant Professor, Biochemistry Department, King Edward Medical University, Lahore.*
[4]*Demonstrator*
[5] *Director Academics and Research, Professor & HoD, Biochemistry Department, Azra Naheed Medical College, Faculty of Medical Sciences, Superior University, Lahore.*
[6]*Lab Technologist, Biochemistry Department, CMH Lahore Medical College & Institute of Dentistry, Lahore.*

[1]amannoor432@gmail.com, s.shah10@alumni.imperial.ax.uk

Corresponding Author: *
Prof. Dr. Syed Imran Ali Shah
DOI: https://doi.org/10.5281/zenodo.17949189

Received	Accepted	Published
16 October 2025	29 November 2025	16 December 2025

ABSTRACT

Background:
Chronic Hypertension, Gestational Hypertension, Preeclampsia, Eclampsia and Chronic Hypertension with superimposed Preeclampsia or Eclampsia are the categories for hypertensive disorders of pregnancy (HDP). Pakistan has a significant burden of hypertensive diseases, as seen by the 9.3% reported prevalence of HDP. The mutation of clotting factor 5 gene called factor 5 Leiden (FVL) has been linked to HDP; For various HDP subgroups, there is currently no information available regarding this mutation.

Material & Methods: *It was a case-control study conducted in the Department of Biochemistry. Time taken for this study was 10 to 12 months from the start of sample collection till final analysis of the data. 56 patients with HDP as cases, and 56 normotensive pregnant controls were taken from a tertiary care hospital, Lahore. DNA was extracted and allele specific amplification was performed to detect FVL mutation (rs6025). A band on the gel documentation system confirmed the presence of a mutation.*

Results: *Genotypes observed: G/G (wild type) and A/G (mutation carriers). Chi-square test: Association observed ($\chi^2 = 5.23$, $p = 0.022$). OR = 12.089 (CI: 2.65 – 55.20). Carriers of the FVL mutation are more likely to develop HDP*

Conclusion: *The A/G genotype of the Factor V Leiden mutation is linked to a higher risk of pregnancy-related hypertension problems in our study sample. Although the mutation was found in just a few percent of patients and not in controls, it suggests a possible hereditary disorder.*

Key Words: *Factor V Leiden, Hypertensive disorders, Preeclampsia, Thrombosis*

INTRODUCTION

Pregnancy is a constantly evolving journey of metabolic and hemodynamic variations throughout each trimester and progressively reverts to normal after delivery.(1)

Hypertensive disorders of pregnancy (HDP) represent a significant health concern that poses substantial risks to both mothers and newborns. HDP has multiple groups with

different characteristics and outcomes. Gestational hypertension, preeclampsia, eclampsia, chronic hypertension and chronic hypertension with preeclampsia or eclampsia significantly increase mother and fetal morbidity and mortality.(2, 3)

HDP affects 10% of pregnancies worldwide, recent data show a considerable increase in the incidence of HDP, with rates ranging from 16.30 to 18.08 million.(4) Hypertensive pregnancy complications cause 15% of perinatal deaths worldwide. Approximately 16% of the 2.6 million stillbirths that occur annually are associated with these illnesses.(5) The incidence of HDP in Pakistan stands at 9.3% posing large burden on national health and also increasing risks of adverse outcomes.(6, 7)

Different risk factors, including nutrition, genetics, immune system, infections, bioactive vascular factors, and additional pathologic processes such as aberrant placentation, oxidative stress, and endothelial dysfunction, are associated with HDP. (8) PE affects 25% of chronic hypertensive women and up to 35% of pregnant hypertensives. Obesity and BMI >35 kg/m² are additional risk factors for hypertensive disorders of pregnancy (HDPs)(9). Genetic thrombophilias, blood clotting problems,poor socioeconomic groups, Black and Hispanic people, and those with a family history of preeclampsia or hypertension had higher rates of hypertensive illnesses.(10)

The Factor V gene encodes the protein coagulation factor V, which plays an important role in the blood clotting cascade. This protein remains inactive while circulating in the bloodstream and its active form (FVa) helps in thrombin formation by interacting with factor X in blood vessel damage.(11) In addition to this protein also helps in the prevention of excess clot formation through cleavage at a specific site by Activated Protein C.(12, 13) Inheritance of Factor V Leiden (FVL) follows an autosomal dominant pattern due to a replacement of a nucleotide at position 1691—guanine (G) to adenine (A)—and single amino acid change (glutamine replacing arginine at amino acid 506) within the factor V gene. As a consequence, the produced FVL variant of factor V exhibits inherent resistance to cleavage by APC.(14, 15) The characters of FVL are given in table 1.

FVL mutation leads to insenstivity to activated protein C and this sustained activation leads to hypercoagulable state. Individuals with FVL mutation poses a risk of harmful thrombosis formation.(16) A variety of disorders, such as clotting disorders, preeclampsia, intrauterine fetal growth limitation, intrauterine fetal death, and repeated fetal loss, can arise as a consequence of hypercoagulability. (17)

Multiple studies, including meta-analyses and case-control research, suggest a considerable association of FVL mutation with the development of HDP, highlighting its role as a latent genetic contributing factor in pregnancy complications.

Table 1 Characteristics of FVL Gene

SNP ID	rs6025
Mutation type	Missense
Position	Chromosome 1
Cytogenetic location	1q24.2
Exon Involved	10
Natural Variant #	rs6025
cDNA position	c.1691G>A
Protein Position	506
Amino acid substitution	Arginine-to-Glutamine

A favourable association has been observed in many studies between the probability of developing hypertensive problems during pregnancy and the FVL mutation.

A case control study was conducted in Sudanese women diagnosed with preeclampsia to find out association with FVL G1691A and prothrombin G20210A polymorphisms and

the results of the study showed a Factor V Leiden variation in 9.6% of cases compared to only 0.6% in controls, with a significant statistical difference (P<0.001).(18)

Limited studies have been performed on FVL mutation in association with preeclampsia. So far, limited data is available for different subgroups of HDP with respect to this mutation particularly in Pakistan. To adress the identified research gap, this study was designed to evaluate the association of clinical risk factors and FVL mutation (rs6025) in Pakistani females with HDP.

RESEARCH METHODOLOGY

This study was a case-control study, and the target population for this study was the pregnant females attending tertiary care hospital, Lahore, Pakistan. The time taken for this study was 10 to 12 months from the start of sample collection till the final analysis of the data.

Sample size (n) of 112 patients (56 in each group) was estimated by using a level of significance of 5%, and a power of the test as 90% with an expected proportion of factor V mutation in cases as 16.0% and in controls as 0.0%.(19)

Sample size has been calculated by using the formula:

$$n = \frac{\{Z_{1-\alpha/2}\sqrt{2\bar{P}(1-\bar{P})} + Z_{1-\beta}\sqrt{P_1(1-P_1) + P_2(1-P_2)}\}^2}{(P_1 - P_2)^2}$$

Where,

Z1 - β = 90% (power of study)

Z1-α/2 = 5% (level of significance)

P1 = expected population proportion 1 = 16.0%

P2 = expected population proportion 2 = 0.0%

P = P1 – P2

The participants of HDP were diagnosed according to the diagnostic criteria of updated guidelines provided by the International Society for the Study of Hypertension in Pregnancy (ISSHP). Women who were between the reproductive ages of 18 and 49. As controls, those with blood pressure of ≤ 120/80 mmHg over the entire gestational period and no proteinuria or seizures at gestational age greater than 20 weeks were included. Gestational hypertension (≥140/90 mmHg) after 20 weeks of pregnancy without proteinuria, Preeclampsia with Proteinuria, and Eclampsia with proteinuria and seizures. Subjects having past medical history of autoimmune disorders or systemic inflammatory diseases, such as rheumatoid arthritis, ischemic heart disease, diabetes mellitus, gestational diabetes, thalassemia, hemophilia, hepatitis, renal disorders, and systemic lupus erythematosus were excluded from the study. Non-probability convenience sampling technique was used for recruitment of the participants.

The study was approved by the institutional and ethical review board of Superior University, Lahore's in collaboration with CMH Lahore Medical College & Institute of Dentistry, with reference no FRB/BMS/04/01/2024. All laboratory work was performed in the molecular biology laboratory in the department of biochemistry. Informed consent was obtained from the participants on a pre-designed consent form in both English and Urdu before the collection of the blood sample. A blood sample of 3ml was collected through venipuncture following aseptic measures in a purple top vial containing Ethylenediaminetetraacetic acid (EDTA) tubes and stored at -20 degrees Celsius until further use.

DNA extraction from whole peripheral blood was done using a Thermoscientific whole blood Genomic DNA extraction kit (CatLog No. K0781, Vilnius, Lithuania),as per the manufacturer's instructions. The procedure was carried out in multiple steps and was based on the principle of silica membrane–based column binding of DNA. The purified DNA quality and integrity were evaluated by gel electrophoresis. For this purpose, the DNA samples were mixed with loading dye and subjected to electrophoresis, after which the appearance of distinct bands on the 1% agarose gel upon visualization with a transilluminator confirmed successful DNA isolation (Figure 1). The purified DNA samples were stored at –20 °C until further use.

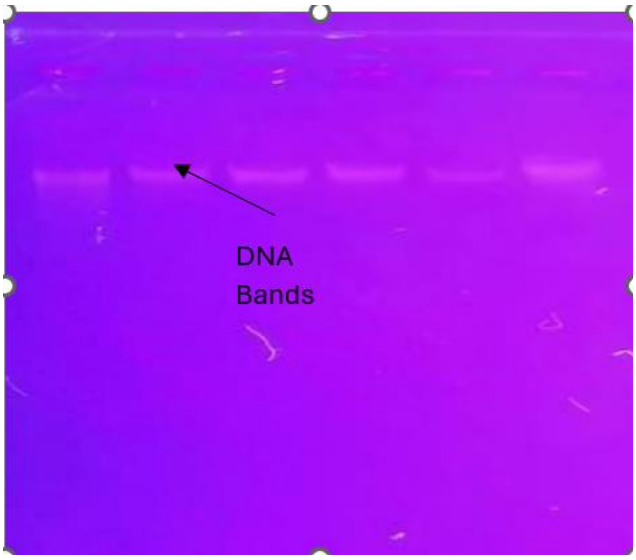


Figure 1 DNA Bands under Ultraviolet Transilluminator

For the detection of 1691 G/A Mutation, allele-specific primers were utilized to amplify the FVL gene. Primers were customized by synbio technologies manufacturer according to verified sequence.(20) (table 2)

Table 2: Primers used for allele specific PCR

Primers	Primers Sequence	Annealing Temperature	Band Size
Forward (A)	GCAGATCCCTGGACAGACA	59°C	152
Reverse Common	GGACTACTTGACAATTACTGTTCTCTTG		
Forward (G)	GCAGATCCCTGGACAGACG		

PCR amplification for detecting the FVL (G1691A) mutation was performed in a total reaction volume of 50 µl. Each reaction combination comprised PCR Master Mix (25µl), allele-specific forward primers(2µl), a universal reverse primer(2µl), template DNA (2µl), and nuclease-free distilled water (19µl).
Genotyping was performed using a G-Storm Thermocycler (Model: GS04822).The PCR tubes with prepared samples were mixed thoroughly and placed in the thermocycler. PCR was performed using following conditions:
Initial Denaturation: 95 °C for 5 minutes (1 cycle)
Amplification (35 cycles)
Denaturation: 95 °C for 30 seconds
Annealing: 59 ° C for 50 seconds
Extension: 72 ° C for 30 seconds
Final Extension: 72 ° C for 5 minutes (1 cycle)

The agarose gel was made by mixing 2g of agarose powder into 1x TAE diluted buffer in a clean, dry glass jar. The mixture was heated in micro wave oven until an homogenous mixture was formed. 3µl of Thermo Scientific Ethidium Bromide Thermo scientific CAS 1239-45-8 gel stain was added to the gel mixture, later on the gel was poured into gel casting tray with pre-positioned comb. The setting time of the gel was 25 to 30 minutes. After that comb was removed the gel was placed into an electrophoresis tank containing TAE buffer solution.
In the first well 13µl of 50bp DNA ladder was loaded and 13µl of the amplified samples were poured into the remaining wells. The gel was run for 45 minutes at 90 volts of current.
After that the gel was removed from the tank and observed under the UV transilluminator to observe the results. Presence of a single band of 152bp on the gel indicated the normal

genotype while mutated allele shows additional band indicating the mutated genotype.

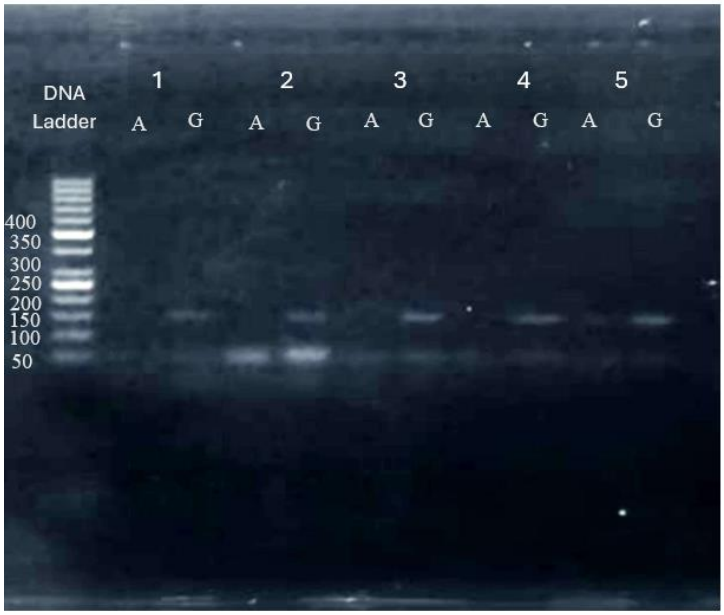


Figure 2 Normal (GG) FV Gene Bands under Transilluminator

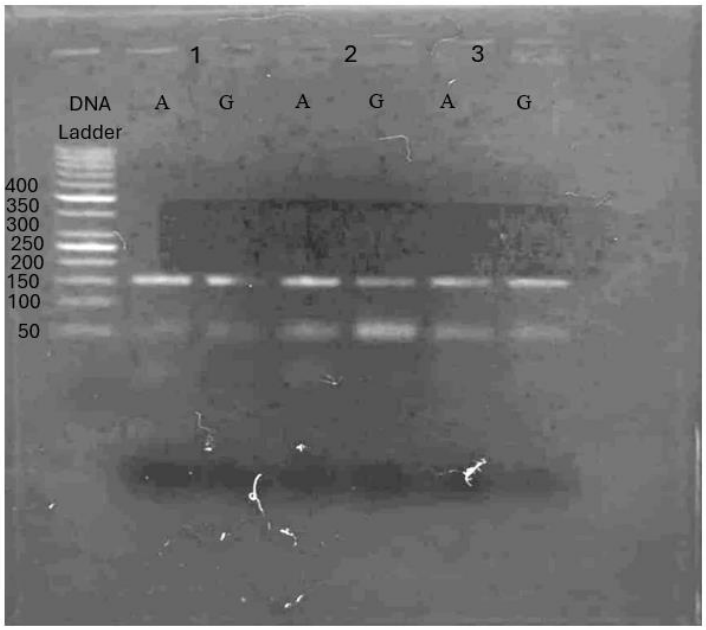


Figure 3 FVL Bands under Transilluminator

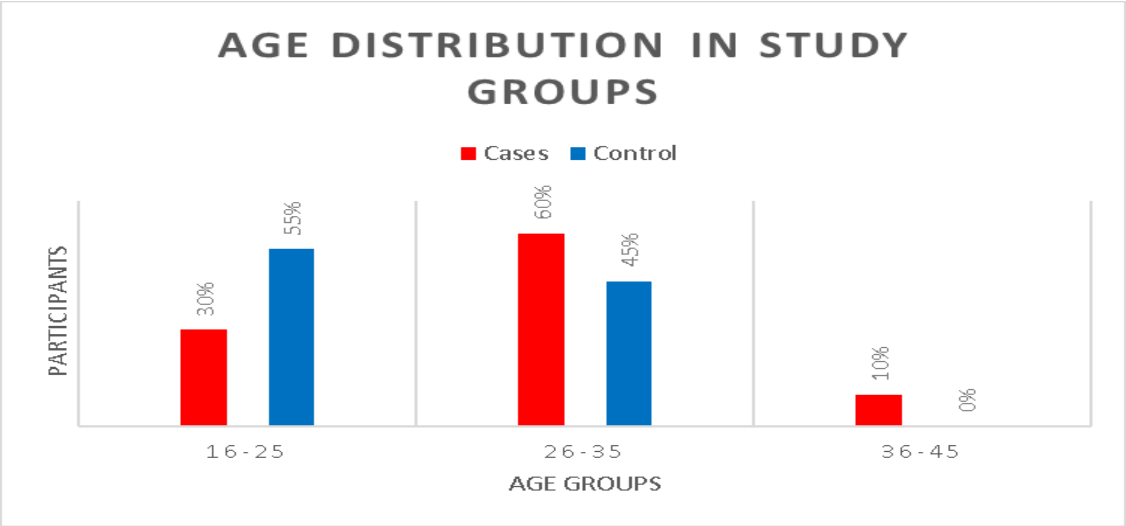
RESULTS

The mean age difference between cases and controls was 3.71 years (95% CI [2.012 to 5.416]), indicating that the case group had a higher mean age than the control group.

Variables	Case (n=56) M±SD	Controls (n=56) M±SD	t value	p value
Age(years)	28.46±5.07	24.91±3.98	4.119	0.011*

*p<0.05 is considered significant

when the age of patients was categorized in different groups, the chi-square test ($\chi 2$) revealed a significant age difference between patients and controls ($\chi 2 = 11.18$, $p = .004$). The highest proportion of cases (58.9%) occurred in the 26-35 age group, followed by the 16-25 age group. The highest proportion of controls (55.4%) was observed in the 16–25 year age group, followed by the 26–35 year age group. It was concluded that women with hypertensive disorders of pregnancy (HDP) were older than their healthy pregnant counterparts.



Genotype	Cases (56) n(%)	Controls (56) n(%)	Cases vs Controls
GG (Normal)	51(91.07)	56(100)	$\chi^2 = 5.23$ p = 0.022* OR = 12.089 (CI: 2.65 – 55.20)
GA (Hetrozygous Mutated)	5(8.93)	0(0%)	
AA (homozygous mutated)	0(0%)	0(0%)	N/A

*p<0.05 is considered significant

The association of the FVL mutation with HDP was evaluated through a Chi-square test. The 51(91.07) normal homozygous gene (G/G) and 5(8.93) heterozygous (A/G) mutations were seen among the 56 women with HDP, while the other 56 healthy pregnant females carried only the normal (G/G) gene.

The significant results indicate an association of mutation and disease. ($p = 0.022$, $\chi^2 = 5.23$) The odds ratio was calculated by adjusting for the 0.5 in controls. The carrier women of the mutated gene (A/G) had an increased risk of developing HDP. (OR=12.089).

DISCUSSION

Females with HDP were included in this study as it is associated with the risk of several chronic diseases, including heart problems like stroke, coronary artery disease, cardiac arrhythmias, renal disease, and multimorbidity, affecting maternal and neonatal health.(21)

In the current study average age of females having HDP was 28.63±5.03 years, while the mean age of healthy pregnant females was 24.91±3.98 years. Maximum number of cases (55.4%) were found to be in 26-35 year age group, while maximum number of controls (60.7%) were found to be in 16–25 year age group. According to this data , the incidence of HDP appeared to increase with advanced age(P=0.004).

Studies have shown that women over the age of 35 have a higher prevalence of HDP compared to younger women. (22)

A large cohort study performed in Japan observed that the likelihood of hypertensive disorders of pregnancy (HDP) was observed to be elevated in women aged 35 years or older compared to those under 35 years of age.(23) FVL is the most common type of thrombophilic mutation associated with potential complications in maternal and fetal health. A comprehensive study of randomized control trials (RCTs) demonstrated the increased likelihood of pregnancy complications including preeclampsia, RPL, and IUGR, with the FVL mutation.(24)

The findings of this study support the hypothesis and highlight that the Factor V Leiden mutation heterozygote (A/G) gene has an association with HDP as compared to control females. Regarding the mutated (A/G), it exhibited an occurrence rate of 8.9% within the diseased group, whereas it was absent (0%) in the control group. The adjusted calculated odds ratio showed that this thrombophilic mutation had a twelve -fold increased likelihood of developing hypertension in cases than controls. The findings of this study align with previous research aimed at identifying any significant association with HDP. Similarly, case-control research identified a strong association of FVL with PE in population of Sudan. (P = 0.0058. OR: 20.20). (19) In 2019, a meta-analysis studies carefully reviewed 50 qualified studies with 6041 cases and 8364 controls and their results showed that FVL mutation was significantly associated with HDP in the overall analysis (OR = 1.97, P < 0.00001).(25)

Opposite data also present that the FVL was insignificant in the preeclampsia of Southeastern Indian women because the heterozygous condition was more prevalent in the controls. In the case group, the frequency of the Leiden variant was 5.3% (8 out of 150), while in the control group, it was 6.7% (10 out of 150). (26) This difference may be due to different ethnicity, disease cohort, small sampling size and sampling method.

According to our study FVL heterozygous mutation A/G has association with HDP but further studies are required on a large scale to improve the quality of maternal and fetal health.

Conclusion

This study was the first study in Pakistan to find out association of FVL mutation with HDP. These data substantiate the complex etiology of HDP, encompassing maternal age, f. The association between the FV gene A/G mutation and HDP is particularly significant, contributing to the increasing evidence linking genetic risk factors to the onset of HDP.

REFERENCES

1. Lee R, Brandt JS, Joseph K, Ananth CVJP, epidemiology p. Pregnancy-associated mortality due to cardiovascular disease: impact of hypertensive disorders of pregnancy. 2024;38(3):204-15.
2. Lourenço J, Guedes-Martins LJJoCD, Disease. Pathophysiology of maternal obesity and hypertension in pregnancy. 2025;12(3):91.
3. Okikiade A, Kanu C, Iyapo O, Omitogun OJMLJ. The pathophysiological spectrum of maternal complications of pregnancy-induced hypertension. 2025;19(3):10.
4. Wang W, Xie X, Yuan T, Wang Y, Zhao F, Zhou Z, et al. Epidemiological trends of maternal hypertensive disorders of pregnancy at the global, regional, and national levels: a population-based study. 2021;21(1):364.
5. Basta M, Hanif K, Zafar S, Khabazeh A, Amin F, Sharif Khan S, et al. Impact of Hypertensive Disorders of Pregnancy on Stillbirth and Other Perinatal Outcomes: A Multi-Center Retrospective Study. Cureus. 2022;14(3):e22788.
6. Khan ZU, Khan SU, Tariq M, Mustafa W, Saleem H, Akbar A, et al. Relationship Between Cardiometabolic Index in Early Pregnancy and Hypertensive Disorder Complicating Pregnancy. Cureus. 2024;16(1):e51598.
7. Poon LC, Nguyen-Hoang L, Smith GN, Bergman L, O'Brien P, Hod M, et al. Hypertensive disorders of pregnancy and long-term cardiovascular health: FIGO best practice advice. 2023;160:22-34.

8. Johnston A, Smith GN, Tanuseputro P, Coutinho T, Edwards JDJP, Epidemiology P. Assessing cardiovascular disease risk in women with a history of hypertensive disorders of pregnancy: A guidance paper for studies using administrative data. 2024;38(3):254-67.
9. Kawwass JF, Badell MLJO, Gynecology. Maternal and fetal risk associated with assisted reproductive technology. 2018;132(3):763-72.
10. Umesawa M, Kobashi GJHR. Epidemiology of hypertensive disorders in pregnancy: prevalence, risk factors, predictors and prognosis. 2017;40(3):213-20.
11. Ahmadi M, Nasiri M, Ebrahimi A. Thrombosis-Related Factors FV and F13A1 Mutations in Uterine Myomas. Zahedan Journal of Research in Medical Sciences. 2016;In Press(In Press).
12. Perez-Pujol S, Aras O, Escolar GJT. Factor V Leiden and inflammation. 2012;2012(1):594986.
13. Favaloro EJ, Arunachalam S, Dean E, Salwa M, Ahuja M, Connelly L, et al. Activated Protein C Resistance Testing: An Update From Australasia/Asia-Pacific. 2025;47(4):720-9.
14. Abdi F, Moghadam ZB, Yazdkhasti M, Darooneh T, Rostami SJAoAiB. The Putative Role of Factor V Leiden and Prothrombin Mutations in Pregnancy Complications. 2019;10(2):63-77.
15. Albagoush SA, Koya S, Chakraborty RK, Schmidt AE. Factor V Leiden Mutation. StatPearls [Internet]: StatPearls Publishing; 2023.
16. Abdi F, Mehraban M, Banihashemi F, Roozbeh N. Potential role of Factor V Leiden mutation in adverse pregnancy outcomes: An updated systematic review. Biomedical Research and Therapy. 2017;4(12).
17. Alsheikh AM, Elsadek AM, Gebreel SAJIJoMA. Prevalence and clinical significance of factor V Leiden mutation in Egyptian preeclamptic women. 2021;3(1):1033-8.
18. Li Y, Ruan YJJoO, Research G. Association of hypertensive disorders of pregnancy risk and factor V Leiden mutation: a meta-analysis. 2019;45(7):1303-10.
19. Ahmed NA, Adam I, Elzaki SEG, Awooda HA, Hamdan HZ. Factor-V Leiden G1691A and prothrombin G20210A polymorphisms in Sudanese women with preeclampsia, a case-control study. BMC medical genetics. 2019;20:1-5.
20. Elzein HO, Saad AA, Yousif AA, Elamin E, Abdalhabib EK, Elzaki SG. Evaluation of Factor V Leiden and prothrombin G20210A mutations in Sudanese women with severe preeclampsia. Current Research In Translational Medicine. 2020;68(2):77-80.
21. Arsov T, Miladinova D, Spiroski MJCmj. Factor V Leiden is associated with higher risk of deep venous thrombosis of large blood vessels. 2006;47(3):433-9.
22. Zhao W, Di J, Huang A, Yang Q, Hu HJCCw. Incidence and risk factors of hypertensive disorders of pregnancy—8 provinces, China, 2014–2018. 2021;3(22):476.
23. Uchinuma H, Tsuchiya K, Horiuchi S, Kushima M, Otawa S, Yokomichi H, et al. Advanced maternal age elevates the prevalence of hypertensive disorders in women of Japanese, independent of blood pressure: a study from the Japan Environment and Children's study. 2025;48(3):904-13.
24. Garovic VD, White WM, Vaughan L, Saiki M, Parashuram S, Garcia-Valencia O, et al. Incidence and long-term outcomes of hypertensive disorders of pregnancy. 2020;75(18):2323-34.
25. BARBU S-A, BĂLĂNOIU L-G, FLOREA R-M, MAMBET CJHT, Syndromes A. The extent of Factor V Leiden thrombophilia in maternal and fetal health. Risk of placenta-mediated pregnancy complications-a systematic review. 2023;1(1).
26. Changalvala K, Kotur P, Shetty M, Kumar KP, Jagadish T, Balakrishna S, et al. Maternal Factor V Leiden Mutation in Preeclampsia: A Case-Control South Eastern Indian Tertiary Care Hospital Based Study. Journal of ClinicalDiagnostic Research. 2020;14(2).