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Evaluation of Factor V Leiden Mutation among COVID-19 vaccinated Sudanese individuals and complications of COVID-19 Vaccines

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Abstract

Background: Factor V Leiden (FVL) (G1691A) is a modified version of human factor V that may increase the risk of venous thromboembolism (VTE). It is generally accepted that the COVID-19 vaccinations have contributed to lowering the transmission of the virus and its severity and fatality. Among Sudanese people who received the COVID-19 vaccination, the research sought to examine the mutation of factor V Leiden (FVL) as well as any side effects.

Methodology: Between June 2022 and March 2023, this case-control experiment was carried out in Khartoum State to examine the mutation of factor V in Sudanese people who received the COVID-19 vaccine as well as side effects of the vaccine. The research included 150 participants who had been vaccinated against COVID-19 and 150 healthy, non-vaccinated individuals as a control group. Both men and women above the age of 18 years were participated in the research, which took place at Hayatt University College and Exon Laboratory in Khartoum, Sudan. The presence of FVL in blood samples was determined by real time PCR after DNA extraction. The best study design for accomplishing our objectives with regard to COVID19 problems and side effects was a direct interview utilizing a questionnaire for people who have received one or two doses of the Pfizer, AstraZeneca, or Johnson-Johnson vaccination.

Results: Among the 150 volunteers included in this study, 7 cases (4.7%) had FVL mutation (6 (4.0%) were heterozygous and 1 (0.7%) were homozygous), with an allelic frequency of 0.026, while no mutation was found in any of the healthy controls. This indicates that factor V Leiden was slightly frequently found in vaccinated individuals than in controls (P-value < 0.05).

The most prevalent side effect was pain at the injection site (32.6%), followed by general fatigue (28.7%), headaches (22.7%), fever (21.3%), joint pain (13.3%), dizziness (11.3%), swelling at the injection site (9.3%), shivering (5.6%), abdominal pain (4.9%), palpitations (4.7%), and thrombosis being the least common (0.7%).

The most common related disease in terms of comorbidities include hypertension (13.5%), obesity (12,8%), diabetes mellitus (12.0%), allergy (8.6%), heart disease (6.1%), asthma (5.3%), and renal disease (3.7%).

The majority of participants (47%) received the Pfizer vaccine, followed by Astra-Zeneca (31%), and Johnson-Johnson (22%). About (58.8%) received single dose, while (41.2%) received two doses, whether from Pfizer, Astra-Zeneca, or

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Johnson-Johnson. The prevalence of respondents who experienced side effects after taking the vaccination was (47.9%). COVID-19 vaccine side effects had lower rates in the participants who received the Pfizer vaccine ($p< 0.05$).

Conclusions: We conclude that the presence of the Factor V Leiden (FVL) in COVID-19 vaccinated individuals in Sudan is very low and does not lead to the incidence of thromboembolism (VTE). The study suggests that COVID-19 vaccines cannot impact or attributed in FVL mutation. It considers various factors other than COVID-19 vaccines that may lead to this mutation.

The study found that the incidence of side effects of the COVID-19 vaccine was lower in those who received the Pfizer vaccine. The study revealed the most important complications of taking COVID-19 vaccines among Sudanese population. It takes into account many elements that may contribute to the development of complications. We found that COVID-19 vaccination has only few complications. Therefore, we expect that this study will help in removing tension about dangerous complications of the COVID-19 vaccine.

Keywords: Factor V Leiden Mutation; COVID-19; COVID-19 Vaccine; COVID-19 Vaccine Complications

1. Introduction

In The factor V Leiden mutation (FVL), named after the Dutch city of Leiden where it was discovered, is a point mutation (G1691A) in the gene that codes for the human clotting cofactor V (FV). As a result of this genetic change, the translated FV molecule has an 'arginine' amino acid at amino acid residue 506 of the molecule instead of the usual 'glutamine' (Arg506Gln).(1,2) It was discovered that this seemingly innocuous point mutation results in a disorder known as activated protein C resistance, which causes carriers of the mutation to experience a severe clinical condition known as hypercoagulability or thrombophilia, which frequently manifests clinically as venous thromboembolic diseases (VTE).(3–6) With an annual incidence of 1 out of 1000, VTE is a major cause of morbidity and mortality in many countries.(3,4,7,8) Our earlier research, however, relied on traditional PCR and limited fragment length polymorphism approaches. In this study, we employed real-time polymerase chain reaction (PCR) (RTPCR) to analyze FVL, as it may be more sensitive than the standard PCR/restricted fragment length polymorphism approach. The COVID-19 vaccinations used in the United States were Moderna [mRNA1273], Janssen [Ad26. COV2. S], and Pfizer [BNT162b2] are typically safe and effective. However, vaccine-induced thrombotic thrombocytopenia (VITT) syndrome is a rare but serious thrombotic complication primarily associated with adenoviral vector-based vaccines. (9,10)

People with hematological illnesses and hypercoagulability conditions, according to the literature, are more susceptible to COVID19 infection due to the elevated likelihood of negative health consequences and death. However, the effect of the COVID-19 vaccine has not been thoroughly studied in individuals with hematologic illnesses such as glucose 6-phosphate dehydrogenase (G6PD) deficiency, thalassemia, sickle cell disease (SCD), pyruvate kinase enzyme deficiency (PKD), thrombophilia, hypereosinophilic syndromes, Glanzmann syndrome, sticky platelet syndrome, immune thrombocytopenia, and antithrombin deficiency.(11,12) Even though it According to the Centers for Disease Control and Prevention (CDC), it is reasonable to presume that every vaccine has risks, that serious negative outcomes are uncommon, and that the majority of minor side effects go away in a few days. (13) Fatigue, widespread pain, headaches, chills, and fever are among the potential systemic adverse effects. Pain, redness, and swelling are among the possible negative effects at the injection site. The United States Food and Drug Administration (FDA) also guarantees that COVID19 vaccination will lessen the severity of coronavirus illness by 50%. (14)

2. Materials and Methods

2.1. DNA extraction and real time-polymerase chain reaction (RT-PCR) analysis

This case-control study was conducted in Khartoum State to investigate the mutation of factor V among COVID-19 vaccinated Sudanese individuals and complications of COVID-19 vaccines during the period of June 2022 to march 2023.The study was conducted in 150 COVID-19 vaccinated individuals and 150 healthy non-vaccinated individuals as control group. The study was conducted in Hayatt university college and Exon laboratory, Sudan – Khartoum, and included both males and females above the age of 18 years. Genomic DNA was extracted from peripheral blood collected in EDTA tubes, 200 µl blood taken according to the manufacturer's instructions using the MN NucleoSpin® Blood kit and we followed the company protocol. A total of 150 µl of elution buffer was used to improve DNA yield.

PCR reactions were performed using the FV Leiden Real Time PCR Kit (SNP Biotechnology, Cat. No: 102R-10-01), which includes pre-aliquoted wild type and mutant master mixes in a ready-to-use format optimized for 5' nuclease PCR

detection. Prior to amplification, 5 µl of DNA (30 ng) was added to each PCR tube containing 20 µl of FV Leiden Wild Type or Mutant master mix (for factor V Leiden mutation G1691A), and 5 µl of control DNA was used in control reactions. Reaction mixtures were gently mixed by pipetting and centrifuged briefly.

2.2. Real-time PCR thermal cycler

Amplification was carried out using a real-time PCR thermal cycler under the following cycling conditions: Initial denaturation: 95 °C for 3 minutes, Amplification (30 cycles), Denaturation at 95 °C for 15 seconds, Annealing/extension at 60 °C for 1 minute. Fluorescence signals were detected using FAM and HEX/JOE channels. Data analysis was conducted using the ABI7500 system, and interpretation was based on amplification curves and Ct values as specified by the kit instructions (FV LEIDEN REAL TIME PCR KIT Cat. No: 102R-10-01).

Results were analyzed by the RT-PCR machine's software to indicate the presence of one of the following in each DNA sample: wild-type alleles only (normal), mutant alleles only (homozygous FVL) or both alleles (heterozygous FVL).

For COVID-19 complications and side effects the optimal study design for achieving our goals were direct interview using a questionnaire. We disseminated the survey to 150 individuals from the period of June 2022 to march 2023 in Khartoum state - Sudan, to males and females above the age of 18 who have been vaccinated by either Pfizer, AstraZeneca, or Johnson and Johnson in one dose or two doses.

2.3. Statistical analysis

Independent samples t-test was performed by SPSS V.28 to compare the percentages of FVL among vaccinated individuals and healthy non-vaccinated controls, and to study COVID-19 complications and side effects. A P-value < 0.05 was considered as statistically significant. Allelic frequencies were calculated by a mathematical equation that includes number of heterozygous cases, number of homozygous cases, and total number of cases.

3. Results

This case-control study was conducted in Khartoum State to investigate factor V Leiden mutation among COVID-19 vaccinated Sudanese individuals and complications of COVID-19 vaccines during the period of June 2022 to march 2023. The volunteers have no previous history of COVID-19 infection. The study was conducted in 150 COVID-19 vaccinated individuals and 150 healthy non-vaccinated individuals as control group. The study involved both males (45.3%) and females (54.6%) above the age of 18 years who have been vaccinated by Pfizer, AstraZeneca, or Johnson-Johnson in one dose or two doses.

Among the 150 volunteers included in this study, 7 cases (4.7%) had FVL mutation, (6 (4.0%) were heterozygous and 1 (0.7%) were homozygous), with an allelic frequency of 0.026, while no mutation was found in any of the healthy control group. This indicates that factor V Leiden was slightly frequently found in vaccinated individuals than in controls (P-value < 0.05).

These findings confirm that the prevalence of factor V Leiden mutation is lower among vaccinated individuals. The low prevalence of factor V Leiden mutation in Sudanese vaccinated individuals can, at least in part, account for the lower frequency of deep venous thrombosis and VTE reported in Sudanese vaccinated individuals. So COVID-19 vaccines cannot be the main causes and cannot impact or attributed in FVL mutation.

Table 1 The findings regarding the FVL mutation show that, at position 1691 of the FV gene, there can be either the normal (G) allele presents on both chromosomes (normal wild-type GG), the mutant FVL (A) allele present on both chromosomes (homozygous AA), or one chromosome carrying a normal (G) allele and the other with a mutant FVL (A) allele (heterozygous GA)

Mutant Genotypes	N = 150	FVL (n, %)	Allele frequency
GG	143	95.3 %	0.026
GA	6	4.0 %	
AA	1	0.7 %	

The majority of participants (47.0%) received the Pfizer vaccine, followed by Astra-Zeneca (31.0%), and Johnson-Johnson (22.0 %).

The most prevalent side effect was pain at the injection site (32.6%), followed by general fatigue (28.7%), headaches (22.7%), fever (21.3%), joint pain (13.3%), dizziness (11.3%), swelling at the injection site (9.3%), shivering (5.6%), abdominal pain (4.9%), palpitations (4.7%), and thrombosis being the least common (0.7%).

The most common related disease in terms of comorbidities include hypertension (13.5%), obesity (12,8%), diabetes mellitus (12.0%), allergy (8.6%), heart disease (6.1%), asthma (5.3%), and renal disease (3.7%).

The majority of participants (47%) received the Pfizer vaccine, followed by Astra-Zeneca (31%), and Johnson-Johnson (22%). About (58.8%) received single dose, while (41.2%) received two doses, whether from Pfizer, Astra-Zeneca, or Johnson-Johnson. The prevalence of respondents who experienced side effects after taking the vaccination was (47.9%). COVID-19 vaccine side effects had lower rates in the participants who received the Pfizer vaccine ($p< 0.05$).

According to the study's findings, pain at the injection site is the most common complications, followed by general fatigue, while thrombosis is the least frequent. Additionally, we found that the Pfizer vaccine recipients experienced low rate of COVID-19 vaccine complications. The complications from the COVID-19 vaccine were more likely in people who did not have any comorbidities. The findings also indicated that the most prevalent comorbidities were hypertension (13.5%), followed by obesity (12,8%), diabetes mellitus (12.0%), allergy (8.6%), heart disease (6.1%), asthma (5.3%), and renal disease (3.7%).

4. Discussion

A single point mutation in the factor V gene (guanine to adenine at nucleotide 1691) causes factor V Leiden, sometimes referred to as factor VR506Q and factor V Arg506 Gln, which results in a single amino acid alteration (replacement of arginine with glutamine at amino acid 506). This eliminates the Arg506 cleavage site for activated protein C in Factor V and Va. The factor V Leiden mutation raises the risk of thrombosis because activated protein C, a natural anticoagulant, cannot bind and inactivate factor V because the binding site on factor V for activated protein C is mutated. As a result, the fact that factor V is not inactivated contributes to its ongoing activity and raises the possibility of thrombosis. (15)

The single-point mutation in the factor V gene that causes factor V Leiden is from guanine to adenine at nucleotide 1691) that would cause the substitution of arginine with glutamine at amino acid 506. This eliminates the activated protein C Arg506 cleavage site in factors V and Va. The effect is a decrease in the anticoagulant function of factor V and an increase in the procoagulant function of factor Va. (15)

The factor V Leiden mutation is the most prevalent inherited thrombophilia in the unselected White population (prevalence, 1% to 5%) and is thought to be the most common inherited thrombophilia in people with venous thromboembolism (prevalence of roughly 10% to 20%). The lifetime risk of thrombosis is increased by around seven times by heterozygosity for this genetic variation, whereas homozygosity (which is uncommon) increases the risk by about twenty times. Heterozygosity of factor V Leiden does not appear to raise overall mortality, even though the risk of VTE has increased. (16) Research has patients with factor V Leiden showed only a slight rise in the risk of coronary artery disease. The factor V Leiden mutation has been found to raise the risk of stroke, particularly among younger people, smokers, and women. (17)

However, in this study, we discovered that vaccinated people had a very low prevalence of these mutations (FVL) at 4.7% each, and that none of our typical control subjects carried these mutations, whereas none of the healthy controls did. In Sudanese vaccinated people, the most important contributing factors to venous thromboembolism (VTE) are not Factor V Leiden mutations. Prior research on Thai people has demonstrated that factor V Leiden is not a significant contributor to venous thromboembolism (VTE), and our findings support this conclusion. (18)

This research covered the negative effects of the Pfizer, AstraZeneca, and Johnson & Johnson vaccines in Sudanese vaccinated people. Common side effects discovered by our research, such as general pain, fatigue, flu-like symptoms, fever, injection site pain, and edema, are not cause for concern. These minor adverse effects were verified by the Saudi Ministry of Health and the centers of disease control (CDC) in January 2021. (19, 20) In a randomized controlled trial, Polack et al. found similar adverse effects and a low rate of severe side effects. (21)

In terms of vaccine type, patients who received the Johnson & Johnson and AstraZeneca vaccines were more likely to experience adverse effects from the COVID-19 vaccination compared to those who received the Pfizer vaccine. Two different articles by Almughais et al. and Alzarea et al. (22,23) provided similar findings. The reverse transcription of nasopharyngeal swab samples was used to identify the COVID19 nucleic acid reverse transcription loop-mediated isothermal amplification, thrombotic microangiopathy (TMA), or transcription polymerase chain reaction (RT-PCR) testing. The gold standard for COVID19 testing is RT-PCR, which is commonly used in diagnosis. On the other hand, TMA uses a different set of enzymes and reagents to amplify the viral RNA, thus identifying COVID19 similarly to RT-PCR. Cases with a low viral burden may produce false negatives when the TMA technique is used, which may lead to erroneous conclusions not be accessible in certain healthcare settings. Vaccines are the most important public health measure to protect people from COVID-19, because SARS-CoV-2 is highly contagious. (24,25) Vaccine trials involve several stages, including preclinical testing on animals to assess safety and effectiveness, followed by clinical trials involving human participants. These trials are conducted in multiple phases, starting with a small group of volunteers and gradually increasing the number of participants to gather more data on safety and efficacy. (26)

5. Conclusion

We conclude that the presence of the Factor V Leiden (FVL) in COVID-19 vaccinated individuals in Sudan is very low and does not lead to elevated incidence of thromboembolism (VTE). The study suggests that COVID-19 vaccines cannot be the main causes and cannot impact or attributed in FVL mutation. It considers various factors other than COVID-19 vaccines that may lead to this mutation.

The study found that the incidence of side effects of the COVID-19 vaccine was lower in those who received the Pfizer vaccine. The study revealed the most important complications of taking COVID-19 vaccines among Sudanese population. It takes into account many elements that may contribute to the development of complications. We found that COVID-19 vaccination has only few complications. Therefore, we expect that this study will help in removing tension about dangerous complications of the COVID-19 vaccine.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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