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Impact of Factor V Leiden (G1691A) Variant in Saudi Women with Gynecological Disorders

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ABSTRACT

Gynecological disorders are defined as a condition which disturbs the female reproductive organs. Recurrent Pregnancy Loss (RPL) is one of gynecological disorder which is ostensibly defined as more than couple of consecutive miscarriages before 20 weeks of gestation. After the abnormalities found in chromosomes, thrombophilia is known to be the one of the major genetic factors that may prone the RPL disease. Factor V Leiden (FVL) mutations are well-known for one of the thrombophilia and eminent for essential clotting factor in coagulation cascade. The aim of this study was to investigate the genetic association of G1691A mutation in FVL gene at RPL in the Saudi women. In this study, 113 women were involved with RPL. Collected blood was stored in an EDTA tube to extract the genomic DNA using kit-based method. Real-Time Polymerase chain reaction was carried out using the probes. The mean age of the involved 113 women were 34.4±5.79 years and Body Mass Index were established as 30.06±7.05. In this study, 99.1% of genotypes has confirmed as GG genotypes, heterozygous (GA) was documented in single RPL women (0.09%) and AA was not documented in any women. In conclusion, this study showed only single heterozygous in G1691A mutation in FVL gene. This could be due to the small sample size.

KEY WORDS: RECURRENT PREGNANCY LOSS; FVL, G1691A, THROMBOPHILIA.

INTRODUCTION

Gynecological disorders are defined as a condition which disturbs the female reproductive organs. Endometriosis, polycystic ovarian syndrome, Fibroids, female infertility (FI), ovarian cysts, premenopausal syndromes are some of gynecological disorders and recurrent pregnancy loss (RPL) is categorized under FI. Nearly 1:20% of reproductive women experiences couple of consecutive miscarriages and <1% experience triple or more miscarriage completely is known to be RPL (Park et al. 2020; Rohilla 2020; van Dijk et al. 2020). RPL can be not essentially for spontaneous demises of pregnancy; although 15% of first trimester pregnancies ends in miscarriages (Leduc-Robert et al. 2019). It has been assessed that 01-02% of second trimester pregnancies involves miscarriages before 24 weeks of gestation (Maddirevula et al. 2020).

RPL aetiology involves numerous factors like chromosome abnormalities in the couples, infections, uterine alterations, endocrinological disorders, autoimmune diseases and randomly, 50% of RPL cases remains to be idiopathic(Arias-Sosa et al. 2018). The major reason for developing RPL is due to the genetic and non-genetic factors (Dean et al. 2019). However, maternal-paternal ages, endocrine, uterine anatomic abnormalities, sperm-quality, infections, metabolic/hormonal disorders, environmental and immunological factors have been associated with RPL(Bhatt et al. 2020).

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Maternal age and previous histories of miscarriages at outset are confirmed to be couple of major and important risk-factors for consequent miscarriage. Maternal age is also connected with the risk of miscarriage when the women turns to be 35 Years (11% risk in 20-24 years and 51% risk in 40-44 years) (Bhatt et al. 2020). The prevalence of RPL is in between 1-5% and documented in reproductive medicine and modern diagnostics. Both maternal and paternal inheritances are connected with in development of RPL in the initial stages of the pregnancies(Trifonova et al. 2019). RPL are categorized as primary RPL is defined as women without any effective pregnancies and secondary RPL is denotes as women with fruitful pregnancy interm of live new-infant(s) with the histories of miscarriages(Michita et al. 2019). After the abnormalities found in chromosomes, thrombophilia is known to be the one of the major genetic factors that may prone the RPL disease (Fesahat et al. 2020).

Thrombophilia is a generic term defines an increased propensity towards thrombosis and its associated morbidities (Favaloro 2019a). Factor V Leiden (FVL) mutations are well-known for one of the thrombophilia and eminent for essential clotting factor in coagulation cascade. FVL acts as co-factor for permitting factor X to rouse, conversion of prothrombin to thrombin (Ajmeri et al. 2020). Activated protein C is known as active anticoagulant which extends the clotting through terminating factor V and lowers the thrombin formation (Heeb et al. 2009). Factor V Leiden (FVL) epitomizes single nucleotide polymorphism (SNP) in the F5 gene causes missense mutation; substitutes from Guanine-Adenine at 1691 position and amino-acid modifies from arginine-glutamine (Kamineni 2015). Heterozygous mutation in G1691A is popularly known to increase relative risk of thrombosis between 1.8-2.6 folds of increase in common population and significance of FVL mutation with RPL disease is controversial. Global studies showed significant and non-significant associations between RPL and FVL mutation (Reddy et al. 2019). There are no studies which have been carried out in the capital city of Saudi population and current study aims to investigate the general association between G1691A mutation in FVL gene and RPL in the Saudi women.

MATERIAL AND METHODS

In this study, 113 cases of RPL cases have been recruited from Gynecology Dept in King Saud University (KSU) Hospital. In this study, only RPL womens were recruited and the selection of RPL is defined as per the prior study carried out in the Saudi population(Turki et al. 2016). Ethical approval was obtained from IRB at KSU (E-19-4445) and inform consent was signed by the women who has participated in this study. Clinical details were obtained from the involved women and 4 mL of the EDTA blood was collected and used for molecular analysis. Genomic DNA was extracted using DNA isolation kit as per the company's instruction. DNA was quantified with NanoDrop, a spectrophotometer and 20ng/ul of each genomic DNA was used to perform the real-time polymerase chain reaction (RT-PCR) using Roche (LightCycler, version 2.0) instrument was used with VIC and FAM probes was used to analyse the alleles for G1691A mutation in FVL gene. The protocol of RT-PCR was performed as per the company protocol. Statistical analysis was applied using Openepi software (Khan et al. 2019). Variable data was expressed in mean±standard deviation. Allele and genotype frequencies were distributed in the form of percentages.

RESULTS AND DISCUSSION

The current study involves 113 women confirmed with RPL from Dept. of obstetrics and Gynecology from KSU clinic. The baseline characteristic details were involved in Table 1. The mean age of the involved 113 women were 34.4±5.79 years. All the involved subjects were women with 85.8% were documented as Saudi nationalities. BMI was confirmed as 30.06±7.05. The overall prevalence of chronic disease was found to be 44.2% and 5.3% was documented as the prevalence of diabetes. The prevalences of Hypertension, hypothyroidism, asthma, anemia, PCOS and stroke were found in the RPL women was 1.7%, 15.1%, 4.4%, 0.9%, 7.1% and 0.9% respectively. None of the women were found to be the prevalence for dyslipidemia (0%) and 92.9% of involved women were had the miscarriages. 7.9% of RPL women had family history. Genotype and allele frequencies of G1691A mutation of FVL gene in RPL women has been documented in Table2. GG genotype was confirmed as homozygous; GA as heterozygous and AA as homozygous variants. In this study, 99.1% of genotypes has confirmed as GG genotypes; GA (heterozygous) was documented in single RPL women (0.09%) and AA was not documented in any women and the prevalence was found to be 0%. The prevalence of G allele was 0.96 and 0.04% was found to be A allele.

Thrombophilia is documented as one of the common causes for RPL which can be observe in 40-50%. Thrombophilia in mother could ripen the hypercoagulable state of pregnancy; which generates prethrombotic vasculopathy at the placental level. This hypercoagulable stage becomes more worst and impair blood flow by the maternal veins, further which leads to deep vein thrombosis. This will clot in the vessels of placenta which leads to fetal growth restriction or demise (Favaloro 2019b; Garrido-Barbero et al. 2019). Thrombophilic gene polymorphism is known to be a risk factor in RPL women and FVL gene is one of thrombophilic gene (Farahmand et al. 2016). In this study, G1691A mutation from FVL gene has been performed in RPL women and 99.1% of GG and 0.09% of GA genotypes has been documented in 113 RPL women. AA genotype was not documented in this study. However, Turky et al (Turki et al. 2016) studies showed prevalence of heterozygous variant as 14.9% and homozygous as 0.5% in Saudi population with RPL couples. The present study was carried out in RPL women in 85.8% of Saudi women.

RPL is a real disenchantment for couple who failed to conceive the child. Most of the studies have confirmed

RPL as cytogenetic and molecular abnormalities which leads to further recurrent miscarriages and pregnancy demises (Jain and Malik 2014). RPL in general was named habitual abortion; defined as minimum of 3sequential miscarriages before 20th week of gestation. This definition was confirmed by both Royal college of obstetrics and gynecologists and European society of human reproduction and embryology. Spontaneous miscarriages occur randomly in 15% of clinically confirmed pregnancies in <35 years of age. The prognosis in RPL couples is not confirmed through single parameter but established with risk factors along with the precise characteristics. Maternal age is connected with RPL through the cellular mechanisms governs meiotic spindle formation and function have the huge rate of error. It was assumed that 30% of embryos are aneuploid in the women whose age is 40 (Koifman et al. 2016). RPL is known to be multifactorial in nature and numerous risk-factors have been linked up with its pathogenesis (Bahia et al. 2020).

Numerous global studies have been linked up with the G1691A mutation and RPL globally. This case-control studies have been carried out in various ethnic populations in the global world. Maximum studies have been performed with PCR-restriction fragment length polymorphism method. However, present study was performed with RT-PCR which is known to be one of the strengths of this study. The difference between thermal PCR and RT-PCR are; thermal PCR require the validation to cross-check the study results, whereas, RT-PCR studies doesn't require any validation. The results were found to be accurate by involving couple of probes. These probes are known to be labelled fluorescently in DNA oligonucleotides and bind to the sense and antisense primers. Global studies have showed both the positive and negative associations (Balajewicz-Nowak et al. 2015; Farahmand et al. 2016; Jusic et al. 2018; Karadag et al. 2019; Kardi et al. 2018; Kashif et al. 2015; Reddy et al. 2019; Sharma et al. 2015; Turki et al. 2016).

The global results may vary depends on the ethnicity of specific countries. Meta-analysis studies should be implemented for the accurate results. Meta-analysis of case-control data can be expanding the association of confirmed analysis from the prior studies (Khan et al. 2016). A couple of meta-analysis studies have been performed in G1691A mutation in FVL gene with RPL and both the studies shows the significant association (Kovalevsky et al. 2004; Sergi et al. 2015). So, G1691A mutation has a prominent role in RPL globally. The strength of the present study was implemented in the RPL women and genotyping was performed with RT-PCR analysis. The limitation of this study was skipped the control subjects, performed only single SNP and lower subjects were involved. In conclusion, this study showed only single heterozygous in G1691A mutation in FVL gene. This could be due to the small sample size. Future studies should be performed with large sample size in various ethnic populations.

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