**The Effect of Oral Contraceptive Pills on the Gene Mutation of Factor V Leiden among Sudanese Women**

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**Abstract:** Oral contraceptive pills are problems for women, often have many effects and may cause several diseases. The purpose of this research was to determine the effect of oral contraceptive pills on factor V sufferers.

This case control study was conducted in Khartoum, Sudan, during the period from April to November 2018. The study included 50 women who used oral contraceptive as a case and 50 women who did not use oral contraceptive pills as a control, all of whom were verbally informed of the study and approved for participation. The PCR was done for each sample.

The results obtained from cases show that the mean age is 30±5.5 and divided into three groups less than 20 with a lower frequency of 4 % (2/50), (20-35) with a higher frequency of 80 % (40/50) and a higher frequency of 16 % (8/50) for more than 35 years. Most cases use oral contraceptive pill for more than 1 year at a frequency of 60 % (30/50) with a mean of 2±0.8.The most frequent oral contraceptive pill use was levonorgestrel 88%(44/50) followed by desogestrel 12% (6/50).

The study concludes that there is no significant difference in gene mutation between case and control. There was also an insignificant association between mutation and demographic data

**Keywords:** Factor V Leiden; Oral Contraceptive; Mutation; polymerase chain reaction (PCR).

**INTRODUCTION**

 Oral contraceptives (OCs) also known as pills are the most popular form of contraception (Dhont, 2010). The key mechanism of action is ovulation suppression and, in addition, the oral contraceptive produces endometrium that is not receptive to ovum implantation and cervical mucus that has become dense and hostile to sperm transport (Hall et al., 2010).

Various types of oral contraceptives exist; combined oral contraceptives (COCs) containing estrogen and progestin (Kiley and Hammond, 2007), and Progestin-only contraception (POPs) containing progestin but no estrogen containing this drug referred to as a mini pill (Adamopoulou & Vgenopoulou, 2015; Renner & Edelman, 2016).

Oral contraceptive estrogen raises the amount of clotting factors (II, VII, XII, VIII) in plasma (van Rooijen et al., 2002), Fibrinogen and thrombin activable fibrinolysis inhibitors by influencing gene transcription of different proteins (Hoppe, 2014). Estrogen crosses a cell membrane for a specific target tissue within a cytoplasm attached to a nuclear receptor (Bassett et al., 2003). Estrogen – a nuclear receptor complex then moves to the nucleus where it identifies and binds to unique recognition sites that contribute to gene transcription by allowing RNA Polymerase II to transcribe proteins to that DNA region in this case, new proteins are the coagulation factors (Trenor et al., 2011; Aranda & Pascual, 2001).

Factor V is one of several substances that helps blood clots to increase blood clotting (Fogelson, & Neeves, 2015), The clotting action of factor V is regulated by anther protein called the activated protein C dose that does not function well with the abnormal factor V suffering protein factor V (Levi & van der Poll, 2013) and increase the chance of developing deep vein blood clot (DVT) in the leg or lung (pulmonary embolus) (Gruber and Bull, 2012).

**METHOD AND MATERIAL**

 This was prospective case control study, conducted at women use oral contraceptive during period from April to November 2018.fiftywomen use oral contraceptive (case group) and 50 women did not use anycontraceptive (control group) were enrolled in this study. As well as any women had coagulation problem, last time exposure to bleeding, thrombosis or take vitamin effect on coagulation system were excluded from this study**.**

**Sampling and data collection**

 Three ml of venous blood was collected from each participant in study using disposable sterile syringe, after disinfected the collection site with70% alcohol then the bloodwas dispensed in a sterile EDTA blood container.Data was obtained by direct interviewing using a well-structured questionnaire.

**DNA extraction**

 DNA was extracted from whole blood using Guanidine hydrochloride, 4 ml of red blood lysis buffer (RCLB) were added to each sample, then centrifuged for 5 min at 6000 rpm, this step was repeated 2 times until a clear pellet of white blood cell were appeared, the supernatant was discard and(2 ml of white blood lysis buffer (WCLB), 1ml of guanidine hydrochloride, 300 of ammonium acetate (NH4) and 10µl of proteinase K) were added, then were incubated over night at 37Cº.After overnight incubation the sample were cooled at room temperature, and then 2ml of pre chilled chloroform were added, after that samples were centrifuge for 5 min at 6000rpm, upper layer were collected to new falcon tube contained 10 ml of cold absolute ethanol and then were incubated overnight at -20Cº.Afterovernight incubation the sample were centrifugated for 10 min at 6000rpm, the supernatant weredrained, pellet were washed with 4ml 70% ethanol, then were centrifugatedfor 10 min at 6000 rpm and supernatant were poured off and pellet were allowed to dry, then pellet were dissolved in 100µlof double distilled water (DDW)and incubated at 4Cºuntil used (Chomczynski et al., 2006).

**PCR reaction**

 Each PCR reaction was performed in a final volume of 20 containers (2.5Mm dNTPs, 2 target DNA.1(10pmol) of each primer(C, N, M) and 2.5U (5U/) of taq polymerase, followed by thermal cycler (Eppendorf master cycler) samplesThe PCR reaction was performed by ARMS for Factor V Leiden, the wild-type primer is 5, GGACAAATACCT GTATTCC3, the mutant primer is 5, GGACAAATAC CTGTATTCCTT3 and the common primer is 5CTTTCA GGCAGGAACACC3. Thermal cycling conditions consisting of 5 min denaturation at 95Co followed by 35 denaturation cycles at 95Co for 30s, annealing at 60oC for 30s, extension at 68Co for 60s, then final extension at 72Co for 5 min, maintained at 4Co for use. The PCR reaction was treated with ethidium bromide on 2% agarose gel at 150 V for 45 minutes (Dajani et al., 2013).

**Ethical considerations**

 Ethical approval was obtained from the College of Medical Laboratory Committee, Ethical approval No (MLS–IEC-02-17).

Participants were verbally informed, in their simple language, about the research, its benefits and the method of collecting the samples, and then their participation approval and publication of the data were obtained.

**Statistical analysis**

 The statistical analysis of the result was conducted using the Quantitative Package for Social Sciences (SPSS) version 16 for the interpretation of the results, a significant difference of less than 0.05 was calculated using the Chi square method.

**Result and discussion**

 The study conducted to determine the subsequent effect of oral contraceptive on factor V gene includes 50 women using oral contraceptive age of the participating patient and a corresponding group of fifty apparently healthy women who do not use oral contraceptive pills or other contraceptives.

Both participants have no drug effect on haemostatic parameters and have no prior thrombosis or family history of bleeding or thrombosis.

Participants were classified in less than 20, (20-35) and more than 35 years by age in three groups.

Based on the results, mean ± StD (30± 5.5 and 29±8.0) in case and control respectively, with insignificant P.value=0.52.

The overall frequency in case group showed that, the higher frequency 19 (38.5%) were in women with more than three children (multi grand), while the lower frequency 3 (6.5%) were in women with one child (primary). In contrast, the higher frequency 19 (38.5%) were in women with two (secondary) and three (multi) children for each among control group (table 1). Women those used Levonorgestrel were more frequent 44 (88.5%) than those were used Dosogestrel 6 (12.5) (table 1).

The age group of (20-35) years was the most frequent, while the less than 20 years were lowest frequent (table. 1)

The result of PCR showed that, all sample give reaction in wild type (normal), the mutant type was absent in all participant (figure 1).

Table1: Distribution of Demographic Data among Subjects

|  |  |  |
| --- | --- | --- |
| **Variable** | **Case (%)** | **Control (%)** |
| **Gravity** |
| Primary (one child) | 3 (6.5) | 4 (8.5) |
| Secondary (two child) | 14 (28.5) | 19 (38.5) |
| Multi (three child) | 14 (28.5) | 19 (38.5) |
| Grand multi (more than three child) |  19 (38.5) | 8 (16.5) |
| **Type of oral contraceptive** |
| Levonorgestrel | 44 (88.5) | - |
| Desogestrel | 6 (12.5) | - |
| **Age group** |
| >20 | 2 (4.5) | 6 (12.5) |
| 20-35 | 40 (80.5) | 31 (62.5) |
| < 35 | 8 (16.5) | 13 (26.5) |



**N**

**M**

**N**

**M**

**N**

**N**

**M**

**M**

**M**

**N**

**PCR**

**Product**

**250 bps**

Figure 1: The PCR product for participant, the lane 1 ladder 50 pb,N= normal (wild type), M=mutant

The factor V gene is located on chromosome 1q23 and contains 25 exons. The factor V gene defect occur in exon 10 where there is a G-A substitution at nucleotide 1691, this study showed that, there no significant change on factor V, this agree with study of Ibrahim found there was no mutation in FV, but disagree in that most significant affect patient in age 18-45 was oral contraceptive and in this study there no significant affect in age (*P.value*=0.5243) and there no correlation with mutation and other risk factors (age, gravity)[8].Also agree with study done by Piparva, on deep vein thrombosis in women taking oral contraceptive and notice that woman taking oral combined contraceptive for 3-5 months, developed DVT thrombosis of left leg, hereditary and acquired cause of DVT were excluded (there no mutation in factor V) and believe that risk of blood clot due to dose of estrogen (Piparva et al., 2011).

In present study duration of use oral contraceptive found most cases use oral contraceptive for more than one year this mean use of oral contraceptive for long time act as protection by decrease risk of thrombosis this agree with study of Martinelli that was divided case group into three (short less than year, long (1-5) year, very long more than 5 years) and found the risk of VTE in oral contraceptive user decrease over time (Martinelli et al., 2016)

In our study most of cases use levonorgestrel that mean this type of oral contraceptive not cause or have lower effect, this agree withVinogradova study risk of venous thromboembolism and exposure to combined oral contraceptive and found that type of progestogen hormone (drospirenone, desogestrel, gestodene and cyproterone) were associated with increased risk of venous thromboembolism when compared to pill containing older progestogen (levonorgestrel and norethisterone) (Vinogradova et al., 2014).

Effect of oral contraceptive increased probability of VTE development depend on dose in medication and type of contraceptive used (Wolski, 2014).

The study conclude that, oral contraceptive pill that not had effect on factor V and there no correlation between mutation and other risk factor (age, gravity).

**Aknowlogment**

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