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# Association between Plasminogen Activator Inhibitor Type I 4G /5G Genes Polymorphisms with Menorrhagia among Sudanese Females in Gezira State

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***Abstract: Background:*** *Menorrhagia is a heavy periods due to menstrual problems (the regularity, frequency or character of bleeding), leading to seeking medical help. Menorrhagia is an excessive menstrual blood loss that interferes with the woman's physical, emotional, social, and material quality of life.* ***Aim:*** *The study aimed to find out association between plasminogen activator inhibitor-1(4G/5G gene) polymorphisms and menorrhagia amongst Sudanese females from Gezira State. In addition, to study association between the plasminogen activator inhibitor -1 (4G/5G gene) polymorphisms and the different risk factors (duration of menorrhagia, amount of menorrhagia, regulation of menstruation, family history, and clinical features).* ***Methodology:*** *This descriptive case control study was done during the period from 2016 to 2020. A 90 blood samples were collected from adult females with menorrhagia as cases and 90 blood samples from normal healthy females as control. Their age ranged from 19 – 43 years with mean 31 ± 5.88 years. DNA was extracted and analyzed for detection of plasminogen activator inhibitor -1 (4G/5G gene) polymorphisms, by using Allele Specific Primer-PCR (ASP-PCR) method with specific primers.* ***Results:*** *16% of PAI-1 genotypes in cases groups and 7% in control groups are recessive gene with polymorphism (4G/4G), 46% of PAI-1 genotypes in cases groups and 43% in control groups are 4G/5G polymorphism, 38% of PAI-1genotypes in cases groups and 50% in control groups are 5G/5G polymorphism (84% of PAI-1 genotypes in cases group and 93% in controls group are wild types with polymorphism (4G/5G) and (5G/5G).* ***Conclusion:*** *The plasminogen activator inhibitor type -1 (PAI-1) (4G/5G) polymorphism had significant association with menorrhagia amongst Sudanese females in Gezira State.*

**Keywords:** plasminogen activator inhibitor type -1, 4G/5G, Polymorphism, Menorrhagia, Women, Sudan.

## Introduction

*Menstruation is the cyclic, orderly sloughing of the uterine lining, in response to the interactions of hormones produced by the hypothalamus, pituitary, and ovaries (1). The menstrual cycle is divided into two phases: follicular or proliferative phase, and the luteal or secretory phase (2). The length of a menstrual cycle is the number of days between the first days of menstrual bleeding of one cycle to the onset of menses of the next cycle (3). The median duration of a menstrual cycle is 28 days with most cycle lengths between 25 to 30 days (4, 5). There are some factors to control the amount of normal menstrual cycle flow like especial hormones (estrogen and progesterone), growth of the endometrial layer of the uterus, increased amounts of stroma and glands, and increasing the depth of the arteries (6). Vasoconstriction of spiral arterioles is desirable at this time to limit blood flow, that meaning that a small increase in vessel radius will dramatically increase the amount of blood flowing (7). Also decreased constriction of endometrial vessels at the time of menstruation will contribute significantly to increased menstrual blood loss (8). Heavy menstrual bleeding (HMB) can be caused by abnormal blood clotting, disruption of normal hormonal regulation or uterine pathology (e.g., fibroids, adenomyosisui). It is important to diagnose the underlying cause in order to determine the best treatment option (9). Causes of menorrhagia include coagulopathy, ovulation dysfunction, or iatrogenic. Up to 20% of women with HMB will have an underlying inherited bleeding disorder (IBD) (10 - 12). Bleeding disorders have been recognized as important etiologic or contributory factors in women with heavy menstrual bleeding. Fibrinolysis in the endometrium plays a role in heavy menstrual bleeding (13). It is unknown whether increased systemic fibrinolysis might also increase the risk of heavy menstrual bleeding (14). Plasminogen activator inhibitor type-1 is a main regulator of the endogenous fibrinolytic system (15). It inhibits fibrinolysis activity of the tissue plasminogen activator, which produces active plasmin from plasminogen that cleaves fibrin, so that the deficiency of plasminogenactivater inhibitor type-1 can lead to menorrhagia, according to continuous of fibrinolysis (16). Plasminogen activator inhibitor type 1 (PAI-1) is an important component of the coagulation system that down-regulates fibrinolysis in the circulation (17). Reduced PAI-1 levels may result in increased fibrinolysis and an associated bleeding diathesis (18).*

## Methodology:

The study was done in Wad Medani City, Gezira State in East Central Sudan. 50% of cases participants were selection from Wad Medani Obstetrical and Gynecology Teaching Hospital and the others participants were selected from Faculty of Medical Laboratory Sciences, University of Gezira. All control participants that selection as control for genetic analysis was from Faculty of Medical Laboratory Sciences, University of Gezira (matching in age and region with cases participant). 180 samples were collected from 90 females with menorrhagia as cases (90 samples during the menstruation and 90 samples post the menstruation). 90 samples were collected from females with normal healthy menstruation females as control. Both cases and control gave consent and agreement to participate in this study.

Screening for PAI-1 among study subjects and controls using Allele Specific Primer-PCR Sample collection and DNA extraction peripheral whole blood samples were collected and preserved in -20ºC until used. DNA extraction was conducted as instructed (innuPREP DNA kit cat#845-KS-1041250, Analytik Jena GmbH, Germany). PCR setting Allele Specific Primer-PCR (ASP-PCR) method was used for screening of PAI-1 genotypes among study subjects. 4G or 5G allele specific reverse primers were used in combination with a single forward primer in separate PCR reactions (Table 1). A nucleotide mismatch was introduced in allele specific primer to increase the specificity of the primer. Internal control primers pair targeting human beta globin (HB) gene sequence was co-amplified to check for successful PCR amplification (Supplied by Macrogen, Korea).

**Table 1: PAI-1 genotypes primers:**

| Target | Forward | Reverse | PCR fragment |
|---|---|---|---|
| **PAI 4G /5G** | 5\TGCAGCCAGCCAGCCACGTGATT 3\ | ASP 4G : 5\ GTCTGGACACGTGGGGA 3\ | 138 |
| | | ASP 5G: 5\ GTCTGGACACGTGGGGG 3\ | 139 |
| **Internal control** | Hb-1: 5\CAACTTCATCCACGTTCACC 3\ | Hb-2: 5\GAAGAGCCAAGGACAGGTAC3\ | 268 |

Amplification reactions of PAI-1 were performed in two separated PCR reactions using 20µL volume of PCR contained 5µl of genomic DNA, 1µl of allele- specific primer 5G or 4G (reverse primers), 1 µl of common primer (forward primer), 0.5 µl of internal control Hb-1and 0.5µl of Hb-2, 10µl of ready master mix solution (APSMAG TAQ 2XPCR Master mix) and 7µl of H2O molecular biology grade. DNA was denaturated at 94◦C for 5 min, followed by 29 cycles of denaturation at 94◦C for 1min, annealing at 60◦ C for 1min,and extension at 72 ◦C for 1min, with a final extension step for 5 min at 72 ◦C, by using Amp PCR system 9700 (Applied Biosystem).

The electrophoresis was done for separation of fragments of nucleic acids, the amplification reaction was visualization on 2% agarose gel; a100 bp DNA ladder was used as a marker. Samples were scored 4G when a PCR product of 138 bp could be detected and 5G when a PCR product of 139 bp could be detected.

Ethical approval of the study was taken from both Research and Ethic Committee in Ministry of Health, Gezira State, and Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan. Written informed consent was taken from each participant.

The data was collected by structural a questionnaire and then analyzed using statistical package for social science (SPSS) computer version (16). Analysis also was done by SNP States (descriptive statistic for single SNP analysis).

**Results:**

**Table 2. The genotypes frequencies of PAI-1 (4G/5G) polymorphisms in cases and controls (percentage of typed samples 90/90).**

| PAI-1 Groups | Genotypes | | |
|---|---|---|---|
| | **4G/4G** | **4G/5G** | **5G/5G** |
| **Case** | 14(16%) | 42 (46%) | 34 (38%) |
| **Control** | 6 (7%) | 39 (43%) | 45(50%) |
| **P.value** | 0.14 | | |

**Table 3. The alleles frequencies of PAI-1 (4G/5G) polymorphisms in cases and controls (percentage of typed samples 90/90).**

| PAI-1 Group | Genotypes | | Alleles | |
|---|---|---|---|---|
| | **4G/4G** | **4G/5G + 5G/5G** | **4G** | **5G** |
| **Case** | 14(16%) | 76(84%) | 39% | 61% |
| **Control** | 6 (7%) | 84(93%) | 28% | 72% |
| **P.value** | 0.047 | | | |

(Odd Ratio = 2.58; 95% CI 0.944 – 7.049).

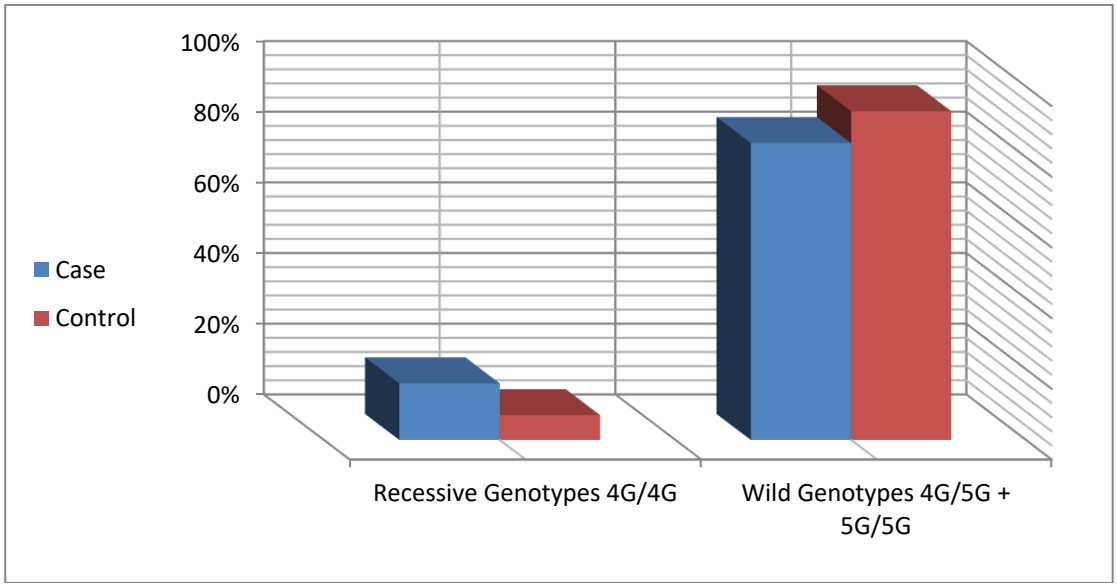


**Figure 1. The frequencies of recessive genotypes and wild genotypes of PAI-1 (4G/5G) polymorphisms in cases and controls groups.**

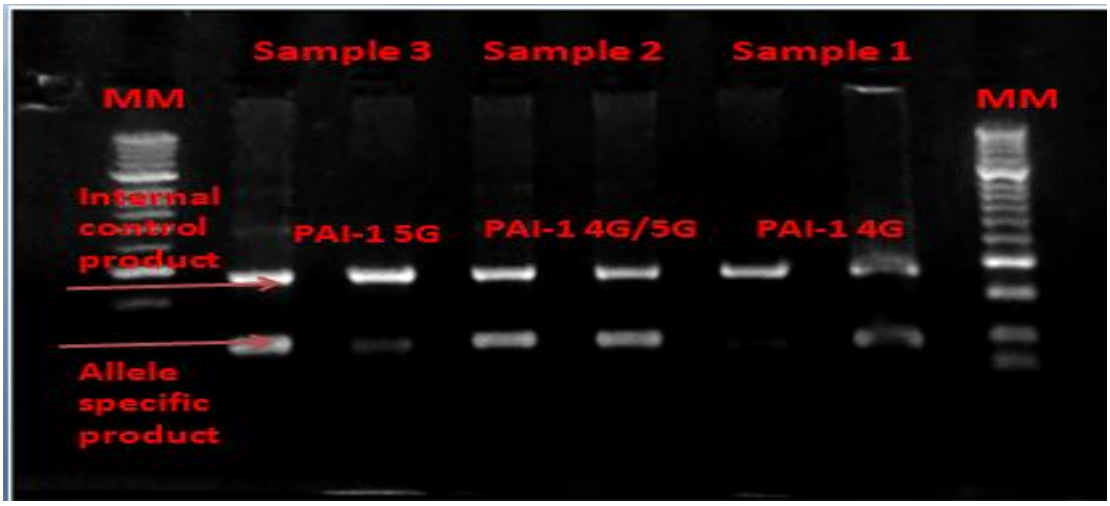


**Figure 2. Electrophoresis of amplified DNA forplasminogenactivterinhibitors type 1 from female monarrhagia in 2% agarose gel by using (APSMAG TAQ 2XPCR Master mix) and visible by UV light.**

**Table 4. Relationship between PAI-1 (4G/5G) polymorphisms and risk factors:**

| PAI polymorphism | Family history of menorrhagia | | Clinical features | | Regulation of menstruation | | Amount of menstruation | | Duration of menstruation | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Yes | No | None | Bleeding tendency | Regular | Irregular | Normal | Excessive | Up to 7 days | More than 14 days |
| 4G/4G | 4% | 11% | 13% | 2% | 2% | 13% | 4% | 11% | 9% | 6% |
| 4G/5G | 7% | 40% | 42% | 5% | 25% | 22% | 26% | 21% | 21 % | 26% |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **5G/5G** | 9% | 29% | 28% | 10% | 14% | 24% | 14% | 24% | 19 % | 19% |
| **Total** % | 20% | 80% | 83% | 17% | 41% | 59% | 44% | 56% | 49 % | 41% |
| **Sig** | 0.414 | | 0.263 | | **0.039** | | 0.152 | | 0.938 | |

**Discussion:**

Menorrhagia is a gynecological condition which concern with excessive uterine bleeding occurring at regular or irregular intervals, or prolonged bleeding more than seven days, although a large amount of blood loss (exceed 80 ml blood loss) is the main reason.Menorrhagia or Abnormal bleeding intensity is one of the most common manifestations of an inherited bleeding disorder and associated with many hemostatic abnormalities (12).

This study reports the associated between tissue plasminogen inhibitor type I 4G/5G. Epidemiological studies suggested that multifactor associated with menorrhagia.

Among menorrhagia females there are 41% (37/90) with regular menstruation and 59% with irregular menstruation, when compare with study in Gezira State in Sudan in 2015 (5) that it was observed 52% of the girls had regular menstrual cycle while 48% had irregular cycles; I observe that menorrhagia can appear with both regular or irregular menstruation according to the factors that causes menorrhagia; according to literature review done by Duckitt *et al*., 2015 (19).

About 16% of PAI-1 genotypes in cases groups and 7% in control groups are recessive gene with polymorphism (4G/4G), 46% of PAI-1 genotypes in cases groups and 43% in control groups are 4G/5G polymorphism, 38% of PAI-1genotypes in cases groups and 50% in control groups are 5G/5G polymorphism (84% of PAI-1 genotypes in cases group and 93% in controls group are wild types with polymorphism (4G/5G) and (5G/5G). When compare the genotypes of PAI-1 between case and control groups the P. value was (0.140). This study is agree with Khosravi and his team in 2014 (16) associate between plasminogen activator inhibitor 1 gene mutation and different subgroups of recurrent miscarriage and implantation failure, which included the percentages of homozygosity and heterozygosity for the 4G allele in all case subgroups were significantly higher than those in the controls. By contrast, the proportions of homozygosity for the 5G allele in all case subgroups were lower than those in normal individuals. In the otherwise Saes and his group found that there are relation between the menorrhagia and PAI-1 deficiency and they suggested that PAI-1 and the accompanying proteolytic process play a role in degradation of the follicular wall during ovulation.

From reference SNP (rs) report (db SNP ncbi.nlm nil. Gov/snp/) when encoded by rs1799889 the mutation 4G was 0.35 among the global groups, but when compare to my study; the allele frequency of 4G was 0.39 and 0.28 in case and control respectively. The frequency of 5G was 0.61 and 0.72 in case and control respectively. Otherwise when compare the recessive gene and wild types between case and control participants the significant different (0.047) this explain that the recessive gene (4G/4G) has relation with menorrhagia.The frequency of PAI-1 recessive mutant genotypes (4G/4G) in cases groups are higher than one in the control groups. The relationship between plasminogen activator inhibitor type -1 (PAI-1) polymorphism gene and risk factors by using Chi square cross tabulation test in statistical analysis found That there are no relationship appears between plasminogen activator inhibitor type -1 (PAI-1) polymorphism and risk factors (Family history, clinical feature, amount and duration of menstruation) with P. values (0.414, 0.293, 0.152, and 0.938) respectively. But there are relation between the PAI polymorphism and the regulation of menstruation (Significant 0.039), and the frequency of cases that have irregular menstruation are higher than regular, this explicate that the irregular menstruation is the early feature for diagnosis the menorrhagia.

**Conclusion**

The plasminogen activator inhibitor type -1 (PAI-1) (4G/5G) polymorphism had significant association with menorrhagia amongst Sudanese females in Gezira State.

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