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Vitamin D receptor *Fok1* polymorphism and its relationship with premenstrual syndrome

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ABSTRACT

Background: The term premenstrual syndrome (PMS) encompasses a variety of physical and psychological symptoms that are related to the menstrual cycle. The likelihood of developing this syndrome, along with its potential severity has been found to be linked to many environmental, nutritional, and genetic factors.
Objective: We aimed to evaluate the frequency of vitamin D receptor (VDR) - *Fok1* Restriction fragment length polymorphism in young women complaining of PMS.
Materials and methods: The current study included evaluation of 340 female students by a validated questionnaire, anthropometric measurement, followed by collection of venous blood samples and DNA extraction. VDR-*Fok1* polymorph was investigated by PCR then fragmentation by *Fok1* restriction enzyme and finally detection of genotypes using agarose gel electrophoresis.
Results and conclusion: We observed a significant association between VDR *FokI* polymorphism and PMS. However, on subgroup analysis by ethnicity, the FF genotype was more predominant among Arab women. ff mutation was associated with a significantly higher risk for PMS [OR = 3.72, 95% CI = 1.65–8.35, $p \leq 0.05$] among non-Arabs.

1. Introduction

Vitamin D exerts its biological effects through interaction with its specific receptor in the body. The vitamin D receptor (VDR) is expressed in most of the tissues including the brain (Wang et al., 2012). Earlier studies (Zou et al., 2008; Penninx, 2008) have linked 1, 25(OH) $_2$ D3 serum concentration with many neurological, behavioral, and emotional characteristics in both humans and animal subjects.

Several genetic mutations have been found in the VDR in any given population. These sequence variations are known as 'polymorphism' and can have biological effects. The genetic variation of the vitamin D receptor includes the *Fok1* single nucleotide polymorphisms among many others (Gross et al., 1996; Iqbal and Khan, 2017). This variation has been linked to developing an increased susceptibility to a variety of diseases including cancer (Iqbal and Khan, 2017; Baxa-Daguplo et al., 2011; Deuster et al., 2017), osteoarthritis, diabetes, autoimmune (Liu et al., 2014; Mukhtar et al., 2017; Horst-Sikorska et al., 2008), infectious and cardiovascular disease (Hajj et al., 2016; Magee et al., 2017).

The coding sequence for the human VDR gene starts at the centromere, extending to the proximal long arm of chromosome 12 [12cen-q12]. It consists of 11 exons and intervening introns. *Fok1* restriction fragment length polymorphism can be found in exon II (Baxa-Daguplo et al., 2011). This polymorphism (rs10735810) involves a C/T transition at the initiation site, leading to the generation of a protein that is shortened by three amino acids (424 amino acids versus 427 amino acids) (Gross et al., 1996).

Glocke et al. (Glocke et al., 2013) suggested a potential influence of VDR-*Fok1* polymorphism on the mood and daily activity of a geriatric population. Moreover, it was also found to be linked to gynecological disorders as uterine leiomyoma (Güleç Yılmaz et al., 2018) as well as polycystic ovary (Dasgupta et al., 2015). According a meta-analysis by Tang et al. (Tang et al., 2009), VDR-*Fok1* polymorphism was found to be a susceptibility marker for breast cancer in the European population.

The role of VDR *Fok1* gene polymorphism in premenstrual disorders has not been previously explored. Pre-Menstrual Syndrome (PMS) can

Abbreviations: VDR, vitamin D polymorphism; PMS, premenstrual syndrome; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism

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be defined as a collection of emotional and physical symptoms that develop shortly before the menstrual cycle and resolve with the start of menstruation (Hofmeister and Bodden, 2016). It is estimated that 80% of menstruating women worldwide report one or more of the physical or psychiatric symptoms that can be attributable to PMS during the luteal phase of their menstrual cycle (Hofmeister and Bodden, 2016).

PMS is diagnosed if the patient reports at least one of the following symptoms that occurs during the 5 days before the onset of menses and is present in at least three consecutive menstrual cycles. These symptoms may be affective (e.g., depression, angry outbursts, irritability, anxiety, confusion, social withdrawal) or physical (e.g., breast tenderness, abdominal bloating, headache, and swelling of extremities). Symptoms are characteristically relieved within 4 days from the onset of menses, without recurrence until day 13 of the menstrual cycle. The symptoms are present in the absence of any pharmacologic therapy, hormone ingestion, or drug use. (Anon, 2001)

PMS has a negative impact on quality of life, especially among the young women population; a substantial decrease in quality of mental health and general wellbeing has been documented by previous studies (Hofmeister and Bodden, 2016; Taghizadeh et al., 2008; Balık et al., 2015; Buddhabunyakan et al., 2017).

Due to the lack of knowledge on the precise etiology of this syndrome, there have been several postulated theories for the underlying cause. Some studies (Rizk et al., 2006; Jahanfar et al., 2011) have suggested a genetic influence, while the role of a hormonal association was stipulated in others (Coulson, 1986).

PMS has been largely underdiagnosed and undertreated. The symptoms can be caused by an imbalance in the complex interactions between hormones, neurotransmitters, psycho-social stresses, and essential nutrients (Pérez-López et al., 2009). Suggested evidence-based treatments include nutritional and lifestyle modifications such as lowering caffeine intake, cessation of any smoking habits and psycho-behavioral therapy to enhance self-esteem. Pharmacological treatments that have been recommended include anti-inflammatory drugs to reduce the cramping and breast tenderness; and anti-depressants such as serotonin reuptake inhibitors. Additionally, due to the considerable overlap between the symptoms of hypocalcemia and PMS, calcium supplementation may be considered as a modality of treatment (Pérez-López et al., 2009; Maddineshat et al., 2016).

In addition to this, vitamin D levels have been shown to fluctuate within the cycles of a female in the reproductive age, but its relationship to PMS and premenstrual dysphoric disorder (PMDD) remains unclear (Subramanian and Gernand, 2017). Ovarian hormones influence the metabolism of many micronutrients, including calcium and vitamin D. Estrogen has a specific regulatory effect on calcium absorption and parathyroid gene expression and hormone secretion – this may explain the fluctuation seen in the female's reproductive cycle (Thys-Jacobs, 2000).

A previous study (Muhairi et al., 2013) conducted in the United Arab Emirates in 2013 estimated the prevalence of either vitamin D deficiency or insufficiency to be 65.1% among healthy adolescents. There was also a higher percentage of females with vitamin D deficiency compared with males (28%, and 10% respectively). It's also worth noting that VDR-*Fok1* polymorphism was proved to affect calcium absorption, kinetic and bone mineralization during puberty (Abrams et al., 2005). According to Ames et al. (Ames et al., 1999), f allele may interfere with calcium absorption, as they found that FF homozygous had a mean calcium absorption that was 41.5% greater than ff homozygous, and 17% greater than Ff heterozygous.

To the best of our knowledge, the present study is the first report of an investigation regarding the potential relationship between the VDR polymorphism and the occurrence of PMS. This association was selected in the light of the aforementioned correlations between vitamin D, premenstrual syndrome, and a possibility of a genetic linkage between the two.

2. Material and methods

The current study was conducted during 2016–2017, and included 340 female students from Dubai Medical University, with ages ranging between 17 and 25 years and a mean age of 19.6 ± 1.4 years. A trained physician performed all the anthropometric measurements. A questionnaire was prepared using the American College of Obstetricians and Gynecologists criteria for diagnosing PMS and was pilot tested among 40 students. All participants were asked to complete the questionnaire, detailing their medical history and blood samples were collected for DNA extraction. The study was undertaken in the Biochemistry Department of Dubai Medical University and it was approved by the Research Ethics Committee of the University. All participants were informed about the nature of the study and samples were taken in accordance to the Declaration of Helsinki, with informed consent.

2.1. Inclusion criteria

The subjects of this study are young women between the ages of 17 to 25 years who met the American College of Obstetricians and Gynecologists diagnostic criteria (Anon, 2001) for PMS, as well as and having regular menses. Age-matched women without mood and behavioral disorders were recruited as control group.

2.2. Exclusion criteria

Subjects should have no general medical illness that is primary (i.e., appears to be causing the mood disorder). Also, they should not be pregnant or taking any medication for PMS including hormones, vitamin D and calcium supplements, steroids, anxiolytics, and antidepressants during the 3-month period prior to screening and during the study (Halbreich et al., 2007).

2.3. Anthropometric measurements

Prior to measurement of body weight and height, the students were instructed to wear light clothing and no shoes. Measurements were collected using a portable digital scale and a portable stadiometer. Participants were instructed to stand straight with their heads, backs vertically aligned to the height gauge, and their height measurements were taken and rounded to the nearest 0.5 cm. At the same time, the patients' weights were recorded from the digital screen and rounded to the nearest 0.5 kg. Body mass index (BMI) was calculated as weight in kilogram divided by height in meter square. World Health Organization cut-offs were used to classify overweight (25.0–29.9 kg/m^2) and obese ($\geq 30.0\ kg/m^2$) adults.

2.4. DNA extraction

Genomic DNA was extracted from fingertip blood samples collected on Whatmann FTA (Flinders Technology Associates) Cards. A few blood drops were collected onto each FTA, left to dry overnight at room temperature prior to further processing. A 3 mm punch of FTA card was transferred to 1.5 ml aliquot and pretreated using Whatmann recommended protocol. In every sample tube 50 µl of distilled water was then added and incubated for 30 min in 95 °C then the templates were withdrawn and stored in −70 °C till analyzed.

2.4.1. Genotyping Fok1 polymorphism of VDR gene

We have studied single nucleotide polymorphisms *Fok1* (T2C/rs10735810) site in Exon 2, in the VDR gene using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Israni et al., 2009). DNA amplifications were performed in a final volume of 25 µl containing 0.2 µM of each primer (5′AGCTGGCCCTGGCACTGAC TCTTGCTCT 3′ and 5′ATGGAAACACCTTGCTTCTTCTCCCTC 3′), 10 µl of 2× master mix with dual dye contained 100 ng genomic DNA