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Effect of some vitamin D receptor gene variants on the frequency of sister chromatid exchange in Iraqi women with polycystic ovary syndrome

Sanaa J. Kadhim , Ismail A. Abdul-hassan

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies /University of Baghdad , Iraq .

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Abstract: Polycystic ovary syndrome (PCOS) is among the most common disorders of premenopausal women , cause infertility. This study was conducted to investigate whether the vitamin D receptor (*VDR*) gene polymorphisms are associated with the enhancement of sister chromatid exchanges(SCE) . This study was carried out in the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies - University of Baghdad through the period from 1 November 2016 until the end of August 2017. The PCOS samples were taken from the Kamal Al-Samarraee infertility treatment Hospital in Bag hdad. The genomic instability were evaluated by measured the frequency of sister chromatid exchange in the presence of 5-bromodeoxy- uridine (BrdU) and using the Hoechst stain in 32 PCOS patients who have homozygous mutant result in genotyping assay , and from 34 PCOS patients who have heterozygous result assay. as well as 14 apparently healthy women of 50 women as control group. PCOS are classified depending on the results of the genotyping assay. The result showed significant differences in the level of sister chromatid exchange in patients with PCOS for each of *VDR* gene polymorphisms compared with normal women. This study concluded that the SCE as affected by polycystic ovary syndrome and the genotypes of VDR gene.

Keywords: polycystic ovary syndrome, VDR gene , sister chromatid exchange.

Corresponding author: should be addressed (Email: farahsanaa@yahoo.com)

Introduction:

Polycystic ovary syndrome (PCOS) is the most common endocrinological disorder. It is characterized by its reproductive phenotype of hyperandrogenism and disordered gonadotropin secretion (1). It represents a condition in which an estimate of 10 small cysts of a diameter ranging between 2 and 9 mm develop on one or both ovaries and/or the ovarian volume in at least one ovary exceeds 10 ml (2).

Previous studies have demonstrated that the prevalence of the syndrome in different populations ranges from 2% - 20%, depending on which diagnostic criteria are used (3,4,5). It is generally admitted that PCOS is a multifactorial disorder that results from a combination of multiple gene. There are several genes that linked to PCOS susceptibility (6, 7, 8).

Cytogenetic studies have shown that women with PCOS have increased damage in their genetic material (9,10) Additionally, a relation between PCOS and X chromosome aneuploidy, including XX/XO mosaics and high proportion of X

chromosome abnormal segregation has been reported in a limited number of studies (10,11).

The Vitamin D receptor (VDR) is defined as the nuclear steroid hormone receptor resulting in gene expression regulation through binding to specific response elements within the promoter of some genes (12,13). Cellular ligand activated transcription factors are encoded by the *VDR* gene (14).The *VDR* gene is mapped to chromosomal locus 12q12-14.

Cytogenetics provides several biomarkers for chromosomal instability assessment, one of which is the sister chromatid exchange (SCE) frequency in cells. SCE is a natural process that implicates the exchange of homologous genetic segments as a mode of repair mechanism. The methodology of SCEs has been proved to be a very useful tool with predictive value, for detecting harmful effects on DNA, caused by various physical and chemical factors. Increased frequency of this index reflects the existence of genotoxicity in cells and the subsequent failure of repair mechanisms to recover the damaged site (15) .Several studies have reported that SCE analysis is a very sensitive method, able to detect mutagens and/or carcinogens (16), and may be more sensitive than other cytogenetic endpoints, such as micronuclei and chromosome aberrations (17). Additionally, proliferation rate index (PRI) and mitotic index (MI) are sensitive indices of cellular toxicity by antimutagenic and chemotherapeutic agents (18,19). This study aimed to find out the correlation between Vitamin D receptor gene polymorphisms including (rs731236 , rs2228570 and rs7975232) and sister chromatid exchange frequencies among Iraqi women.

Materials and methods:

The study was carried out in the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies - University of Baghdad through the period from 1 November 2016 until the end of August 2017.

Sampling:

Blood samples were collected from PCOS patients, and apparently healthy subjects.

The PCOS patients were taken from the Kamal Al-Samarraee infertility treatment Hospital in Baghdad.

The blood samples were collected during the follicular phase (day 3, 4) of the menstrual cycle from each women of both patients and healthy control. five ml of venous blood samples were collected and divided into two portions:

A. First portion: EDTA containing tubes for DNA isolation (genotyping analyses were evaluated by Real Time PCR in previous study (20).

B. Second portion: Heparin containing tubes for cytogenetic studies ,

Two milliliters of blood were collected by venipuncture from 12, 10 and 10 PCOS patients who have homozygous mutant in genotyping assay for *VDR* gene SNP (rs 7975232, rs 731236 and rs 2228570) respectively and from 10,10 and 14 PCOS patients who have heterozygous mutant for *VDR* gene SNP rs 7975232, rs 731235 and rs 228570 respectively. as well as two

milliliters of blood were collected from 14 apparently healthy women as control each collected blood sample was dispensed into heparinized tubes for cytogenetic studies .

Sister chromatid exchange (SCE):

Sister chromatid exchange (SCE) can be found in any cell that has replicated twice in the presence of 5-bromodeoxy-uridine (BrdU) (21). It is known that SCE represents the interchange of DNA replication products, which maintain their polarity, at homologous loci (22,23**)**.

About 10 µg per ml concentrations of each sample , 24 h after initiation of cultures for two consecutive cell cycles were put in a slide. Slides were stained with Hoechst stain (33258) for the analysis of cell cycle progression and sister chromatid exchange. according to the method of Freshney, (24**).**

The slides were immersed in Hoechst 33258 at a concentration of 20 µg/ml for 10min in a coplin jar. The slides were transferred to a slide rack, and on them (500µl) of 2 x SSC were dropped. The slides were covered with a 22-mm x 50-mm cover slips, and the edges were sealed with a temporary seal, using cow gum, to prevent evaporation. The covered slides in the slide rack (cover slip facing downwards) were placed on a short-wave UV box. A distance of approximately 4cm between the slides were maintained and the UV source. The longer the pale chromatid will become, when the slides were exposed for about 24-60min.The cover slips were removed from the slides, and the slides were washed three times in water for 5 min. The slide holder was covered with aluminum foil, and they were air dried in the dark , and stained in a coplin jar containing 3.5% Giemsa solution in PBS buffer for 3-5min (PH=6.8). Carefully the slides were rinsed in tap water, and drained using a paper tissue and air dried on the bench for 1hour and dipped into xylene. Four drops of DPX mountant were dropped onto the slide and a 22-mm x 50-mm cover slip was lowered, expressing any air bubbles with tissue. The slides were air dried in a fume hood overnight. Figure (1) illustrate sister chromatid exchange.

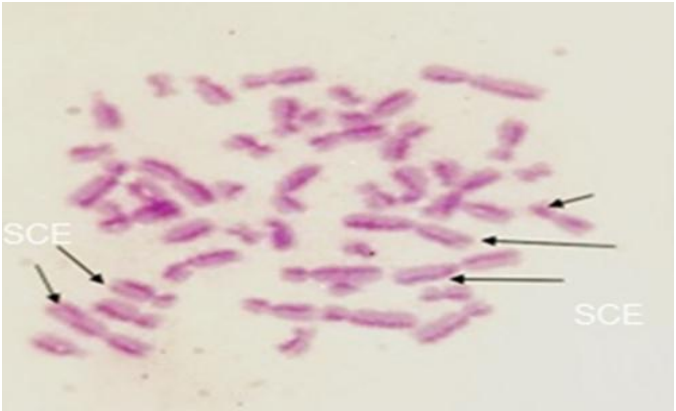

Figure (1): Sister chromatid change (SCE) (X 100).

The Statistical Analysis System- SAS program was used to compare the effect of different factors in this study. Chi-square test was used to significant compare between percentages. Least significant difference –(LSD) test was used to compare between the means of parameter in this study (22).

Results and Discussion:

Sister chromatid exchange :

The results of SCE as affected by polycystic ovary syndrome and the genotypes of *VDR* gene (rs7975232C>A) are shown in table (1)

The SCE frequency was significantly (p<0.0001) increased in PCOS patients compared with apparently healthy subjects (3.42 *versus* 1.79 SCE/1000, respectively). In PCOS patients, the SCE frequency was significantly (p<0.0001) higher in both heterozygous and homozygous mutants (rs7975232 of *VDR* gene) compared with apparently healthy subjects (3.05and 3.78 compared with 1.79 SCE /1000, respectively).

Table (1): Effect of PCOS and VDR polymorphism (rs7975232 C>A) on the number of Sister chromatid exchange (SCE/1000). (Mean ± SE).

Group		SCE frequency(n/1000)
apparently healthy subjects [1]		1.79 ± 0.12 b
PCOS[2]		3.42 ± 0.12 a
p-value		0.0001 **
apparently healthy subjects		1.79 ± 0.12 c
PCOS	Heterozygous(CA)	3.05 ± 0.15 b
	Homozygous (AA)	3.78 ± 0.18 a
p-value		0.0001 **

Different letters refer to a significant difference between means. [1] apparently healthy subjects. [2]Patients with polycystic ovary syndrome.

In addition, SCE numbers were significantly (p<0.0001) lower in heterozygous mutant (rs7975232 of VDR gene) than in homozygous mutant (3.05 versus 3.78 SCE/1000, respectively).

The results of sister chromatid exchange SCE as affected by polycystic ovary syndrome and the genotypes of *VDR* gene (rs731236 T>C) are shown in table (2).

The SCE frequency was significantly (p<0.0001) increased in PCOS patients compared with apparently healthy subjects (3.85 *versus* 1.79 SCE/1000, respectively). In PCOS patients, the SCE frequency was significantly (p<0.0001) higher in both heterozygous and homozygous mutants (rs731236 of *VDR* gene) compared with apparently healthy subjects (3.76 and 3.93 compared with 1.79 SCE /1000, respectively). In addition, no significant differences (p<0.05) in SCE frequency between heterozygous and homozygous mutant (rs731236 of *VDR* gene), (3.76 *versus* 3.93 SCE/1000, respectively) was noticed.

Table (2): Effect of PCOS and VDR polymorphism (rs731236 T>C) on the number of Sister chromatid exchange (SCE/1000). (Mean ± SE).

Group		SCE frequency(n/1000)
apparently healthy subjects [1]		1.79 ± 0.12 b
PCOS[2]		3.85 ± 0.15 a
p-value		0.0001 **
apparently healthy subjects		1.79 ± 0.12 b
PCOS	Heterozygous(TC)	3.76 ± 0.22 a
	Homozygous (CC)	3.93 ± 0.19 a
p-value		0.0001 **

Different letters refer to a significant difference between means. [1] apparently healthy subjects. [2] Patients with polycystic ovary syndrome.

The results of SCE as affected by polycystic ovary syndrome and the genotypes of *VDR* gene (rs2228570 T>C) are shown in table (3).

The SCE frequency was significantly (p<0.0001) increased in PCOS patients compared with apparently healthy subjects (4.11 *versus* 1.79 SCE/1000, respectively). In PCOS patients, the SCE frequency was significantly (p<0.0001) higher in both heterozygous and homozygous mutants (rs2228570 of *VDR* gene) compared with apparently healthy subjects (3.98 and 4.30 compared with 1.79 SCE /1000, respectively). In addition, no significant differences (p<0.05) in SCE frequency between heterozygous and homozygous mutant (rs2228570 of *VDR* gene), (3.98 *versus* 4.30 SCE/1000, respectively).

Table (3): Effect of PCOS and VDR polymorphism (rs2228570T>C) on the number of Sister chromatid exchange (SCE/1000). (Mean ± SE).

Group		SCE frequency(n/1000)
apparently healthy subjects [1]		1.79 ± 0.12 b
PCOS[2]		4.11 ± 0.13 a
p-value		0.0001 **
apparently healthy subjects		1.79 ± 0.12 b
PCOS	Heterozygous(TC)	3.98 ± 0.18 a
	Homozygous (CC)	4.30 ± 0.20 a
p-value		0.0001 **

Different letters refer to a significant difference between means. [1] apparently healthy subjects. [2] Patients with polycystic ovary syndrome.

In general, the frequencies of SCE were significantly higher in PCOS patients than in controls regardless SNP type. Whereas, contradictory results were found in SCE frequencies when compared between heterozygous and homozygous genotypes within each SNP.

Cytogenetics provides several biomarkers for chromosomal instability assessment, one of which is the SCE frequency in cells. SCE is a natural process that implicates the exchange of homologous genetic segments as a mode of repair mechanism. The methodology of

SCEs has been proved to be a very useful tool with predictive value, for detecting harmful effects on DNA, caused by various physical and chemical factors. Increased frequency of this index reflects the existence of genotoxicity in cells and the subsequent failure of repair mechanisms to recover the damaged site. Several studies have reported that SCE analysis is a very sensitive method, able to detect mutagens and/or carcinogens (15) and may be more sensitive than other cytogenetic endpoints, such as micronuclei and chromosome aberrations (17). Evagelia *et al.* (26) used the frequency of sister chromatid exchange as an index of cytogenetic damage and they found a significant increase in SCE levels in women with PCOS compared with controls. Also, Evagelia *et al.* (26) reported a positive correlation between DNA damage and PCOS phenotypes.

To the best of our knowledge this is the first study to investigate a possible association between chromosomal instability and clinical phenotypes of PCOS in Iraq. The results in this study revealed that the DNA of PCOS patients showed significant damage, as shown by the increased SCE frequency in lymphocytes, confirming some reports that deal with this issue (10,27). The high rate of DNA damage in Iraqi PCOS patients suggests that the repair mechanisms are insufficient to genetic changes.

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