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Large Deletion of Sex Hormones Binding Globulin-SHBG Gene in Iraqi Women with Polycystic Ovary Syndrome-PCOS

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Abstract: Polycystic ovary syndrome is the most common, yet complex, endocrine disorder affecting women in their reproductive years. The etiology of PCOS is still unknown, yet, there is increasing evidence to support a major genetic basis, as the syndrome have strong familial predisposition. More than one gene contribute to the heterogeneous phenotype and the clinical and biochemical presentation. Patients with PCOS may present complaining of irregular or unpredictable menstrual cycles, unwanted hair growth, acne or scalp hair loss, or unexplained weight gain or overweight, and infertility. This study explores the investigation mutations of sex hormones binding globulin *SHBG* gene in Iraqi women effected with polycystic ovary syndrome PCOS. Genomic DNA was extracted from these samples. *SHBG* gene was amplified by PCR using specific primer. Then, PCR products were subjected to digestion with *BbsI* restriction enzymes and electrophoresed on 2.5% agarose gel. Hence, the presence of two fragments, 61bp and 30 bp band , indicates normal homozygosity for alleles, the presence of three fragments, 91bp,61bp and 30bp, indicates heterozygosity mutant for alleles and a single 91bp band indicates homozygosity mutant for the alleles. By carrying out the DNA sequencing, the analysis revealed that the PCR products located between the end of the intron 7 and the start of the exon 8 (244174-24184). The analysis also revealed that a novel deletion was recognized in the PCR product of the PCOS patients involving the exon 8 which plays an essential role in PCOS. As a conclusion, PCOS patients could be due to the novel deletion detected by this study.

Keywords: SHBG , PCOS , RFLP , BbsI, Homozygosity, Heterozygous

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Introduction

The polycystic ovary syndrome (PCOS) is the commonest hormonal disturbance to affect women. The main problems that women with PCOS faced are menstrual cycle disturbances (irregular or absent periods), difficulty in controlling body weight and skin problems (acne and unwanted hair growth on the face or body) (1). Although the specific genetic alterations that contribute to the development of PCOS remain unclear, several candidate genes have been proposed including the *SHBG* gen (2).

Human sex hormone–binding globulin (SHBG) transport testosterone and estradiol in the blood (3). Blood concentrations of SHBG are a major determinant of the metabolic clearance of these sex steroids and their access to target tissues; their measurements provide a means of estimating the

amounts of circulating non–protein-bound, or "free," sex steroids (4). Abnormally low serum SHBG levels are frequently found in women with PCOS and contribute to hyperandrogenic symptoms such as hirsutism and acne (5). Serum SHBG levels are also reduced in patients with type 2 diabetes and coronary heart disease (6). The reason that SHBG levels are low in serum samples from many of these individuals is unclear, but it has been reported that SHBG deficiencies are inherited (7). Human SHBG is a homodimeric plasma glycoprotein encoded by a 4-kb gene spanning eight exons on the short arm (p12→p13) of chromosome 17, it is produced by hepatocytes under the control of various hormonal and metabolic regulators (8). The aim of this study is to detect the relationship between PCOS , *SHBG* gene mutations using PCR , REFLP by *BbsI* restriction enzyme and sequencing.

Materials and Methods

The study includes 70 Iraqi women with PCOS and 20 healthy women. Blood samples five ml in EDTA tubes for DNA isolation. The genomic DNA was isolated from whole blood that was collected and frozen in EDTA anticoagulant tubes the extraction of DNA was done using genomic DNA Extraction kits (Bioneer) / Korea . After genomic DNA extraction, agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA (9). Therefore specific primers was designed to amplification (*SHBG*) in this study(PCR) (10). PCR amplicons of the (*SHBG*) gene were subjected to *BbsI* restriction enzyme. The PCR amplicons of the analyzed (*SHBG*) gene and primers were sent to Macrogen Company (U.S.A) for sequencing.

Results and Discussion

Molecular Identification of PCOS

This study focused on the analysis of the extracted DNA from PCOS patients by using specific primers PCR amplification.

DNA Isolation

The genomic DNA, which extracted from blood of polycystic ovary syndrome, showed good concentration 6µg/ml, (Figure 1).

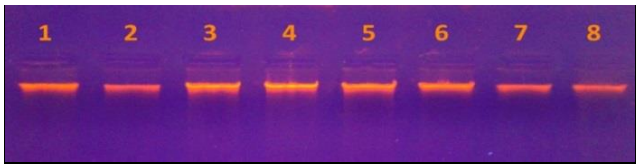

Figure (1): Chromosomal DNA bands on 1.5 % agarose gel at 70 volt/cm for 1 hour. DNA sample were extracted from some PCOS women.

Polymerase Chain Reaction (PCR) Analysis

The PCR results revealed that identical bands related to the *SHBG* gene were present. PCR amplified regions showed a molecular weight of 91 bp (Figure 2) (11).

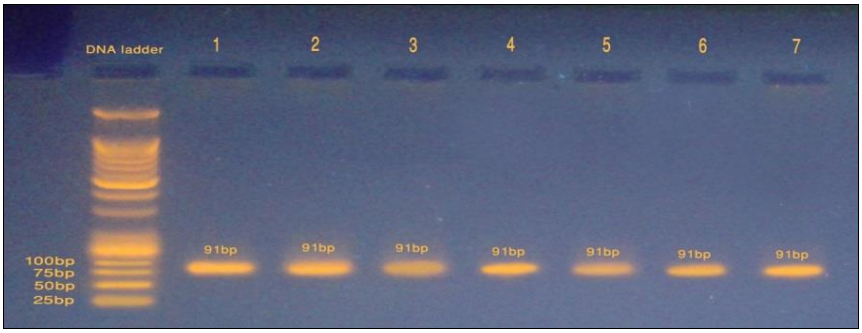

Figure (2): PCR products of *SHBG* gene 2.5% agarose gel at 60 voltages for 60 mint. DNA ladder (25-2000) bp, Lane (1-7) PCR products of the *SHBG* gene of band size 91bp(1-3 are control and 4-7 are patients samples). visualized under U.V light after staining with Ethidium Bromide.

PCR and Enzyme Digestion Products

Restriction Fragment Length Polymorphism (RFLP) Analysis

The restriction fragment length polymorphism (RFLP) analysis was performed to determine genotypes of the *SHBG* gene in PCOS women. The PCR products were digested with *BbsI* enzyme and electrophoresed on 2.5% agarose gel. Hence, the presence of two fragments, 61bp and 30bp band, indicates normal homozygosity (Figure 3) for alleles; the presence of three fragments, 91bp,61bp and 30bp, indicates heterozygosity mutant (Figure 5) for alleles and a single 91bp band indicates homozygosity mutant for the alleles (Figure 4). Among 50 PCOS patients included in the molecular study, 43(86%) of them were heterozygous mutants, 5(10%) were homozygous mutants and 2(4%) were with normal (table 1). These results indicated that POCS could be due to heterozygosity mutations in the *SHBG* gene.

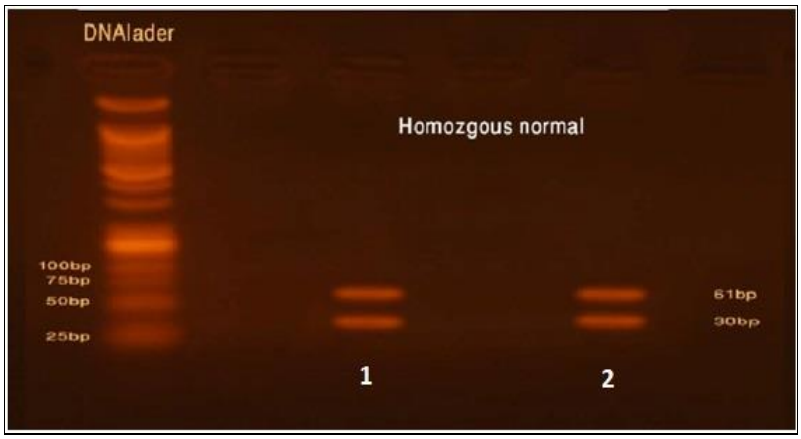

Figure (3): Restriction fragment length polymorphism (RFLP) analysis of the *BbsI* digest of the PCR product that contains the *SHBG* gene separated on a 2.5 % agarose gel electrophoresis. DNA ladder (25- 2000)bp and Lane (1-2) homozygous normal (G/G)(lane 1 is a control and lane 2 is a patient samples) ,the presence of two fragments, 61bp and 30bp band.

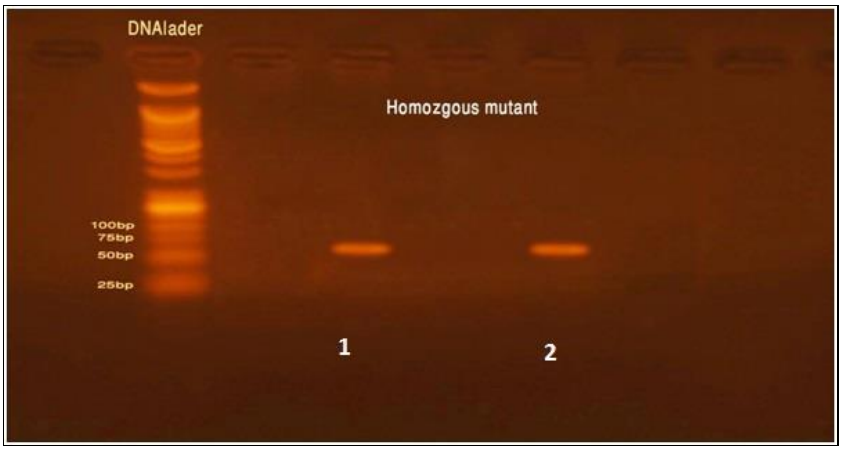

Figure (4): Restriction fragment length polymorphism (RFLP) analysis of the *BbsI* digest of the PCR product that contains position 91 bp of the *SHBG* gene separated on a 2.5 % agarose gel electrophoresis. DNA ladder=(25-2000) bp, Lanes (1and 2) homozygous Mutant (A/A) , alleles and a single 91bp band.

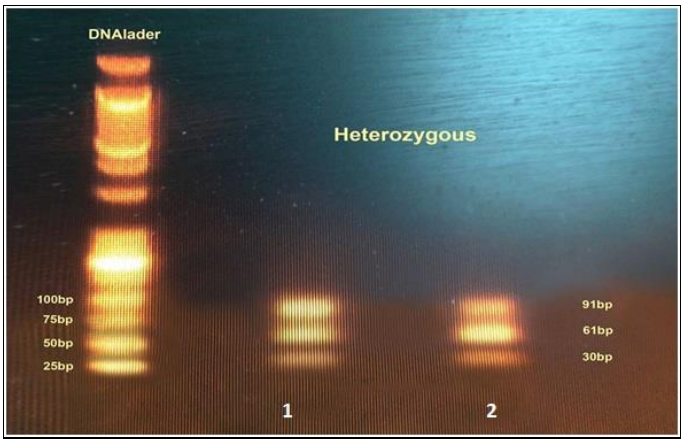

Figure (5): Restriction fragment length polymorphism (RFLP) analysis of the *BbsI* digest of the PCR amplicons that contains the *SHBG* gene separated on a 2.5 % agarose gel electrophoresis. DNA ladder (25-2000)bp; Lanes 1,2Heterozygous mutants (G/A), the presence of three fragments, 91bp,61bp and 30bp.

Table (1): The Genotype Distribution Among PCOS Women

Genotypes	Number of patients	Alleles %
G /G 50	2	4
G / A 50	5	10
A /A 50	43	86

Sequencing of the patients PCR products

The normal complete *SHBG* gene sequence was taken from the NCBI site((http://www.ncbi.nlm.nih.gov/) and Mega 6 was used for sequences analysis. The analysis revealed that the PCR products located between the end of the intron 7 and the start of the exon 8 (from 24091 to 24181)(Figure 6). The analysis also revealed that a novel large deletion was recognized in the PCR product of the PCOS patients involve a part of intron area and a part of the exon area (244174-24184).

24091 tggagg agtg gaaaagtggg gagaagattc tgga **tccgag ccaccttaatgctctaatgccac ctttgc a ctacctccct cta g** 24175 **gag aag act cttc cac ctctttttgc ctgaatggcc tttgg** 24181……..etc.

Normal sequence

Tccgagccaccttaatgctctaatgccacctttgcactacctccctcta g gag aag act cttccaccctcttttgcctgaatggcctttgg

Normal amino acids start with Glycine glutamic acid aspartic acid Leucine proline proline

Mutatan sequence

tccgagccaccttaatgctctaatgccacctttgcactacctccctcta **-deleted area** cttccaccctct ttttgcctgaatggcctttgg

Mutant amino acids start with Leucine proline proline ……

Figure(6) : Intron 7 (24091-24175) and the start of the exon 8 (24176-24181) of the gene *SHBG*.

The PCR product sequence located between 24091 and 24181 and the deleted area located between 24174 and 24184. The recognized deleted sequence showed to include the enzyme ***Bbs*I** cleavage site (GAAGACN2) which explain the homozygosity and heterozygosity mutants existence in patients REFLPs. The detected deletion in PCOS samples may lead to a deletion of three amino acids from the translated protein or causing exon 8 skipping resulting in short polypeptide chain. The mutations of the *SHBG* gene in PCOS patients was also found to result in a completely defected protein (12) and may cause stop codon which truncates the protein synthesis (13).

Mutations in the *SHBG* gene were also detected by other studies. Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting women of childbearing age causing not only reproductive but also metabolic anomalies. PCOS women present with ovulatory dysfunction, abnormal hormones, hyperandrogenemia, obesity, and hyperinsulinemia (14). It is a heterogeneous disorder which results from interaction of multiple genes along with environmental factors (15). For a number of genes altered patterns of expression have been detected, suggesting that the genetic abnormality in PCOS affects signal transduction pathways controlling the expression of multiple genes rather than abnormal expression of a single gene (16). Despite evidence suggesting that *SHBG* may be a candidate gene for PCOS (17, 2). Mutations in SHBG gene which associated with PCOS were detected by several authors. Hogeveen and Hammond (2001) (18) identified a polymorphism in the coding region of *SHBG* that encodes a missense mutation, P156L, in 4 of 482 women with PCOS, hirsutism or ovarian dysfunction while Xita *et al*.(2003) (17) shows evidence of genetic contribution to the decreased *SHBG* levels frequently seen in PCOS women. They investigated the possible association of the functional (TAAAA)n repeat polymorphism in the promoter of the gene with PCOS and lower *SHBG* levels. They also reported that carriers of the longer allele had lower *SHBG* levels within the PCOS group. Other researchers found no association between four *SHBG* SNPs studied and PCOS which are consistent with reported results from other groups investigating associations between *SHBG* gene polymorphisms and PCOS (19, 20). Perry *et al*. (2010) (21)

detected the mutation when they studied the association of *SHBG* SNPs and serum *SHBG* concentrations in POCS women. They found that the association depending on differences in the population under study (i.e. sex, age and menopausal status), the disease under study (i.e. type 2 diabetes, hormone-dependent cancers). Such finding was also detected by others (22,23,24,25,26,27). The (TAAAA)n repeat *SHBG* polymorphism could be one of the factors representing a genetic link to the developmental hypothesis for PCOS (28, 29), by which individuals with genetically determined low SHBG levels may be exposed to high free androgen levels during fetal life, programming their future PCOS hyperandrogenic characteristics. Since longer *SHBG* alleles were found to have lower transcriptional activity in vitro (30), leading to lower serum SHBG levels and consequently to higher levels of bioavailable androgens (17,2). On the genetic level, no association between the (TAAAA)n *SHBG* genotypes and PCOS was observed and the allelic variants were similarly distributed to control patients (17). Other studies observed that *SHBG* polymorphism could be related to serum SHBG level (2). Ferk *et al*., (2007) (20) investigated the association between a microsatellite polymorphism located in the *SHBG* gene promoter region (TAAAA)n and PCOS. Although serum SHBG levels appeared to be strongly influenced by the presence of the (TAAAA)n, the polymorphism was not present in significantly higher rates in women with PCOS compared with normal control women. On the other hand, mutations of other genes in PCOS patients were mostly detected (31,32).

References

1. Balen, A. and Michelmore, K. (2002). What is polycystic ovary syndrome? Are national views important? *Human Reproduction*, 17(9): 2219-2227.
2. Cousin, P.; Calemard-Michel, L.; Lejeune, H.; Raverot, G.; Yessaad, N.; Emptoz-Bonneton, A., *et al*. (2004). Influence of SHBG gene pentanucleotide TAAAA repeat and D327N polymorphism on serum sex hormone-binding globulin concentration in hirsute women. *J. Clin. Endocrinol. Metab*., 89:917–924.
3. Hammond ,G. and Bocchinfuso, W. P. (1996). Sex hormone-binding globulin: gene organization and structure/function analyses. *Horm. Res*., 45 (3–5): 197–201.
4. Lindstedt, G.; Karachalios, A.; Soudan, B. and Hammond, G. (1991). Low sex-hormone-binding globulin concentration as independent risk factor for development of NIDDM. 12-year follow-up of population study of women in Gothenborg Sweden. *Diabetes*., 40:123–128.
5. Blank, S. K.; McCartney, C.R. and Marshall, J. C. (2006). The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. *Hum. Reprod. Update*, 12:351-61.
6. Doi, S.A.; Al-Zaid, M.; Towers, P.A.; Scott, C. J. and Al-Shoumer, K. A. (2005). Ovarian steroids modulate neuroendocrine dysfunction in polycystic ovary syndrome. *J .Endocrinol. Invest*., 28: 882-92.
7. Grishkovskaya, I.; George V.; Avvakumo, V.; Gisela S.; David D.; Geoffrey, L., *et al*. (2000). Crystal structure of human sex hormonebinding globulin: steroid transport by a laminin G-like domain. *EMBO.J*. 19(4): 504-512.
8. Ring, H.Z.; Lessov, C.N.; Reed, T.; Marcus, R.; Holloway, L.; Swan, G. E. *et al*. (2005). Heritability of plasma sex hormones and hormone binding globulin in adult male twins. *J. Clin. Endocrinol. Metab*., 90:3653- 3658.
9. Sambrook, J. (1989). Molecular Cloning: A Laboratory manual. Second Edition. (Plainview, New York: Cold Spring Harbor Laboratory Press).
10. Alison, M.; Dunning, M.; Catherine, D.; Healey, S.; Tee, L.; Robert, N., *et al*. (2004). Polymorphisms Associated With Circulating Sex Hormone Levels in

Postmenopausal Women, *J. N. Center. INST*., 96: 12.
11. Bartlett, J.M. and Stirling, D.A. (2003). Short History of the Polymerase Chain Reaction. *Methods Mol. Biol*., 226: 3-6.
12. Hastings, P.; Lupski, J.; Rosenberg, S. and Ira, G. (2009). Mechanisms of change in gene copy number. *Nat. Rev. Genet*., 10 (8): 551- 564.
13. Isbrandt, D. ; Hopwood , J.; von Figura, K. and Peters, C. (1996). Two novel frameshift mutations causing premature stop codons in a patient with the severe form of Maroteaux-Lamy syndrome. *Hum. Mutat*., 7(4): 361–363.
14. Franks, M.I.; McCarthy, K. and Hardy, (2006). Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *Int. J. Androl*., 29: 278–285.
15. Xita, I.; Georgiou, A. and Tsatsoulis, A. (2002). The genetic basis of polycystic ovary syndrome. *Eur .J .Endocrinol*., 147 : 717–725.
16. Jakubowski , L. (2005). Genetic aspects of polycystic ovary syndrome. *Endokrynol. Pol*.,56(3) :285-93.
17. Xita, N.; Tsatsoulis, A.; Chatzikyriakidou, A. and Georgiou, I. (2003). Association of the (TAAAA)n repeat polymorphism in the sex hormone-binding globulin (SHBG) gene with polycystic ovary syndrome and relation to SHBG serum levels. *J. Clin. Endocrinol. Metab*. ,88: 5976-5980.
18. Hogeveen, K.; Talikka, M. and Hammond, G. (2001). Human sex hormone-binding globulin promoter activity is influenced by a (TAAAA)n repeat element within an alu sequence. *J. Biol. Chem*. 276: 36383-36390.
19. Bendlová, B.; Zavadilová, J.; Vanková, M.; Vejrazková, D.; Lukásová, P., Vcelák, J., *et al*. (2007). Role of D327N Sex hormone-binding globulin gene polymorphism in the pathogenesis of polycystic ovary syndrome. *J. Steroid. Biochem. Mol. Biol*., 104:68–74.
20. Ferk, P.; Teran, N. and Gersak, K. (2007). The (TAAAA)n microsatellite polymorphism in the SHBG gene influences serum SHBG levels in women with polycystic ovary syndrome. *Hum. Reprod*., 22:1031-1036.
21. Perry, J. R.; Weedon, M.N.; Langenberg, C.; Jackson, A.U.; Lyssenko, V.; Sparso, T., *et al*. (2010). Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. *Hum. Mol. Genet*., 19:535–544.
22. Dunning, A. M.; Dowsett, M.; Healey, C. S.; Tee, L.; Luben, R. N.; Folkerd, E. *et al.* (2004). Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J .Natl. Cancer Inst*., 96:936–945.
23. Berndt, S. I.; Chatterjee, N.; Huang, W.; Chanock, S.; Welch, R.; Crawford, E. *et al*. (2007). Variant in sex hormone-binding globulin gene and the risk ofprostate cancer. *Cancer Epidemiol. Biomarkers Prev*., 16:165–168.
24. Escobar-Morreale, H. F. and San Millan, J. L. (2007). Abdominal adiposity and the polycystic ovary syndrome. *Trends. Endocrinol. Metab*., 18:266–272.
25. Ding, E. L.; Song, Y.; Manson, J. E.; Hunter, D.J.; Lee, C.C.; Rifai, N., *et al.* (2009). Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N. Engl. J .Med*., 361:1152–1163.
26. Wickham, E.P.; Ewens, K.G.; Legro, R.S.; Dunaif, A.; Nestler, J.E. and Strauss, J. F. (2011). Polymorphisms in the SHBG gene influence serum SHBG levels in women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab*., 96: 719–727.
27. Sidiropoulou, E.; Ghizzoni, L. and Mastorakos, G. (2012). Adrenal androgens. In: Chrousos G (ed). Endotextorg-Adrenal Disease and Function. South Dartmouth, M.A.: M.D.Text.com., Inc.
28. Abbott, D. K.; Barnett, C. M.; Bruns, D. A. and Dumesic, A. R. (2005). Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Hum. Reprod. Update*, 11 : 357–374.
29. Dumesic, D.H. and Abbott, V. (2007). Padmanabhan. Polycystic ovary syndrome and its developmental origins. *Rev. Endocr. Metab*., 8: 127–141.
30. Hogeveen, K. N.; Cousin, P.; Pugeat, M.; Dewailly, D.; Soudan, B. and Hammond, G. L. (2002). Human sex hormone-binding globulin variants associated with hyperandrogenism and ovarian dysfunction. *J. Clin .Invest*., 109: 973-981.
31. Al-Deresawi, M.S. and AlFaisal, A.H.M. (2015). Two novel missense mutations in exon 9 of *TPO* gene in Polycystic Ovary Syndrome patients with hypothyroidism.

J*ournal of Biotechnology Research Center*, 9(1):30-37.

32. Al-Deresawi, M.S.G.; Al Najar, A.F. and Abdul AlFaisal, A.H.M. (2013). Detection of five substitution TPO mutations in Polycystic Ovary Syndrome (PCOS) and thyroid hormones disturbance patients. J. *Biotechnol. Resaerch center*, 8(2):5-10.