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# MOLECULAR STUDY OF SLC2A9 GENE IN DIABETIC ASTHENOZOOSPERMIC IRAQI PATIENTS

**Sara Harith Abdul-Razaaq* and Prof. Dr. Waleed Hameed Yousif**

Collage of Applied Biotechnology/ Al-Nahrain University/ Baghdad-Iraq.

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***Corresponding Author**
**Sara Harith Abdul-Razaaq**
Collage of Applied Biotechnology/ Al-Nahrain University/ Baghdad-Iraq.

## ABSTRACT

This study was conducted to investigate the molecular aspects of SLC2A9 gene in diabetic patients' type 2 with asthenozoospermia and changes associated with sperm motility. The study included semen and blood samples collected from 44 patients and 20 healthy control. Twenty non-diabetic asthenozoospermic, twelve diabetic asthenozoospermic, twelve diabetic normozoospermic patients and twenty non-diabetic normozoospermic men serving as the healthy control group, from the consulting clinic of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University. Average ages of patients and control group were (20-64) years. Sperm motility was investigated for both patients and control according to (WHO, 2010). Sperm progressive motility showed a significant decrease ($p<0.01$) in non-diabetic asthenozoospermic and diabetic asthenozoospermic. While it significantly increased in diabetic normozoospermic patients compared with control. Sperm non-progressive motility showed a significant decrease ($p<0.01$) in all three groups of patients as compared to healthy control. Moreover, there was a significant increase ($p<0.01$) in immotile sperm of nondiabetic asthenozoospermic and diabetic normozoospermic patients and non-significant differences in diabetic patients when compared to healthy control. Polymerase chain reaction (PCR) was done using a specific primers set to amplify exon (7,8,9) of Solute carrier family 2 member 9 (SLC2A9) gene. It was found after PCR product sequencing that the substitution, insertion and deletion occurs.

**KEYWORDS:** This study was asthenozoospermia control.

## INTRODUCTION

Infertility is defined as failure of a couple to conceive after one year of regular sexual intercourse. It remains both prevalent and problematic among couples worldwide (Jairajpuri *et al*., 2017). It is widely accepted that male factor alone accounts for infertility in about 40% cases, female factor alone in 40% of cases of infertility, and in 20% cases, there is combined male and female factor (Jajoo and Kalyani, 2013).

Diabetes mellitus (DM) is a group of metabolic disorders in which the patient has high blood sugar and this either because cell do not respond to the insulin or pancreas does not produce enough insulin (Mirhoseini *et al*., 2013). Diabetes mellitus is a known cause of male sexual dysfunction but can also affect the endocrine control of spermatogenesis and the process of spermatogenesis itself (Bhattacharya *et al*., 2014).

The SLC2A9 **(**Solute carrier family 2 member 9) gene provides instructions for making a protein called glucose transporter9 (GLUT9). The recently cloned human GLUT9 gene, which maps to chromosome 4p15.3-p16 consists of 12 exon coding for 540 amino acid proteins (Augustin *et al*., 2004).

This study aim to study the sperm motility and investigating the role of SLC2A9 gene in type 2 diabetic patients with asthenozoospermia.

## MATERIALS AND METHODS

The study was done on 20 non-diabetic asthenozoospermic, 12 diabetic asthenozoospermic and 12 diabetic normozoospermic patients from high institute of infertility diagnosis and assisted reproductive technologies, AL-Nahrain University, Baghdad, Iraq between November 2016 to April 2017. Control group of 20 non-diabetic normozoospermic men. The average age of patients and control were 20-64 years.

### Sperm Motility Examination

Motility should be evaluated within 1 hour of collection, and ideally within the first 30 minute to prevent the effect of heat of the microscopic light source on the result. The number of motile spermatozoa in (10) randomly selected fields was counted away from the cover slip edge. The motility of each a spermatozoon encountered was graded as it is shown below:

1. Progressive motile sperm.
2. Non - Progressive motile sperm.

3. Immotile sperm.

**DNA Extraction**

Total cellular DNA was extracted from blood samples by using the gSYNC™ DNA Extraction Kit supplied by geneaid/ Taiwan**,** determination of concentration and purity of extracted DNA was done using nandrop (Techne/UK).

**PCR protocols**

Extracted DNA from blood samples and healthy control was used in PCR for amplification of SLC2A9 gene (S1, S2, S3) primers table (1). Initial denaturation 94℃ for 5 min., 35 cycle contain denaturation 94 ℃ for 1 min., Annealing 55℃, 58℃ and 56℃ respectively for each primers for 30 second, extension 72℃ for 1 min, and final extension 72℃ for 10 min.

**DNA sequencing**

The purified PCR products of amplified SLC2A9 gene region and primers were send by Macrogen Company in Korea for DNA sequencing. The obtained sequences of these samples were aligned using (Mega-7) software. Furthermore, the nucleotide sequences were compared to information in gene bank of the National Center For Biotechnology Information (NCBI) web site databases using BLAST search tool and examined for the presence of SNPs.

**Statistical analysis**

The Statistical Analysis System-SAS (2012) program was used to study the effect of different factors on the study parameters. The Least significant difference (LSD) test was used to significantly compare between the means in this study.

**Table 1: Primers used in this study. (Matsuo *et al*., 2008).**

| NO. | Name | OligoNucleotides | Product Size | Sequence (5`-3`) |
|---|---|---|---|---|
| 1 | S1-F | Forward Primer | 272bp | CAGTCCCCTCAACAATGACC |
| | S1-R | Reverse Primer | | ATTGGCCCAGGTCCCAG |
| 2 | S2-F | Forward Primer | 312bp | CAGGGCCCAGCATTAGAC |
| | S2-R | Reverse Primer | | CACCCTCTGATCCCTCCAG |
| 3 | S3-F | Forward Primer | 238bp | AGAAAATAGATTAAGTCCTTCCACTG |
| | S3-R | Reverse Primer | | TGAACATTCCCACTGTGCTG |

**RESULTS AND DISCUSSION**

**Fasting Blood Sugar**

Table (2) shows the fasting blood sugar in healthy control, non-diabetic asthenozoospermic, diabetic asthenozoospermic and diabetic normozoospermic patients. A significant ($P<0.01$)

increase in fasting blood sugar was observed in diabetic asthenozoospermic and diabetic normozoospermic patients when compared to the healthy control and nondiabetic asthenozoospermic patients. Diabetes results in a defect in the male reproduction. Glucose plays an important role in the process of spermatogenesis and maintenance of cell activity, as well as certain functions such as mature sperm motility and fertilization ability (Ding *et al*., 2015).

**Table 2: Level of fasting blood sugar in healthy control, nondiabetic asthenozoospermic, diabetic asthenozoospermic, and diabetic normozoospermic patients.**

| Groups | No. | Mean ± SE |
|---|---|---|
| | | Fasting blood sugar Mg/dl |
| Healthy control | 20 | 98.84 ± 5.72 c |
| Non-diabetic asthenozoospermic | 20 | 88.69 ± 3.91 c |
| Diabetic asthenozoospermic | 12 | 200.98 ± 16.03 b |
| Diabetic normozoospermic | 12 | 247.38 ± 25.67 a |
| LSD Value | | 43.0178 ** |

**Sperm Motility**

**Percentage of Progressive Motile Sperm**

Table (3) showed the percentage of progressive motile sperm in non-diabetic asthenozoospermic, diabetic asthenozoospermic and diabetic normozoospermic patients as compared to healthy control.

Results showed that the percentage of progressive motile sperm had a significant decreased in non-diabetic asthenozoospermic, diabetic asthenozoospermic patients. These results agree with those described in many studies described by Milardi *et al*. (2012), Shaikh *et al*. (2011), Qian *et al*. (2014), Ramzan *et al*. (2015), and Sharique *et al*. (2016) who found that the progressive sperm motility decreased in asthenozoospermic compared to the healthy controls. Delfino *et al*. (2007), Rama *et al*. (2012), Bhattacharya *et al*. (2014) and Lu *et al*. (2017) found that percentage of progressive motile sperm decreased in diabetic patients compared with the healthy controls. Progressive motile sperm significantly increase in diabetic normozoospermic patients compared with control. These results seem to agree with those obtained by Agbaje *et al*. (2007).

**Percentage of Non-progressive Motile Sperm**

Table (3) showed the percentage of progressive motile sperm in non-diabetic asthenozoospermic, diabetic asthenozoospermic and diabetic normozoospermic patients as compared to healthy control.

Non-progressive motile sperm showed a significant decreased ($p<0.01$) in non-diabetic asthenozoospermic patients, also diabetes caused a significant ($P<0.01$) decrease in non-progressive motile sperm asthenozoospermic and normozoospermic patients when compared with healthy control.

**Percentage of Immotile Sperm**

Table (3) showed the percentage of immotile sperm in non-diabetic asthenozoospermic, diabetic asthenozoospermic and diabetic normozoospermic patients as compared to healthy control.

The percentage of the immotile sperm increases significantly ($P<0.01$) in non-diabetic asthenozoospermic and diabetic asthenozoospermic patients in comparison with the healthy control. The increase in the percentage of immotile sperm in asthenozoospermic patients in the current study is in accordance with other studies (Tavilani *et al*., 2007; Khan *et al*., 2011; Hashemitabar *et al*., 2015). Magoli *et al*. (2013) and Hussein *et al*. (2015) reported that the percentage of immotile sperm increased with diabetic patients compared to the controls. Non-significant ($P<0.01$) changes in the percentage of immotile sperm in diabetic normozoospermic patients as compared to the healthy control.

Sperm motility is an important factor in semen quality and fertility potential, because it is required for transport through the female genital tract and penetration of zona pellucida (Khan *et al*., 2011). Sperm motility increased in men with the highest testosterone level while there was a negative correlation between FSH and LH hormone levels and sperm motility (Meeker *et al*., 2007 and Ghasemian *et al*., 2017).

**Table 3: Sperm motility in healthy control, non-diabetic asthenozoospermic, diabetic asthenozoospermic and diabetic normozoospermic patients.**

| Groups | Mean ± SE | | |
|---|---|---|---|
| | Progressive motile sperm % | Non-Progressive motile sperm % | Immotile sperm% |
| Healthy control | 42.05±2.58 b | 35.84±1.82 a | 22.63±1.29 b |
| Non-diabetic asthenozoospermic | 11.70±0.42 c | 30.45± 2.05 b | 56.05± 2.75 a |
| Diabetic asthenozoospermic | 15.83±0.74 c | 28.50±1.49 b | 55.83± 2.92 a |
| Diabetic normozoospermic | 50.67±2.39 a | 28.83±1.66 b | 20.50±1.42 b |
| LSD Value | 7.027 ** | 3.289 ** | 8.316 ** |

**Amplification of SLC2A9 gene**

All blood samples were subjected to molecular detection through PCR amplification of SLC2A9 gene by using three specific primers. The first primer used was specific for the amplification of exon 7 for SLC2A9 gene with product length (272 bp). The second primer used was specific for the amplification of exon 8 for SLC2A9 gene with product length (312 bp). The third primer used was specific for the amplification of exon 9 for SLC2A9 gene with product length (283 bp).

**Detection of SLC2A9 Gene Mutation by Sequence**

The first DNA sequence of SLC2A9 gene located in exon (7) was taken from blood samples of non-diabetic asthenozoospermic patient and was compared using the NCBI nucleotide blast, as shown in the table (4). Three SNPs are identified. First SNP encoded for a frameshift mutation (deletion of adenine nucleotide in ACA/-C-) in position 29 that converts threonine to deletion. The second SNP also frame shift mutation (deletion of adenine nucleotide in ACA/-C-) in position 31 that converts threonine to deletion. The third SNP encoded for substitution mutation (substituted thymine with adenine ATA/AAA) in position 47 that converts isoleucine to lysine. The second DNA sequence of SLC2A9 gene located in exon (7) was taken from blood samples of diabetic asthenozoospermic patients and was compared using the NCBI nucleotide blast, as shown in table (4). One frameshift mutation (deletion) found in this sequence. In position 235 deletion (deletion of cytosine CCT/C-T), the change is from proline to deletion. The third DNA sequence of SLC2A9 gene located in exon (7) was taken from blood samples of diabetic normozoospermic patient and was compared using the NCBI nucleotide blast, as shown in table (4). Only one SNP occurs with this patient in position 32 (deletion of adenine ACA/-AC) which converted threonine to deletion.

The fourth DNA sequence of SLC2A9 gene located in exon (8) was taken from blood samples of non-diabetic asthenozoospermic patient and was compared using the NCBI nucleotide blast, as shown in table (4). It shows substitution mutation occurring in position 109 (guanine substituted with adenine CGC/CAC) that convert arginine to histidine. In position 274 frameshift mutation occurs (deletion of adenine GAT/G-T) leading to convert aspartic acid to deletion. The fifth DNA sequence of SLC2A9 gene located in exon (8) was taken from blood samples of diabetic asthenozoospermic patient and was compared using the NCBI nucleotide blast, as shown in table (4). Three SNPs are found in this sequence, in position 18 frameshift mutation occurs (deletion of adenine AAA/-AC) that converted lysine to deletion. Substitution occurs in position 20 (adenine substituted with cytosine AAA/-AC) that convert lysine to other. In position 274 deletion occurs (deletion of adenine ATC/-TC) that converts isoleucine to deletion. The sixth DNA sequence of SLC2A9 gene located in exon (8) was taken from blood samples of diabetic normozoospermic patient and was compared using the NCBI nucleotide blast, as shown in table (4). Only one SNPs is located in this automated sequence, in position 273 frameshift mutation occurs (adenine deletion GAT/G-T) that convert aspartic acid to deletion.

The seventh DNA sequence of SLC2A9 gene located in exon (9) was taken from blood samples of non-diabetic asthenozoospermic patient and was compared using the NCBI nucleotide blast, as shown in table (4). Two-substitution mutations are located in this sequence of non-diabetic asthenozoospermic. In position 17 and 18, thymine and cytosine are substituted with adenine TTC/TAA that converts phenylalanine to stop codons. The eighth DNA sequence of SLC2A9 gene located in exon (9) was taken from blood samples of diabetic asthenozoospermic patient and was compared using the NCBI nucleotide blast, as shown in table (4). Two-frameshift mutations are located in this sequence. The first one in position 16 (deletion of cysteine CTC/CT-) lead to change leucine to deletion. The second one in position 19 insertion changes to methionine (guanine insertion AT-/ATG). The ninth DNA sequence of SLC2A9 gene located in exon (9) was taken from blood samples of diabetic normozoospermic patient and was compared using the NCBI nucleotide blast, as shown in table (4). There are two deletion in this sequence: deletion of adenine TCA/TC- in position 15 that converts serine to deletion, also in position 198 (deletion of thymine TGG/-GG) that converts tryptophan to deletion.

**Table 4: Type of polymorphism in *Homo sapiens* solute carrier family 2 member 9 (SLC2A9).**

| Name of primer | Wild sequence | Mutant sequence | Amino acid change | Site of NA | Type of mutation | Effect of translation |
|---|---|---|---|---|---|---|
| S1 | ACA | -C- | Threonine to deletion | 29 | Deletion | Frame shift |
| | ACA | -C- | Threonine to deletion | 31 | Deletion | Frame shift |
| | ATA | AAA | Isoleucine to lysine | 49 | Substitution | Missense |
| | CCT | C-T | Proline to deletion | 235 | Deletion | Frame shift |
| | ACA | -CA | Threonine to deletion | 32 | Deletion | Frame shift |
| S2 | CGC | CAC | Arginine to histidine | 109 | Substitution | Missense |
| | GAT | G-T | Aspartic acid to deletion | 247 | Deletion | Frame shift |
| | AAA | -AC | Lysine to deletion | 18 | Deletion | Frame shift |
| | AAA | -AC | Lysine to threonine | 20 | Substitution | Missense |
| | ATC | -TC | Isoleucine to deletion | 274 | Deletion | Frame shift |
| | GAT | G-T | Aspartic acid to deletion | 273 | Deletion | Frame shift |
| S3 | TTC | TAA | Phenylalanine to stop codons | 17-18 | Substitution | Missense |
| | AT- | ATG | Insertion to Methionine | 19 | Insertion | Frame shift |
| | CTC | CT- | Leucine to deletion | 16 | Deletion | Frame shift |
| | TCA | TC- | Serine to deletion | 15 | Deletion | Frame shift |
| | TGG | -GG | Tryptophan to deletion | 198 | Deletion | Frame shift |

Ding *et al*. (2015) proposed that the impairment in Glut9 transcription is due to the lack of insulin or hyperglycemia and suggested that insulin and glucose are necessary for sperm maturation and play a roles in glucose movement, which in turn control sperm motility during fertilization. Some polymorphisms have been associated with an increased incidence of diabetes in Chinese populations, the adenine/guanine polymorphism in SLC2A9 gene may alter the amino acids polarity affecting of SLC2A9's to glucose and fructose, and this change leads to level change of glucose. (Xing et al., 2015).

## CONCLUSIONS

The adverse changes shown in semen quality (progressive motile sperm, non-progressive motile sperm and immotile sperm) were more pronounced in non-diabetic asthenozoospermic and diabetic asthenozoospermic patients in comparison with the diabetic normozoospermic patients. Theses adverse changes may be related to the mutations occurring in SLC2A9 gene, since more than one phenomena were observed in exons 7,8 and 9 (deletion, substitution and insertion) of the non-diabetic asthenozoospermic and diabetic asthenozoospermic patients when compared to those reported in exons 7,8,9 (deletion only) of diabetic normozoospermic patients. Consequently, mutation in SLC2A9 gene may affect glucose transport via GLUT9, and this, in turn, affect the pituitary-testicular axis, steroidogenesis and sperm parameters.

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