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Investigation of SCP3 Gene Expression in Men with Obstructive Azoospermia

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Abstract

Introduction and goal: In obstructive azoosperm, a person has normal spermatogenesis but no sperm is ejaculated due to obstruction in the sperm from the testes to the ejaculation. Also the disorder of the expression of each of the effective genes in the pathway of spermatogenesis is one of the causes of azoospermia one of these genes being SCP3 and producing a protein in the phase of homologous and synaptic chromosomes. The lack of expression of this gene in mice causes male infertility and reduced fertility of the female sex. Examination of this gene in humans can also help determine the cause of infertility and as a diagnostic factor in determining the progress of spermatogenesis and infertility in the treatment of male patients. Methods: In this descriptive cross-sectional study, the expression of Scp3 gene in healthy and control tissues of men with obstructive visophagus referred to Ebncenna and Mrs. Al-Nabiya infertility in 1396 1397 was evaluated and compared by real time polymerase chain reaction (Real time PCR). Results: In order to determine the gene expression in obstructive oesophageal men which according to our research hypotheses that the absence of this gene causes impaired spermatogenesis and fertility of the individual, the relative level of expression in the control tissue with healthy tissue was the same and no significant changes were observed. Conclusion: In this study, the SCP3 gene was not expressed in patients with obstructive oesophageal disease which made them suitable molecular markers for determining the presence of sperm and examination of spermatogenesis in infertile men without requiring invasive testicular biopsy.

Keywords: Obstructive Osteoporosis. SCP3 Gene, Real Time PCR Technique.

Introduction

Infertility is one of the most common and rising problems in the world that is said to be the inability of couples to achieve successful pregnancy after a year of sexual intercourse without interruption And about 15 percent of couples are involved (Hamada et al., 2012). The causes of infertility can be related to women's , men's or both factors accounting for about half of the causes of infertility related to male factors. Infertility in men can be defined as a multifactorial syndrome that covers a wide range of disorders and the result of all of these is man's inability to transfer her hereditary material to the next generation. Infertile men can not produce a healthy woman (Anawalt, 2013) Along with medical records and accurate physical examinations, semen analysis is the mainstay of studies in male infertility. Infertile men are classified into several groups such as azoospermia in terms of the number, shape and natural motion (health characteristics) of the sperm. Azoospermia is said to be a person without sperm in semen, often with a very low chances of fertility or even sterility. Azoospermia affects 1% of the male population and may account for 10% of the observed cases of male infertility which is categorized as two main types of obstructive azoospermia and obstructive azoospermia (Verheyen et al., 2017). The causes of azoospermia are different and differ in their classification in different sources. Some of the causes of azoospermia are divided into preexemorial (secondary testicular failure) testicular (primary insufficiency) and after the testicles (obstruction). Others believe that in practice and in therapeutic decisions, only two phenomena can be considered for azoospermia which are obstructive and non-obstructive azoospermia and a group proposes the classification of azoospermia based on genetic knowledge but what is most commonly used today is the categorization of azoospermia based on obstructive and non-obstructive causes (Uchechukwu, 2000).

In anesthetic azoospermia, sperm is not made and it is believed that this type of azoospermia is due to a defect in the process of spermatogenesis that can be caused by several causes such as chromosomal aberrations, congenital defects, infectious diseases, radiation therapy and chemotherapy or for unknown reasons. In contrast to obstructive azoosperm a person has normal spermatogenesis but no sperm is ejaculated due to blockage of the sperm from the testicle to the ejaculation (Zhang et al., 2015). Spermatogenesis is a complex process of differentiation and cellular changes derived from a diploid parent cell (spermatogonium), ultimately resulting in a haploid male gamma

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whose germ formation and maturation are regulated by 2300 genes (Xie et al., 2017). The expression of the genes involved in the spermatogenesis process including the expression of the VASA gene of cyclin and protamine has been studied by studies (Haraguchi et al., 2009; Ando et al., 2012). However, the expression of other genes that may contribute to the spermatogenesis process such as the Scp3 gene has not been studied in patients with obstructive azoospermia.

Because for the proper functioning of spermatogenesis, the process of pairing the homologous, synapse and recombinant chromosomes and subsequent chromosomal separation is necessary and the correct implementation of these processes is regulated by the specific protein structures called the protein complex of opnominal sinus. In mammals, the SC proteins comprising the three SCP3 SCP2 and SCP1 groups form the core components called SYCP (Parra et al., 2004). SYCP is performed solely in the puberty of testicular germ cells (in the spermatocyte stage). The absence of this gene disrupts the spermatogenesis and fertility of the individual (Aarabi et al., 2006). Therefore, with regard to the prevalence of infertility and that around 10-15% of couples suffer from infertility around the world and half of them are infertile men studies on improving male fertility are necessary (Izadyar et al., 2003).

On the other hand, 40% of cases of azoospermia are obstructive obstructive azoospermia can be congenital and can be acquired. One of the causes of this is severe genital infections. In obstructive azoospermia artificial insemination is commonly used for childbirth. For this, sperm syringe is prepared from the testes and used for artificial insemination and because of the prevalence of infertility problems in azoosperm patients their sexual dysfunction is underestimated. Particular attention to these problems when pursuing infertility treatment in addition to improving the quality of life will in some cases also improve the treatment of infertility. Also all studies done in Azoospermic men are due to the fact that they may be able to do something that they naturally have. Over the last 15 years using aids to fertility has gained a lot of success and many Azospermic men who had no previous chance of becoming a father. And considering that comprehensive study has not been done so far this study was designed to evaluate the expression of SCP3 gene in obstructive azoospermic patients.

Method of Work

Sample collection

One of the main stages and perhaps the first step in assessing infertile people is the examination of the characteristics of semen. In order to carry out this study, standard methods have been developed by the World Health Organization which are widely accepted by infertility research centers such as Khatam al-Nabia, Ebnecna Research Institutes. For this purpose, the present study which is a descriptive study was an infertile male who referred to the Khatam al-Nabia Institution of Infertility for the treatment of infertility during the months of March to March 2012. After performing physical examinations and testing of semen analysis, after diagnosis of obstructive azoosperm (and healthy sperm samples from the individuals as a control) they were enrolled by the urologist and in order to know the clinical and genetic status of the patients. People who had entry and exit criteria were entered or excluded from the study, inclusion criteria included normal karyotype, no withdrawal in AZF areas and non-use of hormonal drugs. The criteria for exclusion from the study included surgery for varicocelectomy.

After the necessary examinations on the tissue and the therapeutic procedures, the tissue remains to the genetic lab for conducting research work in sterile micro vials. Samples were stored in a liquid nitrogen tank at 196 ° C until RNA extraction.

Extraction of RNA from tissue:

RNA extraction (Qiagene, Rneasy Microarray Tissue Mini Kit) was used according to the manufacturer's instructions for extraction of tissue samples from the commercial kit). During one of the final stages of RNA isolation the samples were exposed to the enzyme Dnase (Rnase-Free Set Dnase, Qiagen) according to the instructions for 15 minutes in order to eliminate the possible remnants of the genomic DNA contained in samples. The quantity and quality of RNA extracted by a nanosecond machine (absorption at 280/280 nm) and electrophoresis on a gel of agarose were evaluated. RNA samples were extracted until further experiments were carried out at -80 ° C.

DNA synthesis competently (cDNA):

RNA samples (from each 500 ng sample) using commercial kinetics of converting RNA to cDNA (Takara Bio, Otsu, Japan)on a normal coclar apparatus in a 30 ° C cyclic cycle for ten minutes and then one enzymatic deactivation step at 70 ° C. The reaction mixture of the above cDNAs was then immediately cooled on the ice. The cDNA samples produced until the next step (Real-time PCR) Real time polymerase chain reaction were stored at -20 ° C.

Real-time PCR:

The cDNA samples produced in the previous stage were first melted on ice, then replicated with the Mastermix (RT2 SYBR Green qPCR Mastermixes, Qiagen) mixture. First, in accordance with the kit instructions the reaction mixture components including Syber green master mix, primer and water-free nucleoside were prepared in a 5 mm tube at ambient temperature. Then, 24 µl of each reaction mixture was added to each well of the Real Time PCR plate, Which was added to them by adding a Microliter of cDNA reaction solution. In this way, the final volume of each well yielded 25 microliter reactions. The plates were centrifuged for 1 minute at room temperature and at a rate of 1000 g to remove the air bubbles in the wells, and the reaction components were well mixed. The reaction mixture was amplified by the (Bioneer, Exicycler ™ 96) Real Time PCR apparatus.

The reaction temperature program included initial heating of the thermal block of the device to 95 ° C for 15 seconds, 60 ° C for 30 seconds and 72 ° C for 10 seconds. For each sample, two wells were assigned and the average of their threshold cycles (CT)was calculated for each sample.The essential primers for scp3 gene include upstream FWD:TGCGGTGTGTTTCAGTCAGG sequence and downstream REV: CATATTACTTAGGTTCAAGTTC sequence and for microglobulin housekeeper gene Microglobulin - β-2 (β2M or Beta-2-microglobulin) (as control) includes upstream FWD: CTCACGTCATCCAGCAGAGA sequences and REV: GTCAACTTCAATGTCGGATGGA downstream sequences that were designed on the site Primer3 after genes were extracted on UCSC genome browser site and And checked using Oligoanalyzer and Generunner (Integrated device technology or IDT) software.

After completion of the reaction, a melting point analysis program was performed to confirm the (specificity) of the PCR reaction for all specimens. Thus, by analyzing the melting curve, the peak of the amplification of A messenger RNA(mRNA) Scp3 gene was determined. The threshold cycle was determined using the device software.

Before performing Real time PCR, the replication efficiency for Scp3 and β2M cDNAs was evaluated using the serial dilutions prepared from them and on that basis the reproduction efficiency was approximately similar. So to normalize the Scp3 mRNA changes among the samples, β2M mRNA was used as an appropriate control and method -2ΔΔCTwas used(Livak & Schmittgen, 2001).

In short, CT sc3 and β2M were first determined in Control tissue and healthy tissue and then their ΔCT was obtained according to the ΔCT CT= Scp3 – CT β2M equation. In the next step, the ΔΔCT value of each item was calculated according to the following relation,

ΔΔCT= ΔCT (Control tissue) – ΔCT (healthy tissue). Finally, the healthy tissue mRNA value was calculated as -2ΔΔCT. According to this method, the amount of Scp3 gene expression is calculated in Control tissue relative to its healthy tissue.

Statistic analysis:

The difference in control and healthy tissue was checked by Wilcoxon test.

Results

In order to evaluate the gene expression in obstructive vasospromic men, based on our research hypotheses that the absence of this gene causes impaired fertility and spermatogenesis, a Real Time PCR test was performed. As seen in these patients, the relative levels of SCP3 expression were compared in healthy control and tissue, the relative expression of SCP3 expression in the control tissue was similar to that of healthy tissue.

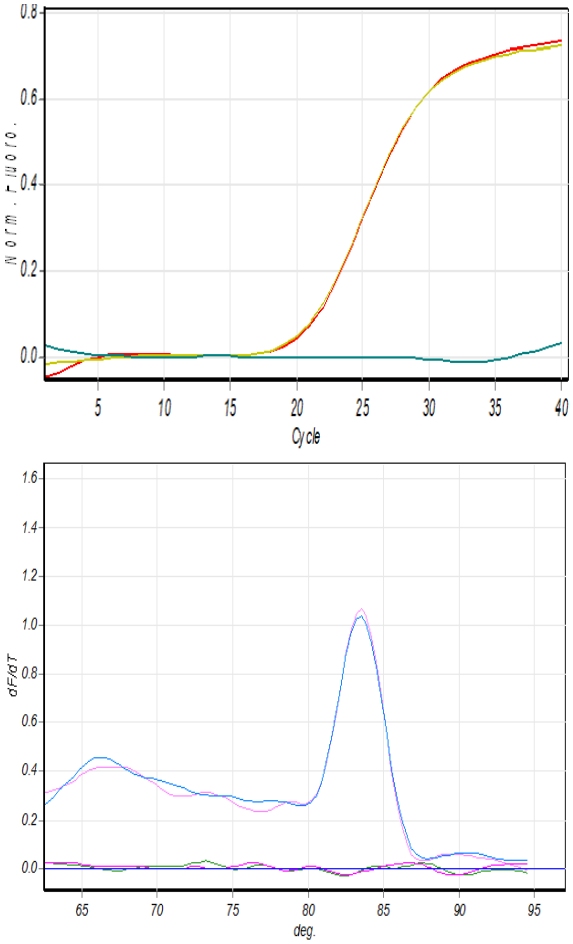

Figure 1. Comparison of relative expression of SCP3 expression with healthy tissue in patients with obstructive azoospermia, the difference between control and healthy tissue was measured by Wilcoxon test.

Discussion

In the past, it was thought that mature sperm is silent in terms of transcription; recent evidence suggests that the sperm nucleus is more active than it was thought to be the research confirms the presence of active mRNA transcription in the sperm (Lambard et al., 2004; Zhang et al., 2008). Studies have shown that the sperm nucleus contains nucleoprotein and nucluohistonic segments, the nucleoconstrictor sections have less compression and are susceptible to nucleases and may play a role in expressing the genes required for sperm function (Galeraud-Denis, 2007). Today we know that part of the contents of the mRNA contained in the sperm is the residues of the mRNA reserves that are synthesized in the terminal stages of spermatogenesis that are transmitted to the sperm and the other part of the mRNA is synthesized by the sperm cell (Lambard et al., 2004; Wang et al., 2004; Wykes et al., 200). On the other hand, recent studies have identified the stage

of the expression of specific testicular genes by using test specimens with a stop in certain classes of spermatogenesis that At what stage does the specific testicular genes begin to express and at what stage the spermatogenesis stops its expression and what stage of the protein is produced from it. For example, in the Arabi study, he expression of the SYCP3 gene was initiated from the initial spermatocyte stage and stopped at the spermatid stage (Aarabi et al., 2006). Therefore, the results of this study on the absence of SCP3 gene in sperm show that all mRNA residues from spermatid to sperm have not been transmitted and transcripts of mature sperm have been actively produced after the spermiogenesis stage. As if their presence in adult sperm is confirmed it can be concluded that this gene is actively expressed in the final stages of spermatogenesis and it can also be said that this gene will not be expressed in non obstructive azoospermic men.

Conclusion

In the present study, the SCP3 gene was not expressed in patients with obstructive oesophageal disease, which would not convert them to appropriate molecular markers for determining the presence of sperm and examination of spermatogenesis in infertile men without the need for an invasive testicular biopsy. And previous studies have shown that the expression of SCP3 gene is present in the primary spermatocyte stage but this gene did not exist in adult spermatozoa. The deletion of these transcripts in the final stages is probably due to the completion of this gene and the absence of adult sperm to this gene for its subsequent functions (Aarabi et al., 2006). Also, more research on patients with obstructive oesophagia and accelerates the use of precise molecular tools for diagnostic procedures. This leads to awareness of the molecular process of fertilization in humans and other mammals as well as the identification of the molecular causes of many infertility cases that are unknown to us today and categorized in the category of infertility with an unknown cause.

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