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Research Article

Immunohistochemical Analysis of Sperm Aquaporin-3 Expression in Men with Varicocele

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Abstract



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Objective: The primary objective of this study is to detect potential alterations in sperm cells in cases of varicocele using immunohistochemical methods.

Materials and Methods: In this study, semen samples obtained with consent from infertile males aged between 25-40 years were analyzed for the presence of varicocele and other determined factors, and were divided into two groups: a normozoospermic control group and a group diagnosed with varicocele. Direct swim-up techniques were utilized to separate spermatozoa with high motility. Immunohistochemical staining methods were employed to visualize protein expression, and the results were photographed at high magnification for documentation.

Results: This research was conducted on sperm samples collected from males diagnosed with varicocele and from a healthy control group. The findings suggest that the presence of aquaporin-3 protein is associated with low or negative expression in sperm cells in the presence of varicocele. Immunohistochemical analyses have revealed a reduced or absent expression of aquaporin 3 in patients with varicocele compared to healthy individuals.

Conclusion: The results of this study clearly demonstrate the adverse effects of varicocele on the structural and functional integrity of spermatozoa. Given that aquaporin-3 protein is critical for the motility of spermatozoa, the reduced expression or absence of this protein sheds light on the molecular mechanisms of male infertility.

Keywords: Sperm DNA fragmentation, immunohistochemistry, Varicocele, protein expression, male infertility, aquaporin-3.

Introduction

Infertility is the inability to achieve pregnancy after one year of regular, unprotected sexual intercourse in individuals of reproductive age. The cause of infertility is attributed to female factors in approximately 40-50% of cases and to male factors in about 30-40% ¹. When assessing infertile males, it is essential to evaluate the man's medical history, physical examination, and semen analysis results. Moreover, the assessment of varicocele in males is of significance ².

Varicocele is the enlargement of veins within the scrotum and can lead to infertility in men ³. Varicocele is the underlying cause in 40% of men with infertility complaints. While varicocele is more commonly observed in the left testicle, it can also occur on the right side. There are three main theories proposed for the formation of varicocele: the anatomical differences between the veins of both testicles, the retrograde flow in the veins, and the partial obstruction of the vein due to pressure exerted on the vein of the left testicle ⁴.

The primary aim of this study is to analyze the effects of varicocele on sperm via immunohistochemical analysis. Thus, the study seeks to determine the impacts of varicocele on males by detecting its consequences through immunohistochemical studies on testicular tissue and spermatogenesis.

Material and Methods

Study Design

This study was conducted on 50 patients aged between 25-40 years with complaints of infertility. Patients with a history of smoking and alcohol consumption, those who had undergone hormone replacement therapy recently, and those with a semen volume of less than 1.0 ml were excluded from the study. The study included semen samples with a volume of 1.5 ml or more, a total sperm count exceeding 15 million, sperm motility above 40%, and sperm morphology above 4%, as well as samples diagnosed with varicocele. Semen samples were obtained by masturbation from individuals who had abstained from sexual activity for 4 days. The samples were collected in a sterile container and allowed to

liquefy for half an hour. All samples were first subjected to macroscopic examination, followed by microscopic examination. Microscopic evaluation assessed the total sperm count, sperm motility, and morphological status.

Determination of study groups

After the liquefaction process, the samples were divided into two distinct groups for the continuation of the study. The first group was selected as the control group, and these samples fell within the parameters considered normal by the World Health Organization in terms of density, count, movement, and morphology. The other group consisted of semen samples from patients diagnosed with varicocele. The fundamental purpose of dividing the samples into two separate groups was to test the study's hypothesis and to obtain accurate data. In both groups, the potential alterations in sperm morphology that could be caused by varicocele were meticulously evaluated.

Sperm Washing

Swim-up Method

To minimize the possibility of contamination, sterile pipettes were used for each sample. A 1 ml sample was transferred from the specimens to sterile centrifuge tubes. A previously prepared 1.2 ml of culture medium was added on top of the semen fluid. Sterile pipettes were placed at the bottom of the culture medium. To allow for the separation of sperm cells in the medium, the tubes

were tilted at a 45-degree angle and incubated at 37 degrees Celsius for 60 minutes. After this process, the tubes were returned to an upright position, separating the medium from the underlying pellet. The sperm cells remaining on top were those with the highest motility. To maintain the viability of these cells, 1.5 ml of culture medium was used for dilution. The sample was then centrifuged for 5 minutes. The resultant sperm pellet was re-suspended in 0.5 ml of medium for assessment and then distributed onto slides.

Immunohistochemical Analysis

In this study, the method used involved washing slides placed in Nunc 4-well culture plates with phosphate-buffered saline. Antibodies for AQUAPORIN-3 protein were applied to each well. To prevent the samples from drying out, stretch film was used to ensure they were airtight. Subsequently, the samples were incubated overnight at 4 degrees Celsius. The samples were washed three times with phosphate-buffered saline to separate unbound antibodies. After washing the slides with water, they were cleaned with alcohol. The samples were examined using a light microscope at 600x magnification and images were captured.

Results

Immunostaining of AQP3 expression in healthy and patients with varicocele were shown in Figure 1-3.

AQP3 Protein Expression in Healthy Individuals

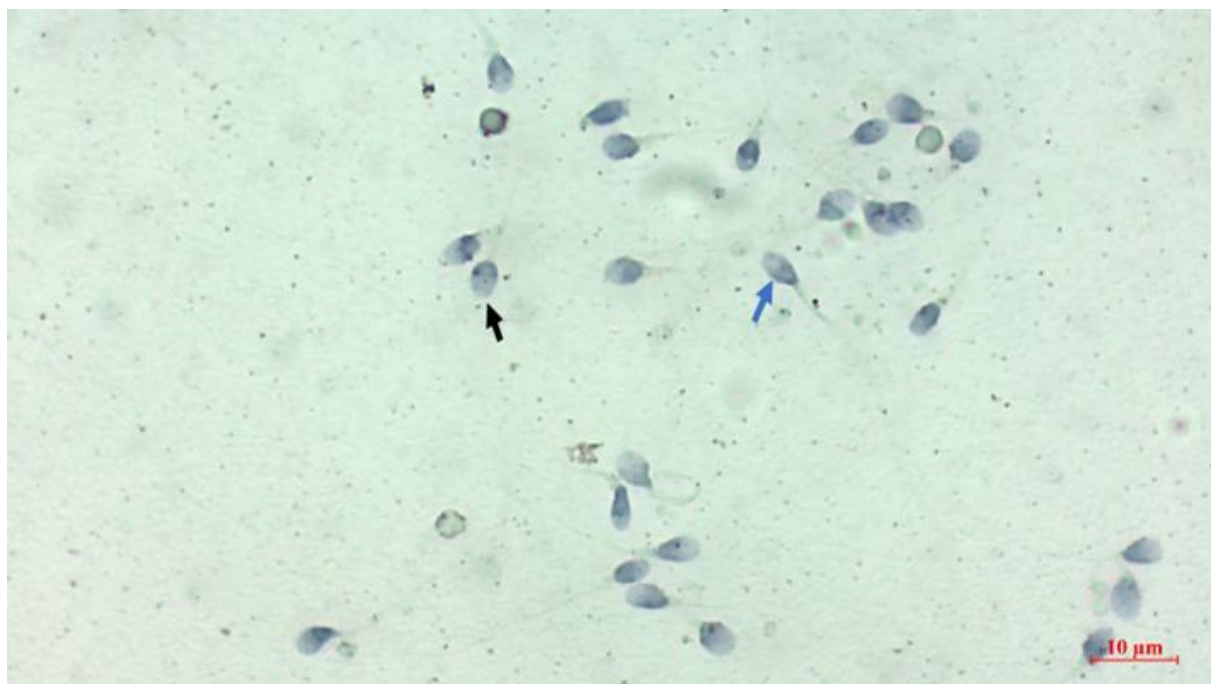


Figure 1: In this image, the expression of aquaporin 3 (AQP3) protein in sperm samples from healthy donors has been examined. Immunohistochemical staining revealed that the cells indicated by the black arrow show intense AQP3 expression, particularly in the head region of the sperm cell. This finding suggests that the AQP3 protein is a critical factor in the water and solute balance of healthy spermatozoa and that the expression of this protein could be a positive indicator of sperm cell functionality. The cells marked with the blue arrow represent areas where this expression is less intense.

Evaluation of AQP3 Expression in Patients with Varicocele

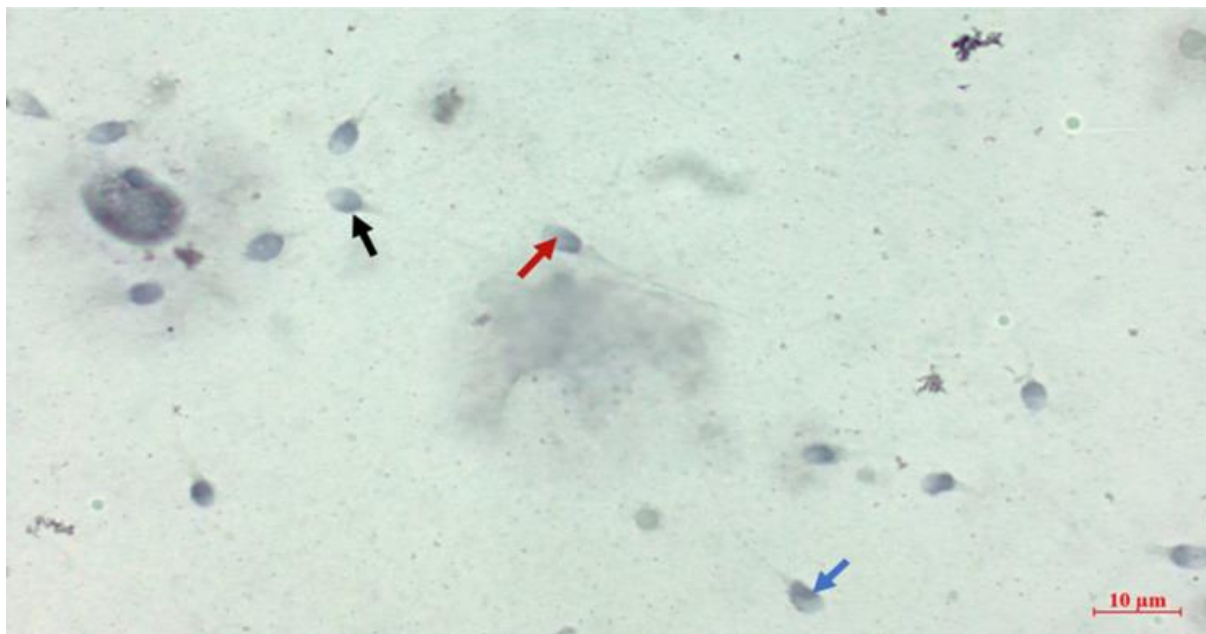


Figure 2: This micrograph demonstrates the expression of aquaporin 3 (AQP3) protein in sperm samples from patients with varicocele. Compared with individuals in the control group, it has been determined that there is a reduction and predominantly negative expression of AQP3 in the sperm of patients in this group. The cell indicated by the black arrow shows a pronounced absence of expression; the cell marked by the red arrow represents an area with little or no presence of AQP3 protein; and the cell pointed out by the blue arrow is another example of negative expression.

Aquaporin 3 (AQP3) Deficiency in Varicocele Patients

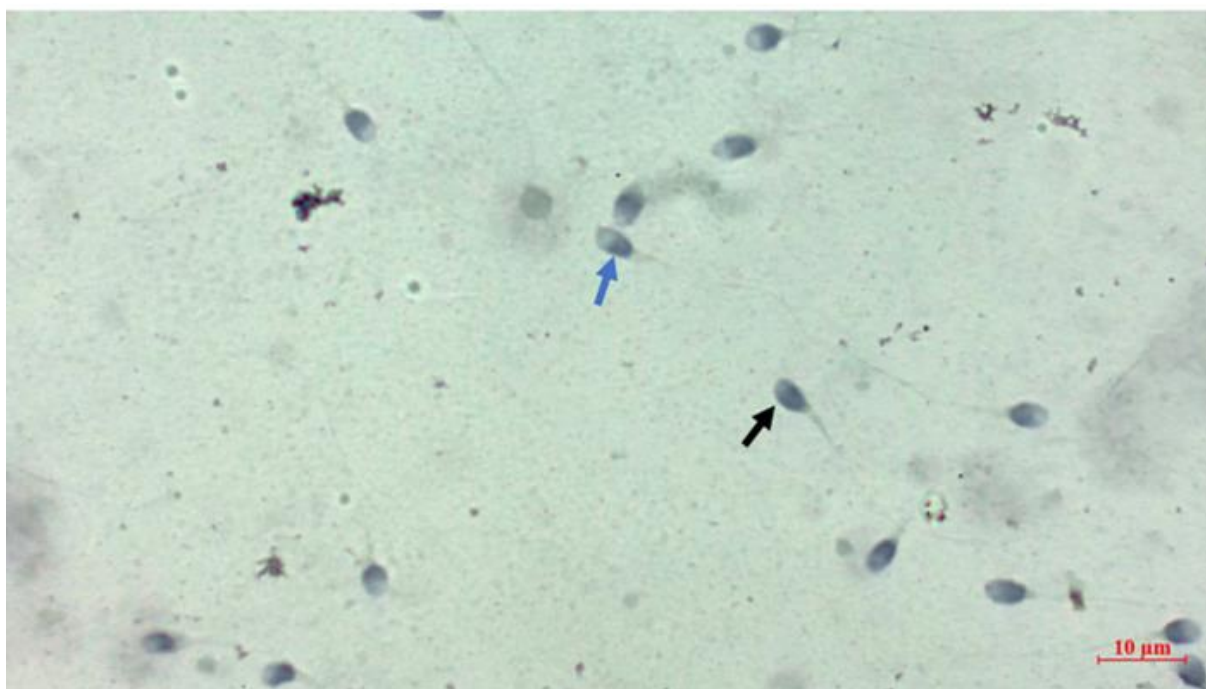


Figure 3: This image presents an evaluation of AQP3 expression in a sperm section from another varicocele patient. The sperm cell marked with a black arrow in the head region has been determined not to exhibit the expected activity of the AQP3 protein, therefore indicating a negative expression. The cell indicated by the blue arrow represents another example of this negative expression. These observations suggest a potential inhibition of varicocele on the membrane structures and water transfer functions of spermatozoa and are considered an important indicator for investigating the effects of this condition on the fertilization capability of sperm.

Discussion

Varicocele emerges as one of the significant factors contributing to male infertility. Varicocele is characterized by the dilation of veins emanating from the testes due to the insufficiency of valves within these vessels; this leads to an increase in temperature and nutritional imbalances in the testes, negatively affecting the tubule cells involved in sperm production. Varicocele can cause damage to the testes, retardation in development, and disruption of spermatogenesis, leading to infertility. Among the effects of this disease on infertility are semen anomalies, reduced testicular volume, and dysfunction of Leydig cells ⁵.

In semen analysis evaluations of men diagnosed with varicocele, approximately 90% of the cases showed a decrease in sperm motility, and in 65% of the cases, sperm concentration was observed to fall below 20 million/ml. However, contrary to popular belief, varicocele does not cause infertility in every case, and it is known that about 80% of individuals with varicocele pathology are fertile ⁶.

In studies on the causes of male infertility, according to the guidelines of the European Association of Urology, the evaluation of 7,057 patients revealed that the most common cause of infertility cases was idiopathic, followed by varicocele with a prevalence of 12.3% ⁶.

Research on the causes and treatments of infertility cases is also necessary. In this context, studying the expression patterns and characterization of certain molecules expressed in sperm cells, which can affect the parameters governing the potential of sperm to fertilize an oocyte, is of critical importance. For this reason, the study evaluated the expression characterization of AQPs 3, 7, and 8 molecules expressed in sperm cells ⁷. AQPs are water transport proteins that facilitate the passage of water molecules across membranes and are essential for maintaining cellular viability and function ^{8,9}.

A hypotonic environment is also significant and may contribute to the formation of sperm morphology and motility. AQPs play fundamental roles in maintaining this environment. In this context, it can be posited that AQP molecules may enhance the viability of the sperm cell and its fertilizing capabilities ^{10,11}.

Since AQP 3, 7, and 8 molecules are localized in the plasma membranes of sperm cells, these molecules can be directly associated with the viability and motility of sperm cells ⁷. Therefore, these molecules can influence the fertilization capacity of sperm cells and may be associated with male infertility. Within the fertile group, we observed this as a slight increase in the immune expression of AQPs.

It has been reported that AQP-3 plays a role in physiological hypotonic stress, which is necessary for sperm motility and regulatory volume decrease, by balancing cell swelling with tail curling, which are common in the sperm of infertile patients and impede sperm motility. Some animal studies have suggested that AQP-3 is associated with sperm cell cryo-tolerance ^{12,13}.

Conclusion

In light of the literature above, it can be noted that certain AQP molecules may be more or less active at different times or stages in the life of sperm cells. We examined only the mature phase of the sperm cell and the same period. AQP expression characterizations may also vary along the female genital tract. Therefore, although we observed a slight increase in the immune expression of AQP molecules in the fertile group, we may not have found a statistical difference between groups.

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