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SPERM DNA FRAGMENTATION INDEX IN A MALE WITH TYPE I GLOBOZOOSPERMIA (TOTAL GLOBOZOOSPERMIA)

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Abstract

Relevance: Total globozoospermia (Type 1) is a severe teratozoospermia leading to complete male infertility. It is an extremely rare spermatozoa pathology caused by disruption of acrosome formation.

The study aimed to determine and compare DNA fragmentation test results in a patient with a total globozoospermia after several sperm processing methods.

Methods: This article describes a case of a male with total globozoospermia. This study examined the DNA Fragmentation Index by Sperm Chromatin Structure Assay (SCSA) of a patient with type I globozoospermia. We compared the test results before and after processing the ejaculate with several sperm processing methods described in the WHO laboratory manual for the examination and processing of human semen (2010).

Results: SCSA showed no abnormality in DNA fragmentation in the patient with total globozoospermia. DNA Fragmentation index did not improve significantly after processing the sperm with a direct swim-up method and discontinuous density gradient.

Conclusion: According to SCSA results, the acrosome absence did not affect DNA stability in a patient with total globozoospermia.

Keywords: type I and type II globozoospermia, acrosome formation, DNA Fragmentation index, Sperm Chromatin Structure Assay (SCSA), direct swim-up, density gradient

Introduction: 15% of all couples suffer from infertility [1]. 50% of them have the so-called male factor infertility caused by sperm pathologies [2]. In 30-40% of the cases, the cause of the infertility is unknown (idiopathic infertility). Infertility has genetic etiology in 5.8% of infertile men [3] and is associated with Y-chromosome mutations in 4.2%. Globozoospermia is among the diseases caused by these mutations. Globozoospermia is a form of primary male infertility characterized by round-headed spermatozoa without a developed acrosome, making them incapable of activating the oocytes. However, those patients have had successful fertilization cases using assisted reproductive technologies [3]. This phenotype is quite rare and registered in only 0.1% of infertile men [4]. It is classified into two types: type I (total) – all spermatozoa in the ejaculate have a round head; type II (partial) – less than 100% of spermatozoa have this defect [5].

The genetic nature of this disorder has been associated with alterations in specific genes [6]. For example, mutations in the PICK1, DPY19L2, and SPATA16 genes lead to type I globozoospermia.

PICK1 is a membrane protein expressed in mouse and human tissues [7]. It interacts with several membrane proteins and lipids and regulates protein transport in the nerve cells [8]. Even though PICK1 knockout mice (KO) were viable and manifested no defects during development [9], male KO mice were infertile and

had globozoospermia [10]. PICK1 is located on proacrosomal vesicles and is involved in transporting the acrosome [11]. A homozygous missense mutation in (G198A) in the thirteenth exon was identified in patients with globozoospermia in China [10].

DPY19L2 is a transmembrane protein specific to testes [12]. In mice, it is localized in the nuclear membrane of the spermatid [13]. It was discovered that globozoospermic patients have homozygous deletion spanning the entire DPY19L2 locus [6, 12]. A large cohort study identified new mutations and deletions in the DPY19L2 locus [14-18]. In addition to that, DPY19L2 knockout mice also had globozoospermia [13]. As such, it is clear that DPY19L2 is one of the primary genes responsible for acrosome formation and its mutations cause the disruption of spermiogenesis in humans.

SPATA16 (previously known as NYD-SP12) is expressed in human testes. It contains a tetratricopeptide repeat (TPR) domain that mediates protein-protein interactions [19]. SPATA16 is localized in the Golgi apparatus and proacrosomal vesicles that merge and form the acrosome during spermiogenesis [20]. Homozygous mutation (848G → A, R283Q) in the fourth exon of the SPATA16 gene was discovered in three brothers with globozoospermia from an Ashkenazi Jewish family. However, this mutation was not found in other 29 globozoospermic patients from Europe and North Africa. These results suggest that SPATA16 is not the leading cause of globozoospermia in humans [21].

Sperm DNA fragmentation index (DFI) is a reliable marker of fertilization and represents the separation or breaking of DNA strands. Its etiology is still unknown, but it is speculated that it has a multifactorial nature. One of the theories suggests that it might be caused by defects of chromatin remodeling, apoptosis, and oxidative processes [22, 23].

DFI equal to or lower than 15% is considered normal; if it is in the range between 15% and 25%, then the results are considered to be undetermined; 25% < DFI < 50% - abnormal, while DFI higher than 50% points at poor sperm DNA integrity.

There are not many publications on the sperm DNA fragmentation index of the patients with total or partial globozoospermia. In this study, we performed a DNA Fragmentation test on a patient with type I globozoospermia using Sperm Chromatin Structure Assay (SCSA) and compared its results after processing the sperm with methods recommended by the WHO laboratory manual for the examination and processing of human semen (5th edition, 2010) – discontinuous density gradients and direct swim-up. SCSA also identifies High DNA Stability (HDS) by measuring the amount of sperm in a semen sample with an increased amount of retained histones due to incomplete protamination [24]. HDS > 25% is thought to influence pregnancy success negatively.

The study aimed to determine and compare DNA fragmentation test results in a patient with a total globozoospermia after several sperm processing methods.

Materials and Methods:

This article describes a case of a male with total globozoospermia. This study examined the DNA Fragmentation Index by Sperm Chromatin Structure Assay (SCSA) of a patient with type I globozoospermia. We compared the test results before and after processing the ejaculate with several sperm processing methods described in the WHO laboratory manual for the examination and processing of human semen (2010) [25].

A married couple came to the “Ecomed-Shymkent” clinic (Shymkent, Kazakhstan) complaining of a possible fertility problem. The couple has been married for 12 years.

The 31-year-old woman had no pregnancies and no surgeries in the anamnesis. Physical, laboratory, and instrumental examinations discovered no diseases or abnormalities. On Day 5 of menstruation, the level of blood hormones was within normal limits: insulin – 11 μ U/ml, prolactin – 217.5 μ U/ml, TTH – 2.0 mU/ml, anti-Müllerian hormone – 0.8 ng/ml. Ultrasound

examination of the pelvic organs discovered no defects. The ovarian reserve was adequate. No sexually transmitted diseases and no blockage of fallopian tubes were registered.

The 47-year-old man had no kids; no surgeries in the anamnesis. His brother had kids. No fertility problems were registered in his immediate family. Physical, laboratory, and instrumental examinations discovered no diseases or abnormalities. Blood hormone levels were within normal limits: FSH – 5.5 mU/ml, LH – 3.5 mU/ml, estradiol – 121.5 pmol/L, testosterone – 20.9 nmol/L, TTH – 0.6 mUI/ml, prolactin – 139.1 mUI/ml, inhibin B – 82.5 pg/ml. The semen analysis revealed the spherical heads without an acrosome in 100% of the sperm cells. The man was diagnosed with total globozoospermia (type I) (Table 1a-d.).

The couple was diagnosed with female infertility associated with male factors (type I globozoospermia) (N97.4).

Table 1a - Results of the microscopic examination of the semen in a man with total globozoospermia (type I), 47 years old

Microscopic examination	Value
Volume	5,8 ml
Viscosity	normal
Color	whitish-gray
Agglutination	no
Aggregation	no
Erythrocytes	no
Leukocytes	0.9 million/ml

Table 1b - Sperm count

Sperm count	Value
Concentration in 1 ml	30 million/ml
Total sperm count in the ejaculate	174 million

Table 1c - Sperm motility

Sperm motility	Value
Class A (rapidly progressive)	3%
Class B (slowly progressive)	22%
Class C (nonprogressive)	47%
Class D (immotile)	28%

Table 1d - Sperm morphology

Sperm morphology	Value
normal morphology, %	0%
acrosome abnormality, %	100%
nucleosome abnormality, %	23%
neck abnormality, %	23%
tail abnormality, %	6%
immature sperm cells, %	4%

Results:

Sperm Chromatin Structure Assay (SCSA) results are provided in Tables 2 and 3.

Table 2 - Sperm DNA Fragmentation test results

Sperm Chromatin Structure Assay	Sperm processing method		
	Native ejaculate	Discontinuous density gradient	Direct swim-up
DNA Fragmentation index	23.08%	24.4%	22.06%

Table 3 – High DNA Stainability index

Sperm Chromatin Structure Assay	Sperm processing method		
	Native ejaculate	Discontinuous density gradient	Direct swim-up
High DNA Stainability (HDS) index	18.89%	16.01%	17.46%

Discussion:

DNA fragmentation index is one of the crucial parameters tested to determine sperm quality. DFI represents the integrity of DNA structure and shows a percentage of sperm in the semen with fragmented DNA. Globozoospermia is a sperm defect caused by the disruption of spermiogenesis that causes the failure to form an acrosome. It leads to the development of round-headed sperm cells that cannot penetrate an ovum. It is thought that this head defect could affect the DNA stability, thus increasing the DNA fragmentation index.

In this study, we determined DFI in a male with total globozoospermia and, contrary to our expectations, test results were not abnormally high, as shown in Table 2. We also wanted to determine whether semen with high motility sperm processed with direct swim-up and density gradient will have lower DFI than the native semen.

Conclusions:

1. Number of studies show a positive correlation between sperm morphology and DNA fragmentation index [24]. We discovered that DFI determined by SCSA in a patient with total globozoospermia was not abnormal despite the head defects.
2. Sperm processing methods (discontinuous density gradient, direct swim-up) did not increase DFI significantly in comparison to the native ejaculate. It leads to the thought that sperm motility should not be the main criterion of sperm selection for intracytoplasmic sperm injection in patients with total globozoospermia. It was previously assumed that due to lack of morphological diversity in spermatozoa (all sperm cells have identical spherical heads), motility could be used as a selection criterion for ICSI [26, 27].
3. Insignificant difference in DFI between density gradient and direct swim-up lets us conclude that these methods have no advantage over each other when processing sperm of patients with type I globozoospermia.

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DOI

ИНДЕКС ФРАГМЕНТАЦИИ ДНК СПЕРМАТОЗОИДОВ У МУЖЧИНЫ С ГЛОБУЛОЗОСПЕРМИЕЙ I ТИПА (ТОТАЛЬНАЯ ГЛОБУЛОЗОСПЕРМИЯ)

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Аннотация

Актуальность: тотальная глобулозооспермия – тяжелая тератозооспермия, приводящая к полному бесплодию. Она является крайне редкой формой патологии сперматозоидов, обусловленная нарушением процессов формирования акросомы.

Цель исследования - определить индекс фрагментации ДНК (DFI) и сравнить результаты теста до и после обработки эякулята несколькими методами.

Методы: В этой статье описывается случай тотальной глобулозооспермии, Мы провели тест на определение индекса фрагментации ДНК методом SCSA до и после обработки семенной жидкости методами, описанными в руководстве ВОЗ по исследованию и обработке эякулята (2010).

Результаты: Результаты SCSA не выявили аномалии в индексе фрагментации ДНК у мужчины с тотальной глобулозооспермией. DFI не изменился значительно после обработки методами прямой swim-up test и градиент плотности.

Заключение: Результаты позволяют сделать вывод, что отсутствие акросомы не оказало значительного влияния на стабильность структуры ДНК сперматозоида у пациента с тотальной глобулозооспермией.

Ключевые слова: Глобулозооспермия I и II типа, образование акросомы, индекс фрагментации ДНК, Sperm Chromatin Structure Assay (SCSA), прямой swim-up test, градиент плотностей

I ТИП ГЛОБУЛОЗОСПЕРМИЯСЫ (ТОЛЫҚ ГЛОБУЛОЗОСПЕРМИЯ) БАР ЕРКЕКТИҢ СПЕРМАТОЗОИД ДНҚ ФРАГМЕНТАЦИЯ ИНДЕКСІ

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Тұжырым

Өзектілігі: Бұл мақала толық бедеулікке алып келетін ауыр тератозооспермия болып табылатын I типті глобулозооспермияға (толық глобулозооспермия) арналған; ол өте сирек кездесетін сперматозоид патологиясы болып табылады және акросоманың құрылу процесстерінің бұзылуымен сипатталады. Зерттеу барысында біз эякулятты өңдеу әдістерін салыстырдық және ДНҚ фрагментация индексін SCSA әдісімен анықтадық. SCSA нәтижелері толық глобулозооспермиясы бар еркекте ешқандай ДНҚ фрагментация индексінің аномалиясын көрсетпеді.

Зерттеу мақсаты - Толық глобулозооспермиясы бар пациентте ДНҚ фрагментация индексін анықтау.

Әдістер: Зерттеу барысында біз эякулятты өңдеу әдістерін салыстырдық және ДНҚ фрагментация индексін SCSA әдісімен анықтадық.

Нәтижелер: SCSA нәтижелері толық глобулозооспермиясы бар еркекте ешқандай ДНҚ фрагментация индексінің аномалиясын көрсетпеді.

Қорытынды: Алынған нәтижелер акросома дефектінің сперматозоид ДНҚ құрылысының тұрақтылығына кері әсер етпейтініне көз жеткіздік.

Түйін сөздер: I және II типті глобулозооспермия, акросома құрылуы, ДНҚ фрагментация индексі, Sperm Chromatin Structure Assay (SCSA), тікелей swim-up test, тығыздық градиенті

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