Research Article

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Semen testis expressed protein 101 and spermatid-specific thioredoxin reductase 3 levels may be biomarkers in infertile male

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Abstract

Objectives: We aimed to determine the differences between normozoospermic and oligozoospermic individuals according to levels of spermatid-specific thioredoxin reductase 3 (SPTRXR3/STRX3/TXNDC8/TXNRD3) and testis expressed protein 101 (TEX-101), and to evaluate the correlations between spermiogram data and biochemical parameters.

Methods: The study was carried out at the Andrology Laboratory of Medicine Faculty of Selçuk University. Two groups were designed: Group 1: Normozoospermia (n=40, sperm concentration ≥ 15 million/mL), Group 2: Oligozoospermia; (n=40, sperm concentration < 15 million/mL). Seminal plasma SPTRXR3 and TEX-101 levels were analyzed with ELISA method. Spermiogram analysis was evaluated according to WHO 2010 Kruger criteria.

Results: TEX-101 protein levels were significantly different in normozoospermia (2.12 ± 0.08 ng/mL) compared to oligozoospermia (1.55 ± 0.04 ng/mL). SPTRXR3 levels (6.98 ± 0.46 ng/mL) were higher in oligozoospermia than normozoospermia (3.07 ± 0.35 ng/mL). Both TEX-101 and SPTRXR3 levels were correlated statistically with most of the spermiogram parameters.

Conclusions: High SPTRXR3 and low TEX-101 levels may be a biomarker in evaluation of male infertility. The

relations between spermiogram parameters indicates that results present a new clinical approach in biology of oligozoospermic male.

Keywords: infertility; male fertility; semen analysis; SPTRXR3; TEX-101.

öz

Amaç: Normozoospermik ve Oligozoospermik bireyler arasındaki farklılıkların Sperm-Spesifik Tiyoredoksin (SPTRX3/STRX3/TXNDC8/TXNRD3) ve Testis Ekspresyon Proteini 101 (TEX-101) düzeylerine göre belirlenmesi ve spermiogram verileri ile biyokimyasal parametreler arasındaki korelasyonların değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Araştırma, Selçuk Üniversitesi Tıp Fakültesi Androloji Laboratuvarı'na gelen kişiler üzerinde gerçekleştirildi. İki grup oluşturuldu: Grup 1: Normozoospermi (n=40, sperm konsantrasyonu 15 milyon/mL), Grup 2: Oligozoospermi; (n=40, sperm konsantrasyonu < 15 milyon/mL). Seminal plazma SPTRXR3 ve TEX-101 seviyeleri ELISA yöntemi ile analiz edildi. Spermiogram analizi WHO 2010 Kruger kriterlerine göre değerlendirildi.

Bulgular: Seminal plazma TEX-101 protein seviyeleri normozoospermik grup (2.12 ± 0.08 ng/mL) ile oligozoospermik grup (1.55 ± 0.04 ng/mL) karşılaştırıldığında önemli düzeyde farklıydı. SPTRXR3 düzeyleri oligozoospermik grupta (6.98 ± 0.46 ng/mL), normozoospermik gruba (3.07 ± 0.35 ng/mL) göre daha yüksekti. TEX-101 ve SPTRXR3 düzeyleri birçok spermiogram parametreleri ile istatistiksel açıdan önemli korelasyon gösterdi.

Sonuç: Yüksek SPTRXR3 ve düşük TEX-101 seviyeleri erkek infertilitesinin değerlendirilmesinde bir biyobelirteç olabilir. Spermiogram parametreleri ile olan ilişkiler, bulguların oligozoospermik erkek biyolojisinde yeni bir klinik yaklaşım sunduğunu göstermektedir.

Anahtar Kelimeler: infertilite; erkek infertilitesi; SPTRXR3; TEX-101; semen analizi.

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Introduction

Today, approximately 15% of couples have infertility problems, and half of this problem is seen only in male individuals. About 10–15% of the couples are identified as infertile. Unexplained infertility is a complex case and there is still no outcome for nearly 1/3 of the infertilities. Unexplained infertile males do not present any previous history of diseases that leads to infertility. They have normal findings on physical examination and genetic, endocrine, and biochemical laboratory testing [1, 2]. The only known truth is; a significant reduction in the number of spermatozoa is detected in the last 50 years.

In recent years, biochemical mechanisms that possibly lead to male infertility and the development of new therapeutic approaches are being started to survey. The main studies show that oxidative stress in the male sperm nucleus leads to DNA damage. DNA damage may be induced in the nuclear and mitochondrial genomes. Therefore, the spermatozoa retain their fertilization capacity. This is a significant finding since meaning that spermatozoa are capable of passing damaged genes onto the embryo [3, 4].

Studies on redoxins and redox regulation take importance in sperm biology [5]. Redox homeostasis is maintained by thioredoxin and glutathione/glutaredoxin systems in the organisms [6]. In somatic cells, elevated levels of ROS are prevented by the presence of a complex enzymatic antioxidant system involving superoxide dismutase (SOD), and catalase (CAT), glutathione peroxidases (GPXs), peroxiredoxins (PRDXs). They reduce both organic and inorganic hydroperoxides and peroxynitrite by coupling with the TRX system [7, 8].

The thioredoxin system consists of thioredoxin reductase (TRXR/TrxR) and its main substrate, Trx/TRX (thioredoxin). TrxR, is a selenite-reducing enzyme, plays a central role in selenium pathophysiology [9]. The selenol group of TrxR acts as a primary sensor for mutagenic H_2O_2 and initiates a signal cascade leading to the transcription of genes encoding antioxidative proteins, then by play a role in protection against oxidant injury [10–12].

Three isoenzymes of Trx are identified; TrxR-1 is found in the cell cytoplasm, TrxR-2 is located in mitochondria, and TrxR-3 is expressed in testis of the mammalian [13]. Tissue-specific Trx such as SPTRX-1 and -2 are expressed in male germ cells with an important role in spermatogenesis [14]. Mammalian germ cells are endowed with Trx which is a specific structure of spermatid and sperm tail. SPTRX-3 is the first thioredoxin specific to the Golgi apparatus, and its function might be related to the post-translational modification of proteins required for acrosomal biogenesis [9].

Interestingly, whereas SPTRX-3 could not be detected in completely differentiated normal spermatozoa of various animal species, it is retained in the superfluous cytoplasm and is wrapped around the sperm tail mid pieces in morphologically abnormal human spermatozoa [9, 15].

TEX-101 is a cell membrane protein expressed only by testicular germ cells and spilled into seminal plasma [16, 17]. Fujihara et al. [18] showed that males with disrupted TEX-101 gene produced normal-looking but fertilization-incompetent spermatozoa.

Regarding all previous findings, TEX101 may reflect the number of the fertile sperms and the function of sperms. Whereas TEX-101 involves in acrosome reactions, SPTRXR3 is an important protein involves in fertilization during the capacity process.

Based on the idea, these protein analyses may be useful in evaluation as a biochemical parameter in unexplained male infertility. Seen from this aspect, the levels of TEX-101 were analyzed to determine the relations between spermogram and infertility status. and figure out whether TEX-101 gives some hints in morphologically sperm anomaly rates. In addition, SPTRXR3 was analyzed in a sample of normospermic and oligozoospermic individuals to elucidate the biochemical mechanism, and to assess the correlation between spermogram data.

Materials and methods

Individuals

The present study was carried out on semen samples taken from volunteers aged 18–44 years who were admitted to the Andrology Laboratory of Medicine Faculty at Selcuk University. This study was approved by Selcuk University Faculty of Medicine Clinical Research Ethics Committee (No: 2018/103, decision date: 21/03/2018). All the volunteers signed the “Informed Patient Consent Form” as written informed consent conformably to the ethical standards and the Declaration of Helsinki Principles.

The study was composed of two groups:

- Group 1 (n=40): Normozoospermia individuals with a mean age of 31.63 ± 2.15 years old (sperm concentration ≥ 15 million/mL)
- Group 2 (n=40): Oligozoospermia individuals with a mean age of 29.33 ± 1.65 years old (sperm concentration < 15 million/mL)

Individuals treated with any medication, chronic illness, azospermic, alcohol consumers, and smokers were excluded from the study.

Collection of sperm samples, storage, and evaluation of spermogram: The semen samples obtained by masturbation at the hospital after 2–6 days of abstinence were collected in a special sterile plastic container. For liquefaction, samples were incubated at 37 °C for 20 min and evaluated within 1 h of collection. Sperm parameters were

assessed according to the World Health Organization 2010 criteria (semen volume 1.5 mL; sperm concentration 15 million/mL; total sperm count 39 million; total sperm motility 40%; progressive sperm motility 32% A ± B; and sperm morphology 4% Kruger criteria) and the results of those were recorded.

Smear preparations from semen samples were prepared and stained with 'Spermac' stain for morphological evaluations. The percentages of normal and abnormal spermatozoa forms were determined by scoring at least 100 spermatozoa per preparation. Spermio-gram analyzes were performed at Medical Faculty Andrology Laboratory within Selcuk University.

Biochemical analysis: For the biochemical analysis, no extra semen samples were taken from the individuals. The remaining semen samples were used for SPTRXR3 and TEX-101 analysis after analyzing of spermio-gram. Semen samples were centrifuged at 1,000 rpm for 20 min. The supernatants were removed to Eppendorf tubes to be stored at -80 °C until for analysis.

Seminal plasma SPTRXR3 (Mybiosource, cat no: MBS9341730) and TEX-101 (Mybiosource, cat no: MBS7606254) concentrations performed by the commercial ELISA test kit. For the analysis, Elisa Reader BMG LABTECH (Germany) and Elisa Washer as Rayto Micro-plate RT-2600washer (China) were used. Semen analysis results were calculated as ng/mL. Biochemical analyzes were performed at Medical Biochemistry and Physiology Research Laboratories within Selcuk University.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (20.0 Data Analysis SPSS) program. The results were described as mean ± SD. The Shapiro-Wilk test was used for comparison and it was checked whether the normal distribution was observed. Independent *t*-test was applied for a *p*-value greater than 0.05, Mann-Whitney *U* test was applied for the value of *p*<0.05. To evaluate the correlations of the parameters, the Pearson correlation coefficient was used for the *p*-value greater than 0.05, whereas the correlation coefficient of Spearman was used for *p* values less than 0.05.

Results

The spermio-gram results of the groups are shown in Table 1. The mean age of the individuals in group 1 is 31.63 ± 2.15 years old and in group 2 is 29.33 ± 1.65 years old (*p*>0.05).

As stated in below Table 2, the levels of TEX-101 (2.12 ± 0.08 ng/mL) in Normozoospermic individuals were significantly higher than those of the Oligozoospermic (1.55 ± 0.04 ng/mL) individuals (*p*=0.000). Furthermore, SPTRXR3 showed statistically significant higher levels in Oligozoospermic individuals (6.98 ± 0.46 ng/mL) than in Normozoospermic individuals (3.07 ± 0.35 ng/mL) (*p*=0.000).

Correlation coefficient values and *p* values of all group has been shown in Table 3. Regarding table III, TEX-101

Table 1: Sperm parameters and incidences of morphologic anomalies in normozoospermia and oligozoospermia groups (mean ± SD).

Semen parameters	Normozoospermia (n=40)	Oligozoospermia (n=40)
Age (years old)	31.63 ± 2.15	29.33 ± 1.65
Volume, mL	3.73 ± 0.45	4.35 ± 0.47
Concentration, million/mL	53.80 ± 6.81	4.47 ± 1.17
Total number, million	195.76 ± 30.74	19.68 ± 5.59
Total motility, %	70.48 ± 4.31	57.30 ± 5.65
Progressive motility, %	56.63 ± 4.49	39.05 ± 5.12
Non progressive motility, %	14.21 ± 1.36	18.25 ± 2.52
Immotility, %	29.53 ± 4.31	43.20 ± 5.88
TPMSS, million	118.07 ± 20.83	8.45 ± 2.89
Normal morphology, %	2.25 ± 0.21	1.38 ± 0.16
Head anomaly, %	88.60 ± 0.59	91.00 ± 0.50
Amorphous head, %	76.93 ± 1.13	79.25 ± 1.40
Acrosomal vacuole	5.90 ± 0.38	6.00 ± 1.86
Nuclear vacuole	2.85 ± 0.63	3.25 ± 0.77
Round head	6.95 ± 0.52	8.63 ± 1.72
Pin head	6.95 ± 2.15	7.25 ± 0.32
Large head, %	4.35 ± 0.72	5.50 ± 1.49
Small head, %	2.33 ± 0.51	2.13 ± 0.56
Long head, %	4.03 ± 0.91	3.63 ± 0.74
Multiple head, %	0.98 ± 0.55	0.50 ± 0.23
Neck-middle piece anomaly, %	12.53 ± 0.76	16.88 ± 1.01
Cytoplasmic droplet	3.68 ± 7.97	5.00 ± 0.68
Neck fracture	2.50 ± 0.77	3.50 ± 0.55
Segmental mitochondrial aplasia	3.75 ± 1.16	4.88 ± 0.93
Tail anomaly, %	14.18 ± 1.08	13.75 ± 0.87
Double tail, %	1.35 ± 0.37	1.13 ± 0.48
Tail stump, %	0.38 ± 0.19	0.38 ± 0.23
Dag defect, %	6.25 ± 0.78	6.38 ± 0.76
Long tail, %	0.35 ± 0.21	0.43 ± 0.24
Short tail, %	5.90 ± 0.55	5.50 ± 0.47
Teratozoospermia index	1.27 ± 0.015	1.31 ± 0.01

Statistical analysis was performed using the SPSS 20.0 program. Results were described as mean ± SD.

Table 2: TEX-101 and SPTRXR-3 values of the groups.

Groups	TEX-101, ng/mL	SPTRXR-3, ng/mL
Group 1 (Normozoospermia)	2.12 ± 0.08*	3.07 ± 0.35*
Group 2 (Oligozoospermia)	1.55 ± 0.04	6.98 ± 0.46

*Mann-Whitney *U* test, Results were described as mean ± SD;

**p*=0.000 ≤ *α*=0.05.

levels were correlated statistically significant with concentration (*r*=0.693), total number (*r*=0.710), total motility (*r*=0.495), progressive motility (*r*=0.528), non progressive motility (*r*=-0.221), immotility (*r*=-0.496), TPMSC (*r*=0.695),

Normal Morphology ($r=0.347$), and teratozoospermia Index ($r=-0.357$). In addition to SPTRXR-3 levels were significantly correlated with sperm volume ($r=0.227$), concentration ($r=-0.706$), total number ($r=-0.690$), total motility ($r=-0.424$), progressive motility ($r=-0.456$), immotility ($r=0.424$), TPMSC ($r=-0.665$), normal morphology ($r=-0.412$), and Teratozoospermia Index ($r=0.301$).

Discussion

In this present study, TEX-101 and SPTRXR3 levels were analyzed, which are thought to be effective in unexplained infertility of the male. We affirmed that these proteins may

be a new potential aspect in male infertility, that most of the researchers also figure out important roles in spermatogenesis and fertilization.

TEX-101 is one of the proteins bound to GPI (glycosylphosphatidylinositol) and is separated from the sperm surface and released into the seminal fluid [19]. Although there is evidence about TEX-101 enzymatically spill from the epididymal sperm surface [20], the enzyme involved in this process is not clearly known until recently.

Interestingly GPI-linked protein is expressed in testicular sperm membrane as testicular angiotensin-converting enzyme (tACE), and then by epididymal maturation occurs in the sperm membrane. tACE not only affects the catalysis of GPI-bound proteins from the sperm surface and also has roles on egg-sperm binding ability during fertilization. These findings led to the assumption that epididymal passage of tACE is responsible for the release of TEX-101 [21, 22]. Nagdas et al. [23] revealed the presence of TEX101 protein in both testicular and epididymal sperm plasma membrane of bovine.

In the present study, we found strong correlations between semen analysis and seminal plasma TEX-101, and a significant difference between the groups. Our findings are consistent with the supports of the studies mentioned above. TEX-101 values were 2.12 ± 0.08 ng/mL in Normozoospermia individuals and were higher than the levels of Oligozoospermia (1.55 ± 0.04 ng/mL) group. Based on these considerations, we can imply TEX-101 have strong relations with infertility status.

As seen in Table 3, when we assessed the relationship between TEX-101 concentrations in semen parameters in the total group, we found statistically important positive correlations between sperm concentration, total number, total motility, progressive motility, TPMSC and normal morphology. There were negative correlations between non-progressive motility, immotility, teratozoospermia index and TEX-101 levels. These results emphasize the TEX-101 levels may be important in the evaluation of, especially motility of the sperm. As the TEX-101 levels increase in semen, the percentage of sperm with normal morphology increases, while teratozoospermia index, that is, the number of morphological anomalies per sperm, decreases. When we compare the TEX-101 concentrations of both groups, there were approximately two-fold differences between each group. Therefore, evaluation of TEX-101 levels in semen may give some hints in morphologically sperm anomaly rates.

Several researchers analyzed seminal plasma protein levels. It is well known that seminal plasma is a mixture of secretion of male gland including testes, epididymis, prostate, seminal vesicles, Cowper's and expresses various

Table 3: Correlations between sperm parameters and TEX-101 and SPTRXR-3 in total group.

Semen parameters (n=80)	TEX-101		SPTRXR-3	
	(r)	(p)	(r)	(p)
Volume, mL	-0.131	0.248	0.227	0.043*
Concentration, million/mL	0.693	0.000**	-0.706	0.000**
Total number, million	0.710	0.000**	-0.690	0.000**
Total motility, %	0.495	0.000**	-0.424	0.000**
Progressive motility, %	0.528	0.000**	-0.456	0.000**
Non progressive motility, %	-0.221	0.049*	0.196	0.081
Immotility, %	-0.496	0.000**	0.424	0.000**
TPMSC, million	0.695	0.000**	-0.665	0.000**
Normal morphology, %	0.347	0.016**	-0.412	0.004**
Head anomaly, %	-0.272	0.061	0.261	0.073
Amorphous head, %	-0.143	0.333	-0.053	0.722
Acrosomal vacuole	-0.084	0.572	-0.072	0.626
Nuclear vacuole	0.099	0.503	-0.115	0.436
Round head	-0.075	0.613	0.261	0.073
Pin head	0.156	0.289	-0.052	0.725
Large head, %	-0.098	0.509	0.139	0.347
Small head, %	0.050	0.737	0.078	0.601
Long head, %	0.079	0.591	0.005	0.975
Multiple head, %	0.106	0.473	-0.077	0.601
Neck-middle piece anomaly, %	-0.260	0.071	0.254	0.078
Cytoplasmic droplet	-0.135	0.362	0.095	0.519
Neck fracture	-0.274	0.060	0.174	0.237
Segmental mitochondrial aplasia	-0.037	0.802	-0.028	0.852
Tail anomaly, %	0.124	0.402	0.010	0.948
Double tail, %	-0.011	0.938	-0.009	0.952
Tail stump, %	-0.166	0.259	-0.038	0.798
Dag defect, %	-0.106	0.472	-0.126	0.393
Long tail, %	-0.250	0.091	0.110	0.460
Short tail, %	0.031	0.832	0.130	0.378
Teratozoospermia index	-0.357	0.013*	0.301	0.038*

Pearson correlation coefficient was used for the $p \geq 0.05$, whereas the correlation coefficient of Spearman was used for $p \leq 0.05$. * $p \leq 0.05$, ** $p \leq 0.001$.

proteins. Seminal plasma includes strong potential biomarker. TEX-101 amount is closed to zero in cases that have no sperm production. The amount of TEX-101 above a certain threshold is an important finding for the presence of mature sperm cells in the testis [24, 25]. In their study, they found just a correlation between total sperm count and TEX-101 ($r=0.83$, $p<0.0001$) [25]. Our findings are consistent with Drabovich et al.'s study. We also found a positive correlation ($r=0.710$, $p=0.000$) as it is shown in Table 3.

Nonetheless, redox regulation is important in the physiology of normal spermatogenesis. It is proven that the redox regulation have a role in some spermatogenetic anomalies. The ability to counteract acute ROS increase in living systems is attempted via achieved by the use of chemical antioxidants present in the cell as a first process [7]. ROS production is related to sperm process, including maturation, capacitation, hyperactivation, acrosome reaction [26, 27].

Smith et al., indicated that the combined inactivation of these thioredoxin domain-containing protein's (Txndc) isoforms did not have an overall impact on spermatogenesis, epididymal sperm maturation, or fertility [28]. However, Txndc deficiency in spermatozoa did lead to age-dependent changes as reflected by accelerated motility loss, high rates of DNA damage, increases in ROS, and impaired protamination of the sperm chromatin. The researchers suggest the sperm-specific thioredoxins are critically important in protecting these cells against the increases in oxidative stress associated with paternal age.

Buckman et al. [15] suggested 51% of infertile males and 20% of men from couples with identified as unexplained infertile; idiopathic infertile have high SPTRX3 levels (>15% SPTRX3-positive spermatozoa). In addition, 14% of men from couples previously diagnosed with female-only infertility have high SPTRX3 levels. They indicated that couples with high SPTRX3 levels lead to fewer two-pronuclear zygotes and have a decreased pregnancy rate [15].

Our findings have indicated that oligozoospermia individuals have significantly higher concentrations of SPTRX3 compared to normozoospermia. SPTRX3 concentration of oligozoospermic subjects (6.98 ± 0.46 ng/mL) has been two times fold higher than normozoospermic individuals (3.07 ± 0.35 ng/mL) ($p=0.000$). The presence of high amounts of SPTRX3 in semen is important in the absence of sperm and insufficiency of sperm capacity for fertilization. Whereas low SPTRX3 protein levels were shown directly proportionally to fertility, high levels were related to infertility. There is no study up to date in which we can compare our results about the correlations of all sperm parameters and SPTRX3. So that, the present study

is important due to the findings of sperm parameters' correlations.

According to Table 3, the positive relationship between SPTRX3 concentrations and semen Volume, sperm immotility, and Teratozoospermia Index were statistically significant. There were negative correlations between sperm concentration, total number, total motility, progressive motility, TPMSC, normal morphology, and SPTRX3 levels. Therefore, SPTRX3 levels show opposite findings compared to TEX-101 levels. Increased SPTRX3 levels may be indicative of sperm rates in semen with one or more morphological abnormalities.

SPTRX3 is a unique marker because it is a well-characterized, germline-specific protein with a different localization pattern in spermatozoa. Measurement of SPTRX3 levels is thought to confirm the clinical diagnosis in male infertility and to reveal undiagnosed male infertility in idiopathic infertility [29].

Conclusion

Based on this information, we attempted to elucidate the possible relationships between the mechanisms of Thioredoxins and the levels of TEX-101, which are unknown and unexplained biochemical pathways in infertility. Determination of TEX-101 and SPTRX3 concentrations supports that they are important parameters in infertile males.

As a result of the study, it is noteworthy that the TEX-101 protein is functional in semen and directly proportional to the presence of sperm in sufficient capacity for fertilization. We believe that our findings suggest that SPTRX3 is related to the production of abnormal sperms and, that the biochemical mechanisms leading to male infertility are related to those pathways. We think these protein's correlations with semen parameters are important. In addition, conducting new studies on the definition of thioredoxin and thioredoxin reductase in relation to male reproductive will give new sights for future studies.

There is a limitation in this study that could be addressed in future research; data of biochemical analysis may be supported by immunohistochemical studies of seminal plasma in order to clarify these mechanisms.

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