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The Correlation Between Semen Parameters and Galectin-3 Levels of Infertile Men

İnfertil Erkeklerde Semen Parametreleri ve Galektin-3 Seviyelerinin İlişkisi

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ABSTRACT

Objective: Galectin-3 (Gal-3) is a ~30kDa protein which plays role in cell to cell interaction, cancer progression, pathogenesis of infections, and immunmodulation. In male reproductive tract, Gal-3 is present in testes, epididymis, vas deferens, prostate, seminal vesicles and in semen. **Material and Methods:** This is a prospective cohort study performed in a university hospital setting. Inclusion criteria were males suffering from infertility who admitted for semen analysis. Gal-3 analysis in semen was performed by chemiluminescent microparticle immunoassay. The semen parameters were evaluated according to World Health Organization criteria (WHO) (2010). Normozoospermia were defined as sperm count ≥15x10^6/ml and oligozoospermia were defined as sperm count <15x10^6/ml. **Results:** The mean age of the patients are 34.67±5.43 (min 24-maks 52) years. Among the participants, 16,4% had oligozoospermia and 83,5% had normozoospermia. The mean Gal-3 levels of patients were 216 ng/mL (min 8,6-max 794 ng/mL), where as the levels were 162 ng/mL in oligozoospermia and 90 ng/mL in normozoospermia group (p>0.05). The Gal-3 levels were found to be negatively correlated with total progressive sperm count in oligozoospermia group (r=-0.479; p=0.024). **Conclusion:** The findings from this study supports the data that Gal-3 levels are increased in cases with faulty spermatogenesis. Moreover, the possible role of Gal-3 in sperm motility is also supported by the negative correlation between Gal-3 levels and total progressive motile sperm fraction. Further studies about this protein to clarify its potential role in spermatogenesis and sperm functions is still needed.

Keywords: Galectin-3; infertility; oligozoospermia

ÖZET

Amaç: Galektin-3 (Gal-3) hücre etkileşimleri, kanser progresyonu, bulaşıcı organizmaların patogenezi ve immunomodulasyonda rol oynayan yaklaşık 30kDa'luk bir proteindir. Erkek üreme sisteminde; , testis, epididimis, vaz deferens, prostat, seminal vezikül ve semende tespit edilmiştir. **Gereç ve Yöntemler:** Bu araştırma bir üniversite hastanesi ortamında gerçekleştirilen prospektif bir kohort çalışmasıdır. İnfertilite şikayeti ve mevcut semen analizi ile başvuran erkekler çalışmaya alındı. Semende Gal-3 analizi, kemilüminesans mikropartikül immünassay testi ile gerçekleştirildi. Semen parametreleri Dünya Sağlık Örgütü kriterlerine (WHO) (2010) göre değerlendirildi. Normozoospermi sperm sayısı ≥15x10^6/ml, oligozoospermi ise sperm sayısı <15x10^6/ml olarak tanımlandı. **Bulgular:** Hastaların ortalama yaşı 34,67±5,43 (min 24-maks 52) yıldır. Katılımcıların %16,4'ünde oligozoospermi ve %83,5'inde normozoospermi vardı. Hastaların ortalama Gal-3 seviyeleri 216 ng/mL (min 8,6-maks 794 ng/mL) iken, seviyeler oligozoospermide 162 ng/mL ve normozoospermi grubunda 90 ng/mL idi (p>0,05). Oligozoospermi grubunda Gal-3 seviyeleri ile total progresif sperm sayısı arasında negatif korelasyon bulundu (r=-0,479; p=0,024). **Sonuç:** Bu çalışmanın bulguları, hatalı spermatogenezli olgularda Gal-3 düzeylerinin yükseldiği bilgisini desteklemektedir. Dahası, Gal-3'ün sperm hareketliliğindeki olası rolü, Gal-3 seviyeleri ile toplam progresif hareketli sperm fraksiyonu arasındaki negatif korelasyonla da desteklenmektedir. Bu protein hakkında spermatogenez ve sperm fonksiyonlarındaki potansiyel rolünü açıklığa kavuşturmak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Galektin-3, infertilite, oligozoospermi

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Galectins are a family of lectins, which binds β-galactosides on glycoprotein and glycolipids.[1] Up to day, 15 different types of galectins are documented in mammalian tissues.[2] According to cell localisation, concentration and post-translational modifications, galectintypes can show different characteristics. Galectin-3 (Gal-3) is the most commonly documented type.[3] Gal-3 is a~30kDa protein which plays role in cell to cell interaction, cancer progression, pathogenesis of infections, and immunmodulation.[4] In male reproductive tract, Gal-3 is present in testes, epididymis, vas deferens, prostate, seminal vesicles and in semen.[4]

In animal models and in humans the expression of Gal-3 is documented in testes in Sertoli cells.[5] The secretion of Gal-3 is under hormonal control mainly by FSH.[6] Nevertheless, the function of Gal-3 is not clearly documented yet. The previous data showed that the secretion of Gal-3 is triggered one month after germ cell death.[6] This indicates that Gal-3 might have a potential pro-survival role of Gal-3 in the testes as suggested by Deschildre et al. previously.

In this study, we aimed to document Gal-3 levels in semen of infertile males for the first time in the literature. The correlation between Gal-3 and semen parameters were also analyzed.

MATERIAL AND METHODS

This is a prospective cohort study performed in a university hospital setting between June 2017 and December 2018. All the patients who accepted to participate signed the informed consent. Etlik Zubeyde Hanim Women's Health Training and Research Hospital, Institutional Review Board granted ethical approval for the study (Date: 15.06.2017, Number: 2017/5). In clusion criteria were males suffering from infertility who admitted for semen analysis. The patients with chronic medical diseases, any abnormality in physical examination, azoospermia, clinical varicocele, urogenital infections, history of cryptorchidism, heavy smokers (>20 cigarattes/day) were excluded. Semen samples were obtained by masturbation after 3 to 5 days sexual abstinence.

After liquefaction in room temperature, seminal plazma were seperated and stored at -80° until analyses for Gal-3. Gal-3 analysis in semen was performed by chemiluminescent microparticle immunoassay based detection via a commercially available kit (Architect Galectin-3 Assay, Abbott) in optic system (ARCHİTECT iSystem, U.S.A. Philadelphia, Malwern).

The semen parameters were evaluated by Macler chamber according to World Health Organizationcriteria (WHO) (2010). Normozoospermia were defined as sperm count ≥15x10^6/ml and oligozoospermia were defined as sperm count <15x10^6/ml. In addition, using strict morphology criteria to evaluate sperm morphology, 4% was accepted as the lower limit of normal.

STATISTICAL ANALYSIS

Data analysis was performed using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Continuous data were described as mean ± SD (Standard deviation) for normal distributions, and median (minimum and maximum value) forskewed distributions. Categorical data were described as number of cases (%). Mean differences between groups were compared by Student's t test whereas Mann Whitney U test was applied for comparison of median values. Nominal data was analysed by Pearson's chi-square or Fisher'sexact test, where applicable. The correlation between the parameters were evaluated by Spearman's rho correlation analysis. A p value less than 0.05 were considered statistically significant.

RESULTS

During the study period 152 patients were eligible for the study. The mean age of the patients were 34.67±5.43 years (Table 1). The semen parameters of the participants are given in Table 1. The mean (±sd) Gal-3 levels of the patients were 216,19 (±153,17) ng/ml [median 178,45 (min 8,6-max 794,4) ng/ml]. According to WHO criteria 25 cases (16,4%) had oligozoospermia and 127 (83%) had normozoospermia. The meanage of the patients with oligozoospermia were 34.87±5.21years whereas the meanage of the normozoospermia group were 33.62±6.55years (p=0,336). The oligozoospermia and normozoospermia were compared for semen parameters and Gal-3 levels as well (Table 2).

TABLE 1: The age and semen parameters of the patients.

Total patients (n)		152
Normozoospermia (n,%)		127 (83,55)
Oligozoospermia (n,%)		25 (16,44)
	Mean (±sd)	**Median (min-max)**
Age (years)	34,67 (5,43)	34,0 (24-52)
Ejaculate Volume (ml)	3,15 (1,14)	3,0 (1-6)
Sperm count (10^6/ejaculate)	169,0 (138,25)	127,75 (3,4-715)
Sperm count (10^6/ml)	53,85 (41,29)	46.0 (1,2-156)
Motility (%)	59,93 (18,85)	65,5 (0-87)
Normal Morphology (%)	3,35 (2,33)	3,0 (0-10)
Progressive motile sperm (%)	13,45 (9,08)	13,0 (0-40)
TPMSC (10^6 /ejaculate)	28,12 (34,99)	15,18 (0-214)

TPMSC: Total progressive motile sperm count.

The Spearman's rho analysis were used to discriminate the semen parameters correlated with Gal-3 levels. The results showed that none of the paremeters were correlated with Gal-3 levels when all participants were included in theanalysis (Table 3, p>0.05). When oligozoospermia group were analysed seperately, the percentage of progressive motile sperm were found to be negatively correlated with Gal-3 levels (Table 3, Figure 1).

DISCUSSION

For the first time in the literature, Gal-3 levels in semen of infertile males are documented in this study. Although not statistically significant, Gal-3 levels of oligozoospermia group is much lower than normozoospermia group. As the infertile males with normozoospermia and oligozoospermia constituted the studygroups, the correlation analyses of Gal-3 with semen parameters were also performed. In subgroup analyses of oligozoospermia group, total progressive motile sperm count was found to be negatively correlated with Gal-3 levels.

The expression of Gal-3 is under control of mature germ cells.[6] In animal models, in rat testes, Gal-3 levels are increased when spermatocytes are depleted.[6] The findings from this study supports this data as Gal-3 levels are increased in cases with faulty spermatogenesis. In our study, in cases with oligozoospermia Gal-3 levels were much higher than normozoospermia group but this was not statistically significant (162,2vs90,0 ng/ml). We infer that significantly elevated levels of Gal-3 might be documented if studied in larger populations. This is a preliminary data about this protein where neither the levels nor the function is clear yet. However, there sults form this study confirms the need for further studies about this protein and also the possible compensatory role in spermatogenesis.

Prostatic fluid and seminal plasma contain secretory granules called prostasomes.[7-9] These are also found in epithelial cells of prostate gland as extracellular vesicules.[7-9] Gal-3 was previously documented in seminal plasmaand in prostasomes found on spermatozoa.[4] The prostasomes regulate the capacitation of spermatozoan, induce acrosome reaction and stimulate sperm motility.[4] Prostasomes fuse with sperm in vitro and the delivery of intra-prostasomal calcium stores to spermatozoa increases sperm motility.[4] The data from our study also supports that Gal-3 might have a role in sperm motility as levels were found to be negatively correlated with total progressive motile sperm fraction. Unfortunately, the study by Jones et al. about Gal-3 and prostasomes did not report the semen parameters or the correlation of Gal-3 with

TABLE 2: The semen parameters and Gal-3 levels of oligozoospermia and normozoospermia groups.

	Oligozoospermia (n:25)	**Normozoospermia (n:127)**	**p**
Ejaculate Volume (ml) Mean (±sd)	2.88±1.01	3.21±1.16	0.207
Sperm count (10^6/ml) Median(min-max)	8 (1.2-14)	56 (15-156)	<0.001*
Motility (%) Median(min-max)	50 (0-80)	67 (9-87)	0.002*
Normal Morphology (%) Median(min-max)	1 (0-6)	4 (0-10)	0.002*
TPMSC Median (min-max)	0.54 (0-8.40)	18.60 (0-214.5)	<0.001*
Galektin-3 Median (min-max)	162.20 (12.5-547.1)	90.00 (7-186)	0.386

TPMSC: Total progressive motile sperm count; *p<0.05: statistically significant.

TABLE 3: The results of correlation analysis between Gal-3 levels and semen parameters in oligozoospermia group and all cases.

	Spearman's rho			
	Gal-3 oligozoospermia		Gal-3 all cases	
	R	P	R	P
Age (Years)	0.084	0.726	0,008	0,932
Ejaculate Volume (ml)	0.019	0.931	-0,081	0,336
Sperm count (10^6/ml)	-0.153	0.475	-0,004	0,961
Motility	-0.322	0.126	-0,018	0,828
Normal morphology	-0.194	0.471	-0,079	0,396
Progressive motile sperm	-0.479	0.024*	-0,030	0,723
TPMSC	-0.395	0.069	-0,035	0,684

TPMSC: Toplam progresif motil sperm sayısı; *p<0.05: statistically significant.

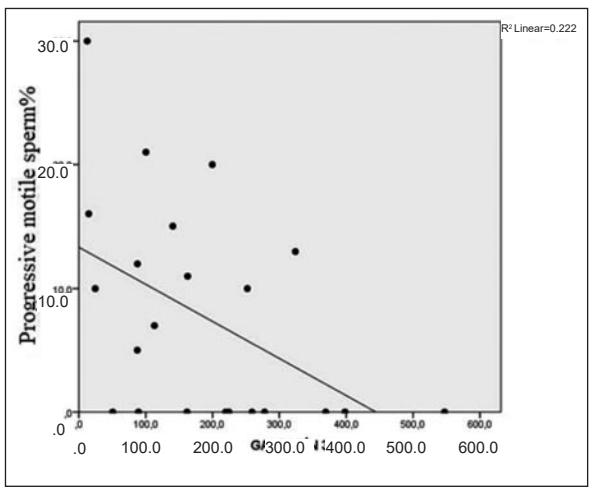

FIGURE 1: The result of Spearman's rho correlation analysis for percentage of progressive motile sperm and Galectin 3 (ng/mL) levels.

semen parameters.[4] But all these data indicate that Gal-3 in prostasomes or in semen might have a regulatory role in sperm motility.

CONCLUSION

In conclusion, the data about the role of Gal-3 in fertility potential of males is very limited. The low number of participants in this study is a limitation to draw strong conclusions. However, the preliminary findings obtained from here are valuable for further research about male infertile populations.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Gamze Sinem Çağlar, Semih Tangal;* ***Design:*** *Gamze Sinem Çağlar, Emre Göksan Pabuçcu;* ***Control/Supervision:*** *Gamze Sinem Çağlar, Metin Yığman;* ***Data Collection and/or Processing:*** *Pelin Menteşoğlu, Emre Göksan Pabuçcu;* ***Analysis and/or Interpretation:*** *Pelin Menteşoğlu, Semih Tangal;* ***Literature Review:*** *Pelin Menteşoğlu;* ***Writing the Article:*** *Pelin Menteşoğlu, Metin Yığman;* ***Critical Review:*** *Gamze Sinem Çağlar, Semih Tangal.*

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