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·*Original Article*·

Assessment of seminal plasma laminin in fertile and infertile men

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Abstract

Aim: To assess laminin levels in the seminal plasma of infertile and fertile men, and to analyze the correlation of laminin levels with sperm count, age, sperm motility and semen volume. **Methods:** One hundred and twenty-five recruited men were equally divided into five groups according to their sperm concentration and clinical examination: fertile normozoospermia, oligoasthenozoospermia, non-obstructive azoospermia (NOA), obstructive azoospermia (OA) and congenital bilateral absent vas deferens (CBAVD). The patients' medical history was investigated and patients underwent clinical examination, conventional semen analysis and estimation of seminal plasma laminin by radioimmunoassay. **Results:** Seminal plasma laminin levels of successive groups were: 2.82 ± 0.62, 2.49 ± 0.44, 1.77 ± 0.56, 1.72 ± 0.76, 1.35 ± 0.63 U/mL, respectively. The fertile normozoospermic group showed the highest concentration compared to all infertile groups with significant differences compared to azoospermic groups ($P < 0.05$). Testicular contribution was estimated to be approximately one-third of the seminal laminin. Seminal plasma laminin demonstrated significant correlation with sperm concentration ($r = 0.460$, $P < 0.001$) and nonsignificant correlation with age ($r = 0.021$, $P = 0.940$), sperm motility percentage ($r = 0.142$, $P = 0.615$) and semen volume ($r = 0.035$, $P = 0.087$). **Conclusion:** Seminal plasma laminin is derived mostly from prostatic and testicular portions and minimally from the seminal vesicle and vas deferens. Estimating seminal laminin alone is not conclusive in diagnosing different cases of male infertility. *(Asian J Androl 2007 Jan; 9: 63–67)*

Keywords: male infertility; semen; seminal plasma; testis; basement membrane; laminin; azoospermia; congenital bilateral absent vas deferens

1 Introduction

The interaction between different cell types within a specific organ has an important role in maintenance and control of both tissue function and growth. Numerous types of cell-to-cell interactions have been classified into environmental, nutritional and regulatory types. Environmental interactions are mediated by the adjacent cell type through components such as extracellular matrix and cell adhesion molecules [1, 2].

Laminin, the most abundant glycoprotein in the basement membrane, is both a structural and a biological ac-

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tive component. It is found in significant quantities in the basement membrane, the thin extracellular matrices that surround the epithelial tissues, nerves and fat cells as well as the smooth, striated and cardiac muscles. All basement membranes contain a common set of proteins includings laminin, collagen IV and various heparin sulphate proteoglycans. In fact, the basement membrane is the first extracellular matrix to appear during embryogenesis and laminin is the first matrix protein to be detected [3]. Laminin is a multi-domain protein with a molecular mass of 800–1 000 kDa. It is composed of three polypeptide chains connected by disulphide bonds [4].

In the testis, the environmental interactions between peritubular cells and Sertoli cells are mediated through a complex extracellular matrix of the basement membrane. The seminiferous tubules are surrounded by lamina propria, which is composed of basement membrane and outer three to six layers of myoid cells, connective tissue and fibroblasts, and the outmost one or two layers are mainly composed of fibroblasts. Xi *et al*. [5] showed that type I and IV collagens and laminin are present in the basement membrane that increases by 50% in the aged. Maekawa *et al.* [6] demonstrated that laminin is produced by Sertoli cells. By using immunohistochemical techniques, laminin has been localized to the epithelial basement membrane, peritubular cell layer and Sertoli cells [7, 8]. Glander *et al*. [9] showed that ejaculated spermatozoa and spermatogenic cells express laminin with an extended intra/inter-individual variation and with different patterns of location. Geipel *et al.* [10] revealed that laminin is elevated in the seminal fluid in comparison to its serum levels and that it exhibits positive correlations with sperm concentrations, age of the individual as well as acrosin content.

In the present study, we revisited such a topic, assessing seminal laminin relationships within different groups of infertile men, to a larger scale.

2 Materials and methods

2.1 Patients

This study enrolled a total of 125 males recruited from the Andrology Outpatient Clinic of Cairo University Hospital. The study plan was approved by the Ethical Committee of Cairo University and informed consent was obtained from all patients. All of the studied cases were married for more than 2 years. They were divided according to their sperm concentration and clinical examination into five equal groups (n = 25 each): fertile normozoospermia, infertile oligoasthenozoospermia, non-obstructive azoospermia (NOA), obstructive azoospermia (OA) and congenital bilateral absence vas deferens (CBAVD). Fertile normozoospermic cases were all recent fathers with normal semen analysis. NOA cases were diagnosed beforehand by testicular biopsy. OA cases were selected from those scheduled for epididymovasostomy operations. They were diagnosed beforehand by normal testicular size, normal serum follicle stimulating hormone, with signs of epididymal obstruction (full epididymis body with nodular tail). CBAVD were clinically verified, and confirmed by absent seminal fructose.

2.2 Samples

A detailed medical history was taken and physical examination was performed for the investigated cases. Ejaculates were obtained in the early morning (7:00–9:30am) after 4 days of sexual abstinence. The samples were examined immediately after liquefaction according to World Health Organizatoin guidelines [11] (normally; sperm count > 20×10^6 sperm/mL, sperm motility >50%; abnormal sperm morphology < 70%; vitality > 75% and leukocytes < 10^6/mL). Azoospermia was verified after three different analyses and centrifugation. Seminal plasma was separated at 1 200 × g immediately after complete liquefaction. All samples were stored at –20ºC until assay.

2.3 Laminin estimation

Laminin was estimated in the seminal plasma by using double antibody radioimmunoassay (BehringWerke AG, Marburg, Germany). The assay is based on laminin fragment P1, which originates from the central portion of this cruciform molecule and accounts for approximately one-third of the molecular mass of the molecule. This Lam-P1 assay is specific for laminin possessing no cross reactions detected with several collagens or fibronectin. The test sensitivity is 0.01 U/mL; intra-assay variation: 0.9–2.6%; inter-assay variation: 1.2–3.9%.

2.4 Statistical analysis

Numerical data were expressed as mean ± SD. Comparisons were performed by the unpaired t-test. Correlations were tested by Spearman's test. Comparisons and correlations were considered statistically significant if $P < 0.05$.

3 Results

Data of different studied groups (mean ± SD) were presented in Tables 1 and 2. Comparison among mean seminal plasma levels demonstrated significant difference between the three azoospermic groups and the fertile normozoospermic group ($P < 0.05$). Also, there was a significant difference between mean seminal laminin levels in the CBAVD group and those in the OA group; other comparisons were nonsignificant. Seminal plasma laminin in both fertile and oligoasthenozoospermia groups ($n = 50$) demonstrated nonsignificant correlation with age ($r = 0.021$, $P = 0.940$), semen volume ($r = 0.035$, $P = 0.087$), sperm motility percentage ($r = 0.142$, $P = 0.615$) and significant correlation with sperm concentration ($r = 0.460$, $P < 0.001$).

4 Discussion

Although matrix components display multiple biological activities and possess important roles, such as cell modulators and sites for binding of activated cytokines, few papers discuss their concentration, functions or their pathological alterations related to male reproduction. In several basement membrane functions, a set of non-collagenous proteins play an essential role in the participation of the collagenous components in the self-assembly of basement membrane among which laminin is the most abundant [12].

Geipel *et al.* [11] demonstrated mean seminal plasma laminin of 1.82 U/mL in azoospermic cases ($n = 5$), 2.13 U/mL in oligozoospermic cases ($n = 48$) and 2.4 U/mL in fertile normozoospermic cases ($n = 26$). In the present study, seminal plasma laminin levels in fertile cases had a mean level of 2.82 U/mL, with significant difference compared to azoospermic cases (NOA, OA and CBAVD): 1.77, 1.72, 1.35 U/mL, respectively. Significant decrease of seminal laminin in NOA cases could be explained by the failure of intact spermatogenic process with disturbed testicular tissue architecture in such cases to produce sufficient levels of laminin. Pöllanen *et al.* [13] showed that the localization of laminin in Sertoli-cell-only (SCO) syndrome NOA cases is different from that in the normal testis. In the normal testis, laminin is localized at the epithelial basement membrane and around the myoid cells,

Table 1. Semen characteristics of the studied groups (mean ± SD). CBAVD, congenital bilateral absent vas deferens; NOA, non-obstructive azoospermia; OA, obstructive azoospermia.

	Fertile normozoospermia	Oligoasthenozoospermia	NOA	OA	CBAVD
n	25	25	25	25	25
Age (years)	39.9 ± 2.7	37.8 ± 2.7	36.6 ± 3.1	35.2 ± 4.1	37.8 ± 3.1
Semen volume (mL)	3.9 ± 1.4	3.8 ± 1.6	2.2 ± 1.1	2.6 ± 1.9	0.7 ± 0.2
Sperm count (10^6/mL)	54.9 ± 5.7	6.5 ± 3.3	0	0	0
Sperm motility (%)	63.7 ± 8.3	24.7 ± 9.3	0	0	0

Table 2. Comparison of mean seminal plasma laminin (U/mL) levels in the studied groups. P_1, comparison of fertile group and other groups; P_2, comparison of oligoasthenozoospermia group and other groups; P_3, comparison of NOA group and other groups; P_4, comparison of OA group and other groups. $P < 0.05$ was considered significantly different. CBAVD, congenital bilateral absent vas deferens; NOA, non-obstructive azoospermia; OA, obstructive azoospermia.

	Fertile normozoospermia	Oligoasthenozoospermia	NOA	OA	CBAVD
Mean ± SD	2.82 ± 0.62	2.49 ± 0.44	1.77 ± 0.56	1.72 ± 0.76	1.35 ± 0.63
P_1		> 0.05	< 0.05	< 0.05	< 0.05
P_2			< 0.05	< 0.05	< 0.05
P_3			> 0.05	> 0.05	
P_4				> 0.05	

whereas in SCO the epithelial basement membrane and the first myoid cell layer are separated by a wide homogenous layer negative for laminin, resulting in the appearance of two concentric rings around the tubular lumen: the inner ring representing the basement membrane and the outer ring, the myoid cell layers. Davis *et al.* [14] and Santamaria *et al*. [15] demonstrated that testicular dysfunction can affect laminin contribution. Kleinman *et al.* [16] pointed to its control over cellular functions by mediating adhesion to anchorage-dependant cells providing signals for direct migration and controlling important cellular function. Also, laminin can bind soluble growth factors, for example, fibroblast growth factor, transforming growth factor and gamma-interferon concentrating the peptides for subsequent interactions with cells [14].

We suggest that the testicular contribution of seminal laminin is approximately one-third of its seminal content, subtracting mean OA levels from that of the fertile group levels as OA cases represent post-testicular laminin without testicular contribution. Consequently, seminal laminin of the CBAVD group revealed that the prostatic contribution with or without ultrafiltration from the serum significantly dropped (approximately half the levels of fertile men) as a result of absence of both seminal vesicular and testicular contributions in these cases [17]. Geipel *et al.* [18] showed that seminal laminin is significantly elevated in fertile men as compared to post-vasectomy men, suggesting that part of seminal laminin is derived from the vas deferens. Our results pointed to a small role of the vas deferens, if any, in seminal laminin contribution.

Nevertheless, seminal plasma laminin levels were significantly correlated with sperm count being significantly higher in normozoospermic than that in oligozoospermic and azoospermic cases. Geipel *et al.* [10] showed that laminin is elevated in the seminal fluid in comparison to the normal serum, with a positive correlation between its level and sperm concentrations, age of the individual, as well as acrosin, a serine protease within the acrosome released as a consequence of the acrosome reaction.

However, laminin was not found to be correlated either with age, semen volume or sperm motility. This indicates that its role in spermatogenesis process and gamete production is mediated most probably through somewhat specific cell to cell interactions more than through sperm activity or kinetic movement. Therefore, it is concluded that estimating seminal laminin alone could be not conclusive in diagnosing different cases of male infertility. Simultaneous measurement of many extracellular matrix components might provide a clue to different inter-relations and/or their possible multifunctional roles.

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