Benha Journal of Applied Sciences (BJAS) **print : ISSN 2356–9751**
Vol.(3) Issue(1) (2019), xxx-xxx **online : ISSN 2356–976x**
http:// bjas.bu.edu.eg

Evaluation of Fibrinogen-Like Protein 2 Level in Semen of Patients with Varicocele

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Abstract

Varicocele is an abnormal enlargement of the pampiniform venous plexus in the scrotum. The adverse effect of varicocele on spermatogenesis can be attributed to many factors such as an increased testicular temperature, increased intratesticular pressure, hypoxia due to attenuation of blood flow, reflux of toxic metabolites from the adrenal glands and hormonal profile abnormalities. FGL2 might play a protective role during sperm maturation in epididymis. FGL2 was found to be secreted from the principal cells into the tubule lumen where sFGL2 binds specifically to the nonviable, but not the viable, spermatozoa. This process forms sFGL2-protein complex that coats and envelops dying sperms to restrict release and spread of detrimental enzymes and immunogenic molecules from defective spermatozoa. The aim of study is to evaluate FGL2 in semen of patients with varicocele. We determined the presence of fgl2 in the seminal plasma via enzyme-linked immunosorbent assay [ELISA], whereas semen analysis was done as a routine investigation before separation of seminal plasma.fgl2 values in 55 infertile patients were significantly higher than in 25 healthy volunteers [p <0.001]. this result demonstrated strong association between infertility and level of fgl2 in the semen, fgl2 level are increased in infertility.

1. Introduction

Fibrinogen-like protein2 [FGL2], also known as fibroleukin, is a multifunctional protein, FGL2 has many functions; it associated with several physiological processes including sperm maturation, embryo development and smooth muscle contraction. Also involved in pathogenesis of viral infections, pregnancy failure, autoimmune disorder, allograft rejections, and tumor growth. Alternations in FGL2 expression or structure are tied to several highly virulent viral infections, including human immunodeficiency virus [HIV] infection, severe acute respiratory syndrome [SARS], and hepatitis B and C, Two distinct forms of the FGL2 protein have been identified, membrane-associated FGL2 [mFGL2] and soluble FGL2 [sFGL2]. MFGL2 integrates with phospholipids of cellular membranes and is expressed as a type II transmembrane protein, while sFGL2 can be secreted into the vasculature, FGL2 might play a protective role during sperm maturation in epididymis. The expression of Fgl2 messenger RNA [mRNA] under normal physiological conditions has been identified in the tubule principal cells of hamster epididymis. FGL2 was found to be secreted from the principal cells into the tubule lumen where sFGL2 binds specifically to the nonviable, but not the viable, spermatozoa. This process forms sFGL2-protein complex that coats and envelops dying sperms to restrict release and spread of detrimental enzymes and immunogenic molecules from defective spermatozoa. This becomes prominent only under certain medical conditions when increased apoptosis of spermatozoa occurs.

2. Methods

Fifty men [30 infertile patients with varicocele, and 20 completely healthy volunteers] had undergone testicular ultrasound to detect testicular size and grade of varicocele in patients with varicocele and complete semen analysis was done before separation of seminal plasma between July 2019 and October 2019, studies were performed at Dermatology and Andrology Department of Benha University Hospital. All subjects participating in the study were asked to sign a consent before inclusion. Then, they were subjected to full history taking and clinical examination. The mean age of all persons was [25.92±4.7] years. Scrotal Doppler US was performed with the patient in the supine position. The examination was performed by a radiology specialist [TA] at Radiology Department, Benha University using GE logic p6 ultrasound device with high- frequency linear array transducers>7.5 MHz. The participants were instructed to collect all semen, Semen analysis was performed according to the WHO manual in a standardized way.. Seminal plasma fibrinogen-like protein 2[FGL2] was assayed using Human fibrinogen-like protein 2[FGL2] ELISA Kit. An antibody specific for fibrinogen-like protein 2[FGL2] has been precoated onto 96-well plates. Standards or samples are then added to the appropriate microplate wells with a biotin-conjugated antibody specific to FGL2. Next, Avidin conjugated to Horseradish Peroxidase [HRP] is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain FGL2, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the

addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of450nm ± 10nm. The concentration of FGL2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.1 Statistical analysis

The Statistical Analysis and presentation of data was conducted using Mean Standard deviation , Median and Inter-quartile range [IQR] , Mann-Whitney's Test, Chi-Square test, Pearson's correlation coefficient and Logistic regression by Statistical computer programs SPSS, version 11.5 for Windows [SPSS, Inc] and MedCalc, version 18.2.1[MedCalc, Ostend, Belgium.

3. Results

Eighty persons were included in our study .Their age ranged from 21to 45 years with a mean of age [mean25.92SD4.7], they were 30 infertile patients with varicocele, 25 infertile patients without varicocele and 25 completely healthy volunteers.

Table (1) Semen count comparison between the groups.

	Group I [30]		Group II [20]		Statistical test [F]	P value
	Mean ±SD	Range	Mean ±SD	Range		
Sperm conc[m/ml]	18.35±20.87	0.5-76	32.16±19.15	2-75	26.24	<0.001**

Sperm concentration was significantly decreased in group I than in group III [p <0.001].

Table (2) Comparison between seminal FGL-2 levels.

	Group I[30]		Group II [20]		Statistical test [F]	P value
	Mean ±SD	Range	Mean ±SD	Range		
FGL-2[ng/ml]	2.37±1.28	0.89-5.1	1.54±0.54	0.83-2.8	13.81	<0.001**

Seminal FGL-2 levels were significantly increased in group I than group II [p value < 0.001].

4. Discussion

This study was conducted to assess the level of FGL2 in semen with its correlation with other seminal parameters and the protective role of fibrinogen-like protein2 [fgl2] in varicocele-associated male infertility. This study included 50 persons [30 infertile patients with varicocele and 20 health volunteers as a control group]. Their age ranged from 21to 45 years with a mean of age [mean25.92SD4.7].

The difference in age and smoking index between the three groups was non- significant. BMI was significantly increased in group II than group I [p value <0.001].

Total sperm count motility and normal sperm forms was compared between the groups.

Our results agree with [5] study on 71 infertile men with varicoceles, 217 fertile men without varicocele. They found that sperm concentration was lower in infertile men with varicocele [33.7 x 10[6]/mL] than in fertile men without varicocele [111.8 x10[6]/mL]. This is also in agreement with [1]

This is in disagreement also with [6] who conducted his study on 30 varicocele patients and found nonsignificant difference in all semen parameters between varicocele
patients and controls. They conducted their study on varicocele patients regardless their fertility potential, while our research groups were infertile either varicocele or non-varicocele patients [groups A and B], and this can explain the discrepancy between their results and ours. Varicocele patients.

Sperm concentration appears to be clinically important. In addition, the correlation between increased testicular volume differentials [10% to 20% and > 20%] with lower total motile sperm counts appeared significant. But they used the testicular volume differential, not the absolute testicular volume.

.K. Wu, [6] investigated 245 men with varicocele, and 103 men with a sperm count < 20 million sperm compared with 142 men with normal sperm count. Men with bilateral hypotrophy were nearly nine times more likely to have a sperm count < 20 million sperm than were men without hypotrophy, and six times more likely than those with unilateral hypotrophy. This supports the hypothesis that testicular volume is positively correlated with sperm count, but they investigated this relation in oligozoospermic and normozoospermic varicocele patients.

Also E. Gary [4] reported that total testis volume and the testicular volume differential are associated with semen analysis outcomes in varicocele men. A total testis volume < 30 cc quadruples the odds of a low total motile sperm count.

These results are inconsistent with the results of [2] M. Cocuzza who found no predictive value of testicular volume measurement with regard to semen analysis. This can be explained by the small sample size of this study.

Many studies investigated the presence of fibrinogen-like protein2 [fgl2] in serum in different inflammatory and autoimmune diseases. It was also investigated in Region-specific Expression and Secretion of the Fibrinogen-related Protein, fgl2, by epithelial Cells of the hamster epididymis and its role in disposal of defective. But no previous studies investigated its presence in seminal plasma. We found that it is present in seminal plasma of normal fertile men at the concentration of 1.18±0.35 nanogram/ml.

Seminal plasma fibrinogen-like protein2 [fgl2] levels were significantly higher in group I than both group II . This result support sperms hypothesis that

occurs in infertile men with or without varicocele that correlates with the protective role of fibrinogen-like protein2 [fgl2] in isolation and protection of viable sperms from harmful antigens and enzymes that result from dead sperms. Increased seminal plasma fibrinogen-like protein2 [fgl2] in infertile varicocele men than infertile non-varicocele men can be explained by increased sperm apoptosis in varicocele.

A study was done on Region-specific Expression and Secretion of the Fibrinogen-related Protein, fgl2, by Epithelial Cells of the Hamster Epididymis and Its Role in Disposal of Defective Spermatozoa [4]. Northern blot analysis demonstrated that fgl2 mRNA is highly expressed by the proximal cauda epididymidis in comparison to other hamster tissues examined, and, *in situ* hybridization analysis of the epididymis revealed that fgl2 mRNA exhibited a region- and principal cell-specific expression pattern. Immunohistochemistry confirmed the association of fgl2 with abnormal spermatozoa in the cauda epididymidis and revealed smaller fgl2-containing particles. Immunoelectron microscopy revealed that fgl2 was distributed throughout an amorphous, "death cocoon," complex assembled onto abnormal spermatozoa and that the smaller fgl2 aggregates consisted of the amorphous material with embedded sperm fragments, organelles, and membrane vesicles. These data demonstrate that fgl2 possesses a specific mechanism to identify and envelop defective spermatozoa with a protein complex. This represents an important protective mechanism not only to shield the viable sperm population from potentially deleterious enzymes released by dying spermatozoa but also to prevent the release of sperm proteins that could initiate an immune response if they escaped the epididymal environment.

5. Conclusion

This study was the first study to evaluate level of fgl2 in the semen of infertile patients. There was significant correlation between infertility and seminal plasma fibrinogen-like protein2 [fgl2]

This result also coincides with data available in literature about the protective role of [fgl2] by formation of protein complex that coats and envelops dead non-viable sperms and isolates them from viable sperms. There was obviously higher levels of fgl2 in patients with varicocele which known as one of the main causes of sperm apoptosis.

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