The Profile of Apoptotic Marker sFas Ligand in seminal plasma of Oligozoospermic men

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Abstract:

Background:

The Fibroblast associated (Fas) system in the testes has been identified as a key regulator of apoptosis, a process that greatly influences the germ cell population of the testes.

Objective:

This study measures the level of soluble Fas Ligand (sFasL) in the semen of oligozoospermic men evaluating the association between seminal plasma sFasL and spermatogenesis.

Methods:

A total 58 oligozoospermic men and 29 normal volunteers were included in this study. semen was evaluated according to World Health Organization 2010 standard parameters. sFasL was measured using ELISA enzyme immunoassay for quantitative determination of sFasL.

Results:

s FasL level was found significantly higher ($P< 0.01$) in seminal plasma of oligozoospermic men,
with weak correlation of the level of this marker with the degree of severity of oligozoospermia.

Conclusions:

The apoptotic marker, sFasL is a novel marker found in the seminal plasma of oligozoospermic men. It's level is higher in cases of oligozoospermia regardless of its severity.

Key words: soluble Fas Ligand, Oligozoospermia, Male infertility.

Introduction:

Male infertility factor is identified in almost 50% of infertile couples, while it is the sole cause in 20-30 % of infertile couples (1). Many factors influence male fertility, and one of the main process involved is apoptosis in male genital tract.

Apoptosis is an active, gene- directed cellular self -destruction which may occur in both physiologic and pathologic conditions (2). It is thought to be one of the important factors in regulating the production of spermatozoa (3).

One factor implicated in sperm apoptosis is the cell surface protein, Fibroblast associated (Fas) factor. The interaction between Fas (CD95/ Apo-1; a type I transmembrane glycoprotein receptor) and a cellular death inducing Ligand (a type II transmembrane glycoprotein; FasL) plays an important role in triggering the apoptotic pathway. Both Fas and FasL exist as a membrane bound and soluble form (4).

Previous reports have suggested that the Fas-mediated system is implicated in the elimination of defective spermatozoa from the ejaculate and shows possible abnormalities that could account for certain forms of male infertility (5).

The aim of this study is to explore the profile of soluble Fas Ligand (sFasL) in the seminal plasma of oligozoospermic infertile males, and to evaluate the correlation between seminal plasma sFasL and the phenomena of oligozoospermia in those males.

Patients and Methods:

This study was performed on 58 males of infertile couples, their age ranged between 21-47 years, in whom the cause of infertility was attributed to male factor as shown by an abnormal seminal fluid analysis profile according to WHO, 2010 standard parameters (6).

The patients were enrolled from the infertility outpatients of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Baghdad / Iraq during the period from October 2013 to March 2014.

These Patients were classified into patients with severe oligozoospermia (sperm concentration ≤5 million / ml) which include 29 patients, and patients with moderate oligozoospermia (sperm concentration ranging between 5-15 million / ml) which includes 29 patients as well.

The control group comprised of 29 men with normozoospermic parameters according to WHO 2010 standard (Normal volunteers) .

The sFasL (sAPO-1L, sCD95L) was measured in the seminal plasma by an enzyme-linked immunosorbent assay ELISA for quantitative detection of human sFasL in human body fluids (US Biological Life Science soluble FasL BioAssay ELISA Kit, F0019-65G,USA).

Principle of the Test

An anti-sFasL monoclonal coating antibody is adsorbed onto microwells. sFasL present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-sFas Ligand antibody is added and binds to sFas Ligand captured by the first antibody. Following incubation unbound biotin-conjugated anti-sFasL is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-sFasL.

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to wells. A colored product is formed in proportion to the amount of sFas Ligand present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven sFasL standard dilutions and sFasL sample concentration determined.

Statistical Analysis:

Statistical Package for Social Sciences (SPSS) software version 18 and Microsoft excel 2007 were used to find:

- The mean ± standard deviation (SD) for each study group (Normozoospermic group; moderate oligozoospermia and severe oligozoospermia).
- Analysis of variance (ANOVA) and Least Significant Difference (LSD) were done to show the significance between the study groups at the level of significant (0.05 and 0.01).
- Correlation coefficient (r) was calculated between (sperm concentration and sFasL concentration) for each study group.

Results:

The seminal plasma sFasL was found to be (0.077±0.019 ng/ml) in normozoospermic males of the control groups. In the group of the moderate oligozoospermia infertile men, it was (0.123±0.07 ng /ml), while it was (0.136±0.0459 ng/ml) in men with severe oligozoospermia.

Analysis of the comparison between the three groups under study, is shown in table (1).

In evaluation of the correlation between the sperm concentration and level of seminal plasma of sFasL, the r-values are illustrated in table (2).

Table (1): Statistical comparison of sFasL levels between the study groups (ANOVA, LSD)

Comparisons	Differences between means	> or <	L.S.D.0.05	L.S.D.0.01	Significant	P Value
Normozoospermic males vs. Severe Oligozoospermia.	0.058	>	0.025	0.033	S.**	P< 0.01
Normozoospermic males vs. Moderate Oligozoospermia	0.045	>	0.025	0.033	S.**	P< 0.01
Moderate vs. Severe Oligozoospermia	0.012	<	0.025	0.033	N.S.	0.33

Table (2): Correlation Coefficient (r-value) between the sperm concentration and level of sFasL within each group of the study.

Correlation between. Sperm concentration & sFasL	r-Value	p- value
Normozoospermic males	0.046104	0.72
Moderate oligozoospermia	0.059669	0.68
Severe oligozoospermia	0.074359	0.64

Discussion:

Fas / FasL interaction play an important role in the regulation of cell behavior and immune system(7).

Under physiological conditions, cells of the testes, which are considered as immune-privileged area i.e. a location in the body that is excluded from immune surveillance, express Fas Ligand to induce apoptosis of the infiltrating lymphocytes. Thus, it is a part of the mechanism employed for establishment and maintenance of the state of immune privilege (8).

The starting finding of this study, was that the level of seminal plasma sFasL was higher in the seminal plasma obtained from oligozoospermic men as compared with healthy control group of men, is in concordance with a recent study by Passadaki *et al.* (9), whom studied sFas and sFasL levels in the seminal plasma and its association with basic parameters of seminal analysis, it was found that sFasL levels in seminal plasma of abnormal seminal samples were slightly higher the normal ones.

However, previous reports which studied soluble form of Fas and FasL concentrations in the seminal plasma of infertile men with varicocele, did not reach a conclusion since the sFasL concentration in seminal plasma was less than the limit of the detection of the chemiluminescence assay employed (10).

Such contradictory results were also observed in the correlation between

sFasL and several diseases conditions. Elevated levels of sFasL was found in patients with acute myocardial infarction, unstable angina pectoris (11), and in patients suffering from diabetic foot (12). While no correlation was detected between levels of sFasL and the clinical variable in chronic obstructive pulmonary disease patients (13), or in cases of congestive heart failure (14).

These finding indicate that sFasL plays different independent roles in different pathological conditions. It's contribution to apoptosis in one condition, depends on the nature of that pathological condition.

In this study, it was established that there was a significant difference in seminal plasma sFasL levels between normal individuals and patients considered Oligozoospermic according to WHO 2010 standard parameters. Again, the recent work of Passadaki *et al.* (9), reached to general conclusion that both factors of Fas system (sFas /sFasL) were detected in seminal plasma, with a proposition that further studies are necessary to shed light into possible role of this system in male infertility.

The significant higher level of sFasL in the seminal plasma of oligozoospermic infertile men was found to be independent of the underlying disease causing or attributing to Oligozoospermia in those men.

On the other hand, it was found that there was no significant difference in the reading of sFasL levels in seminal plasma between sub-groups of oligozoospermia i.e. between the group of moderate Oligozoospermic men and the group of severely oligozoospermic men.

This will lead to the conclusion that an elevated level of sFasL in the seminal plasma, is a novel marker found in the seminal plasma associated with oligozoospermia.

Within the oligozoospermic sub-groups studied, no correlation was found between the sperm concentration /ml and level of seminal plasma sFasL. This reflects the absences of quantitative correlation of the seminal plasma level of sFasL and the severity of oligozoospermia.

The proposed explanation for the result of this study is illustrated below.

It was shown that sFasL is less potent at inducing apoptosis than membrane bound FasL, (15)(16) (17), sFasL deficient in transducting signals upon engagement with membrane Fas as a competitive inhibitor to induce apoptosis (16)(18)(19), and the high level of sFasL in testes reduce the apoptotic rate during spermatogenesis.

According to Tanaka *et al.* (20), sFasL inhibits Fas-mediated apoptosis, and our finding of increased sFasL in the seminal plasma of oligozoospermic men, denotes that the defect of Fas/FasL dependant apoptosis is a contributing factor to the development of Oligozoospermia. Testicular germ cell apoptosis appears to have an essential role in the control of germ cell number in testis (21). This apoptotic wave appears necessary for normal spermatogenesis to develop, probably because it maintains a proper cell number ratio between maturing germ cell stages and Sertoli cells (22).

We consider that the development of new effective research strategies regarding apoptosis, will play an important role in the future management of male infertility caused by oligozoospermia.

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