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RESEARCH ARTICLE

Association of Seminal Plasma Level of NLRP3 Inflammasome Protein with Quality of Seminal Fluid Parameters Among Infertile Men

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Abstract

Background:inflammasome protein govern the inflammatory process. The elevation of NLRP3 inflammasome might be related to bad seminal fluid analysis results.

Objectives: This study aimed to evaluate seminal plasma NLRP3 protein and its relation with seminal fluid analysis parameters before and after *in vitro* sperm activation.

Patients and Methods: a total of 50 infertile patients were collected form higher institute of infertility diagnosis and assisted reproductive technique, there semen samples were processed for seminal fluid analysis before and after *in vitro* sperm activation. NLRP3 inflammasome protein were measured in seminal plasma.

Results:the mean level of NLRP3 protein was 5.12 ng/ml. the higher level of NLRP3 protein (4.5ng/ml) associated with lower sperm concentration, grade A, B, C and higher level of grade D motility. However, 50% of those patients have an improvement in their seminal fluid analysis after *in vitro* sperm activation.

Conclusion: Elevation of NLRP3 protein in seminal plasma may be associated with the increased leukocytospermia, bad sperm quality and unresponsiveness to *in vitro* sperm activation among infertile male.

Keywords:NLRP3, Inflammasome,Seminal fluid analysis and Infertility.

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Introduction:

NLRP3 (NOD-like receptor family, pyrin domain containing 3) inflammasome is a multiprotein complex that orchestrates innate immune responses to infection and cell stress through activation of caspase-1 and maturation of inflammatory cytokines (1).NLRP3 has also been implicated in the pathogenesis of many diseases including arthropathy, ischemic heart and renal disease, vascular disease, diabetes and obesity, Alzheimer's dementia, and others, which are well-reviewed elsewhere (2). It has shown that inflammasome activation and the consequent elevation of seminal proinflammatory cytokines leads to impaired sperm motility in men with spinal cord injury (SCI) (3) and non-SCI men (4–6) .Suggesting leukocyte activation may be key to a relationship between inflammation and infertility (7–9) .Cytokines affect Sertoli cell function, and alterations in cytokine levels in the testis could affect spermatogenesis. One of the mechanisms by which leukocytospermia may lead to sperm dysfunction is related to sperm damage induced by reactive oxygen species (ROS) via activated leukocytes during or after ejaculation (7,10) .

The NLRP3 protein in seminal plasma may be associated with the increased leukocytospermia, bad sperm quality among infertile male and may interfere with pregnancy outcome after different methods of assisted reproductive technologies like: intra-uterine insemination, *in vitro* fertilization and intra-cytoplasmic insemination.

This study aimed to, determine the level of NLRP3 protein in seminal plasma in relation with semen fluid analysis among infertile male. Determine NLRP3 association with improvement of seminal fluid analysis parameters after invitro sperm activation.

Materials and Methods:

Infertile patients and sample processing:

The study included fifty men; their ages were between 20- 45 years who attended the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies / AL-Nahrain University. This prospective study was accomplished through the period from December 2016 till March 2017.

Seminal fluid analysis done according to WHO criteria (2009, 2010), grades of leukocytospermia were evaluated by using Endtz test according to standard method(11) .

Seminal plasma was separated by centrifugation of liquified semen sample at approximately 1000 × g for 20 minutes then aliquots were made and stored at -50°C until assay.

Human NACHT, LRR and PYD domains-containing protein 3 (NLRP3) ELISA:

This sandwich ELISA kit was purchased from Abbexa company UK (abx516996) for quantitative detection of human NLRP3 with detection range is 0.156 ng/ml - 10 ng/ml with Sensitivity: 0.156 ng/ml. the measurement of plasma NLRP3 were done according to manufacturer instruction and optical density read at 450 channels. Linear standard curve was generated, NLRP3 concentration calculated according to the equation of curve.

Statistical analysis:

Data were analyzed by using statistical package for social sciences (SPSS) version 21. Categorical data described as frequency and percentage while, numerical data described means and standard deviation (SD). Chi square test was used to estimate the association between two categorical variables. Level of significance of ≤ 0.05 was considered as significant(12) .

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Results:

The mean age of infertile was 29.44 years old and their seminal fluid analysis can be summarized in table 1. The results demonstrated that there is significant difference found in sperm concentration of positive and negative cutoff values NLRP3 ELISA was significant (p =0.047) and sperm motility before and after activation (Grade A, Grade B, Grade C, Grade D) of positive and negative cutoff values NLRP3 ELISA were showed significant (p =0.041, 0.005, 0.035 and 0.004). The morphologically normally sperm of positive and negative cutoff values NLRP3 ELISA was showed that non-significant (p= 0.139)(table 1).

Parameter		Total (n=50)	NLRP3 ELISA		P value
			Positive cut off (>4.5ng/ml)	Negative cut off (<4.5 ng/ml)	
Age (year)		29.44±5.00	30.2±6.49	29.25±4.63	0.596^{NS}
Volume (ml)		2.55±0.65	2.55±0.6	2.55±0.67	1.000^{NS}
pH		7.36±0.06	7.36±0.07	7.36±0.06	0.816^{NS}
Sperm Concentration (million/ml)		39.26±23.92	31.72±21.69	45.17±24.28	0.047*
Motility (%)	Grade A	8.80±11.05	5.23±7.63	11.61±12.55	0.041*
	Grade B	15.60±9.24	11.59±6.97	18.75±9.68	0.005*
	Grade C	18.40±12.91	14.09±9.71	21.79±14.22	0.035*
	Grade D	57.60±25.92	69.09±19.19	48.57±27.21	0.004*
Morphology (%)		20.48±5.67	19.14±4.53	21.54±6.31	0.139^{NS}
Round cells		10.52±7.25	9.6±8.69	10.75±6.95	0.658^{NS}
NLRP3 ELISA (ng/ml)		5.12±1.85	5.15±1.82	2.11±1.66	≤0.001**
Leukocyte count (million/ml)		2.16±5.41	1.24±2.24	1.64±1.99	0.578^{NS}

Data presented as mean± standard deviation.
Comparison made by independent sample t-test.
NS: None statistical significant difference (p>0.05).
*: Statistical significant difference (p<=0.05).
**: Highly statistical significant difference (p<=0.001).

Relationship between result of ELISA and improvement of sperm motility:

The ratio of positive not improved ELISA was showed (50.00%), the ratio of positive improved ELISA was showed (50.00%). The ratio of negative not improved ELISA was showed (10.71%), the ratio of negative improved ELISA was showed (89.29%) and the total ratio showed (93.33%). The odds ratio of positive and negative ELISA was (8.33) (1.94-35.9). The results can be summarized in table 2.

Table 2. Association between NLRP3 ELISA results and improvement in seminal fluid analysis parameters.

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Relationship between result of ELISA and improvement of sperm motility:
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Table 2. Association between NLRP3 ELISA results and improvement in seminal fluid analysis parameters.

Parameters		Improvement Status		Total
		Not improved	Improved	
ELISA	Positive (≥4.5 ng/ml)	11	11	22
	%	50.00%	50.00%	100.00%
	Negative (<4.5 ng/ml)	3	25	28
	%	10.71%	89.29%	100.00%
Total		14	36	50
%		28.00%	72.00%	100.00%
Odds Ratio (CI)		8.33 (1.94-35.9)		

Correlation of NLRP3 level with seminal fluid analysis:
The results in table 4 showed that positive RADT and quantitative NLRP3 ELISA test were negatively correlated with Sperm concentration, grade B and C and positively correlated with grade D sperm motility indicating that both leukocytes and the presence of NLRP3 have adverse effect on seminal fluid analysis by reducing sperm motility, grade B and C sperm motility and higher rate of grade D motile sperm. Round cells count was positively related count and grade leucocyte indicating that higher rate of false positive round cell count. The results can be summarized in table 3

Table 3: correlation among different methods and change in sperm motility after *in vitro* sperm activation.

Parameters			ELISA (ng/ml)	ELISA (cutoff 5ng/ml)
Volume		r	-0.108	0
		p	0.454	1
pH		r	-0.076	0.034
		p	0.601	0.816
Concentration		r	-0.359*	-0.142
		p	0.010	0.324
Change in sperm motility (%)	Grade A	r	-0.226	-0.215
		p	0.115	0.134
	Grade B	r	-0.346*	-0.077
		p	0.014	0.597
	Grade C	r	-0.304*	-0.121
		p	0.032	0.402
	Grade D	r	0.357*	0.177
		p	0.011	0.218
Morphology(%)		r	-0.167	-0.064
		p	0.248	0.658
Round cells		r	0.037	0.064
		p	0.797	0.658

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Discussion:

The association between inflammation of the male reproductive system has subsequently resulted in development of infertility. It's more likely described as cause and effect relationship. In particular, the quality of seminal fluid analysis could be reduced in case of inflammatory processes through impairment of secretory capacity of sexual glands, obstruction of the sperm, increased pro-inflammatory mediators, increased oxidative stress and dysregulated spermatogenesis(13,14)..To the best of our knowledge, this is the first study that determine the seminal plasma level of NLRP3 protein among infertile men using ELISA method.

The first one is quantitative method performed by Enzyme linked immunosorbent assay (ELISA), the study reported that the mean NLRP3 5.12±1.18 ng/ml and this might reflect an inflammatory state among infertile men. Based on the results of improvement in *in vitro* sperm activation the results showed that 36 infertile patients (72%) were improved in sperm motility and only 14 (28%) were not improved. Furthermore, based on this classification NLRP3 seminal plasma level found to be higher among patients whom not improved in their sperm motility than that improved ones 6.54±2.31 versus 3.77±1.31 respectively. In this study, the results of receiver operating curve showed that 4.5 (ng/ml) as a cutoff value discriminating those whom can improve their sperm motility or not and that 22 (44%) of the patients were positive with equal or more than 4.5 ng/ml and the remainder were negative 28 (56%).

This study highlighted the risk of increasing NLRP3 protein in seminal plasma was 8.33 much more likely to get un improved sperm motility after *in vitro* activation (Table 2). Basically, a lot off researchers highlighted the impact of inflammation on the seminal fluid analysis parameters (reviewed by 15). Several parameters have been applied as indicators for semen inflammation reviewed by La Vignera, *et al.*, 2013. They depend on enzymes(16) and cytokines(17) that participate in inflammatory process of male genitalia(14) .

Group of researchers highlighted the importance of inflammasome protein NLRP3 as a "physiological low level of inflammation" for fertility(6).It had been suggested that low level of NLRP3 is essential for Sertoli cell production of low levels of IL1B to maintain IL1 alpha (18) but higher rate of inflammasome expression will results in higher level of inflammasome activation cytokines such as IL-1B and IL-18(19) and served as a proinflammatory signals generating an inflammatory process in the testis such as leucocyte infiltration and cell death signaling and caspase 1 activation(20–22) .Also, it might activate ASC adaptor protein in both sperm cell and leucocytes in semen resulting in inflammatory cell death(23) .

To date, the primary focus of inflammasome research has been anchored by NLRP3, which is a cytoplasmic protein that is primarily expressed in monocytes, macrophages, granulocytes, dendritic cells, epithelial cells and osteoblasts(24). NLRP3 expression in myeloid cells is highly inducible(25) and other epithelial and Sertoli cells(6). So, the leukocyte counting is not always giving a true picture of inflammation, because of wide range of cells have the ability to respond and considered as a source of inflammatory mediators. This gives an advantage of measurement of NLRP3 as a biomarker for inflammatory processes

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and its targeting is crucial for improvement in sperm cell quantity and quality(26).
The developed kit is user friendly, simple to use and interpret, no antigen extraction procedure is needed. The sample (seminal plasma) was diluted 1:1 with buffer in order to dilute the sample and reducing its viscosity. The reduction of viscosity will facilitate sample absorption and flow across membranes.

Conclusion: Elevation of NLRP3 protein in seminal plasma may be associated with the increased leukocytospermia, bad sperm quality and unresponsiveness to *in vitro* sperm activation among infertile male.

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