



Measurement of Seminal plasma peptidoglycan recognition protein-1 (PGLYRP-1) protein infertile males as a measure of leukocytospermia and bad seminal fluid parameters.

Asawer F. Abdulhussain ¹, Haider F. Ghazi ¹, Muayad S. Abood¹

¹High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.
alrammahi710@gmail.com

ABSTRACT

Human peptidoglycan recognition protein-1 (PGLYRP-1) protein molecule activation in the semen of infertile patients has been suggested to induce negative impact on seminal fluid analysis. This study aimed to evaluate Human Peptidoglycan Recognition Protein 1 for indication of bad seminal fluid analysis parameters. One hundred seventy-six infertile patients were included in this study. Seminal fluid analysis and Endtz test were done; Human Peptidoglycan Recognition Protein 1 in seminal plasma using enzyme-linked immune-sorbent assay technology PGLYRP-1 is evident in seminal plasma of infertile men and statistically different according to the type of infertility. The concentration of PGLYRP-1 in the semen is directly proportional to the presence of leukocytes (Endtz test), which is reflected negatively on the quality of the semen. To the best of our knowledge, this study has not been done in Iraq or elsewhere. The presence of PGLYRP-1 protein in the semen may be evidence of leukocytospermia that may be related to bad quality for semen across infertile men with higher sensitivity than the Endtz test.

Received:
01-Jan 2021
Accepted:
30-May 2021
Published:
06-2021

How to cite:

Asawer F.; Haider F.; Muayad S.; Measurement of Seminal plasma peptidoglycan recognition protein-1 (PGLYRP-1) protein infertile males as a measure of leukocytospermia and bad seminal fluid parameters; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2021); 11 (1): 15-28.
<http://doi.org/10.28969/IJEIR.v10.i1.r4.21>

KEYWORDS

Ketamine, Remifentanyl, Reactive Oxygen Species, Oocyte Quality, General Anaesthesia.

1. Introduction

An approximate 15% of couples worldwide are affected by infertility, totaling to 48.5 million couples (Agarwal et al.^[1]). Male variables account for 20 percent with 50 percent of cases of infertility with infection, and up to 15 percent of male infertility can lead to the genital tract (Jung JH et al.^[2]). As such a predictor of genital tract infection and inflammation, leukocytospermia (LCS) or pyospermia, also characterized as the presence of more than one million leukocytes in 1 mL of semen, has been suggested (Brunner RJ et al.^[3]).

Leukocytes (polymorphonuclear neutrophils and macrophages) provide major effects on male fertility as they trigger sperm damage via the secretion of reactive oxygen species (ROS) via DNA fragmentation (Eldamnhoury EM et al.^[4]). Infectious agents and their innate immune defenses can have a major effect on human sexual ability (Bhushan S et al.^[5]). Peptidoglycan recognition proteins (PGRPs) are pattern recognition receptors

that bind bacterial cell walls peptidoglycans (PGNs) to the innate immune system. All such concentrating molecules, via insects to humans, contribute to host protection against certain Gram-positive and Gram-negative bacteria infections (Guan R et al.^[6]). Mammals have four Protein identification of peptidoglycans (PGRPs or PGLYRPs), which they are natural immunity pattern recognition molecules with effector functions (Wang M et al.^[7]). The Human Genome Organization Gene Nomenclature Committee changed the names of human PGRPs to PGLYRP1, PGLYRP2, PGLYRP3 and PGLYRP4, respectively, and this nomenclature was adopted for all mammalian PGRPs (Yang J^[8]). Proteins expressed in polymorphonuclear leukocytes (PGLYRP1), liver (PGLYRP2), or body surfaces, mucous membranes, and secretions (saliva, sweat) are secreted (PGLYRP3 and PGLYRP4). The bacterial peptidoglycan is recognized by all PGRPs. For both Gram-positive and Gram-negative bacteria, three PGRPs,

PGLYRP1, PGLYRP3, and PGLYRP4 are Specifically bactericidal and have no enzymatic activity, while PGLYRP2 is a N-acetylmuramoyl-L-alanine amidase that hydrolyzes peptidoglycan bacterial cell wall (Dziarski R and Gupta D. ^[9]).

2. Materials and Methods

2. 1. Study design and setting

Correlation study was conducted on 176 infertile males seeking for causes of infertility in the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technique Clinic during September to the end of December 2019. After taking of semen sample according to the standard, seminal fluid analysis will be performed according to the WHO manual.

2.2. Measurement of seminal plasma

Human peptidoglycan recognition protein- 1 (PGLYRP-1)

Human peptidoglycan recognition protein-

1 (PGLYRP-1) ELISA purchased from

Abbexa ® catalog number abx 358442by

sandwich enzyme-linked immune-sorbent assay technology. An antibody specific to PGLYRP-1 is pre-coated onto a well plate. The intensity of the color yellow is proportional to the PGLYRP-1 amount bound on the plate.

2.3. Analysis of statics

The statistical analysis of this comparative study was carried out using the Social Sciences Statistical Package (SPSS) 21.0 and Microsoft Office Excel 2013. Numerical data were described as mean and standard deviation, and analysis of variance (ANOVA) was used for comparison among the three groups.

3. Results

3.1. The basic characteristic for all infertile men:

Table (1): Statistical analysis of semen parameters.

	Mean	Std. Deviation	Range	Minimum	Maximum
Day of abstinence	3.42	1.04	6.00	1.00	7.00
Volume	2.62	1.00	4.50	0.50	5.00
Sperm concentration	31.07	19.88	80.00	0.00	80.00
Grade A	7.85	7.84	25.00	0.00	25.00
Grade B	20.33	9.93	35.00	0.00	35.00
Grade C	27.20	11.27	47.00	0.00	47.00
Grade D	44.49	21.12	80.00	20.00	100.00
Total progressive motile sperm	12.75	14.85	81.00	0.00	81.00
Normal Sperm Morphology	20.31	12.57	40.00	0.00	40.00
Round cells	11.58	6.74	30.00	3.00	33.00
PGLYRP-1 (ng/ml)	38.27	15.39	41.93	3.9	45.83
Endtz Test	6140909.09	24335436.71	160000000.00	0.00	160000000.00

The results in this Figure showed that there is statistically significant difference in the mean of PGLYRP-1 seminal plasma level ($p=0.036$), the lower level found in normozoospermic infertile men (9.43), and the higher levels

found in oligoasthenozoospermia (42.94), Oligoasthenoteratozoospermia (38.12) and Asthenozoospermia (37.09) indicating high level of activity of this receptor.

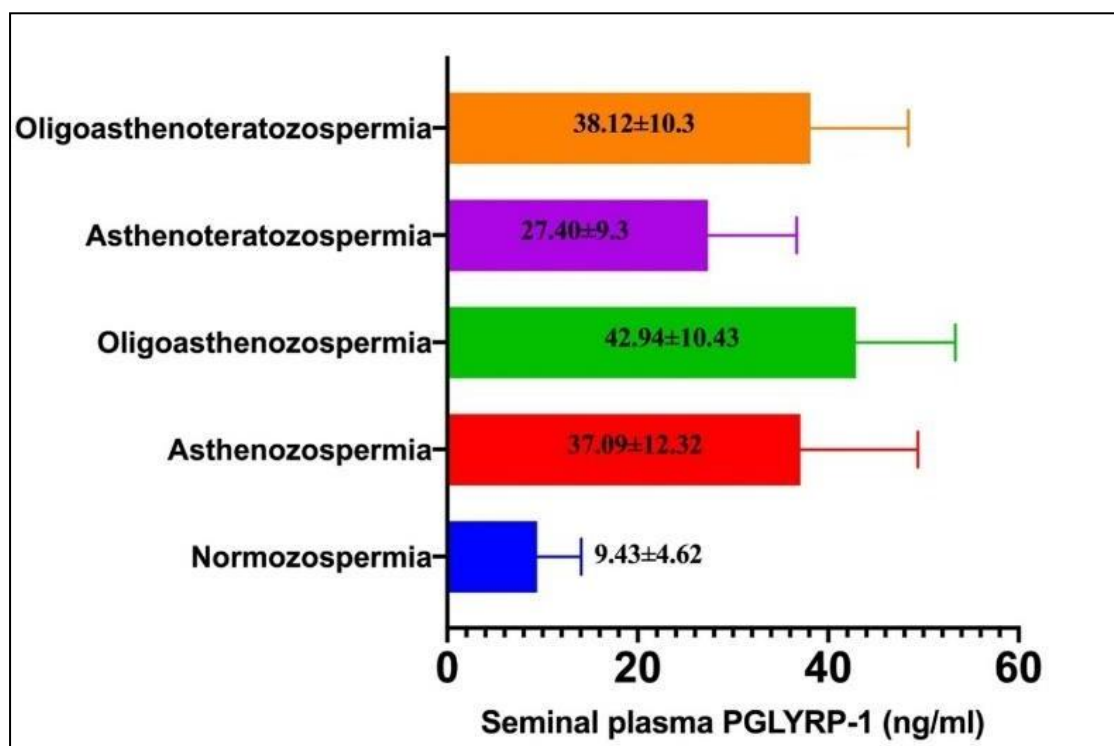


Figure 1: Seminal plasma level of bacterial PGLYRP-1 (ng/ml) among study groups ($p=0.036$)

3.2. Correlation between PGLYRP-1 and seminal fluid analysis parameters

The results in this table showed that PGLYRP-1 concentration and Endtz test in the seminal plasma had a directly relationship with Grade B motility. PGLYRP-1 and grade D motility negatively affect sperm concentration correlated of grade B motile and normal morphology of sperm. The

innate immune receptor PGLYRP-1 was positively related to Endtz results, suggesting its essential role in response to leukocytospermia (**Table 2**).

Table (2): Correlation between PGLYRP-1 and seminal fluid analysis parameters

	PGLYRP-1 (ng/ml)		Endtz Test
Endtz Test	R	0.721**	1.000
	P	<0.001	
Day of abstinence	R	0.217	0.044
	P	0.362	0.687
Volume	R	0.102	-0.177
	P	0.33	0.099
Sperm concentration	R	-0.319	0.064
	P	0.042	0.554
Grade A	R	-0.499	-0.121
	P	0.002	0.262
Grade B	R	-0.239	-0.301**
	P	0.029	0.004
Grade C	R	0.291	0.211*
	P	0.122	0.048
Grade D	R	0.492	0.300**
	P	0.008	0.004
Total progressive motile sperm	R	-0.201	-0.083
	P	0.021	0.442
Normal Sperm Morphology	R	0.238	-0.204
	P	0.073	0.057
Round cells	R	0.360	0.359**
	P	0.009	0.001

4. Discussion

An evidence-based review of seminal plasma PGLYRP1 and (as a tool for in vivo quantitation of bacteria load in the genital tract) and their relationship with seminal fluid analysis is proposed in this research (10). Inflammatory processes can lower the quality of seminal fluid analysis by impairing sexual gland secretory capacity, obstructing sperm, increasing pro-inflammatory mediators, increasing oxidative stress, and modifying spermatogenesis (Sandoval JS et al. ^[11]).

Its clinical association was poorly understood because of few studies have been found. Studies reported that PGPRL-1 as a marker of inflammation in innate immunity (Dziarski R and Gupta D ^[9]) in particular other disorders, like rheumatoid arthritis and Erythematosus chronic epilepsy (Luo Q et al. ^[12]).

Seminal plasma PGRPL-1 was measured using the ELISA method. It was reported that there was a significant difference in the mean of PGRPL-1 among different types of

infertile patients, and the lowest value was found in normozoospermic cases, suggesting an active involvement of this innate immune recognition molecule among patients with abnormal seminal fluid analysis parameters. The results showed statistically significant higher seminal plasma levels of PGPRL-1 in leucocytospermic infertile men than none leucocytospermic infertile men. This might refer to a positive association and evidence for PGPRL-1 expression on leucocytes in semen samples. This suggestion was supported and explained by De Marzi et al., that reported an active involvement of PGPRL-1 in inflammatory responses mediated by increased activation of monocytes (De Marzi MC et al. ^[13], Alzaidi, Z. 2021, ^[14]).

5. Conclusions

Seminal plasma PGLYRP-1 and were evidences in infertile men and statistically different among types of changes in seminal fluid parameters, suggesting a harmful effect on sperms. Seminal plasma

PGLYRP-1 was related to the state of leukocytospermia, indicating the active role of leukocytes in the inflammation.

Acknowledgment

We would like to acknowledge Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding.

Author Contribution

Abd Alhussain, A. performed the study, examined and reviewed results, and manuscript writing with the help and supervision of Reshan, RG, and Mossa, HAL.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

References

- [1] Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol*. 2015 Apr.13 (1):1–9. Doi: 10.1186/s12958-015-0032-1
- [2] Jung JH, Kim MH, Kim J, Baik SK, Koh S-B, Park HJ. Treatment of Leukocytospermia in Male Infertility: A Systematic Review. *World J Mens Health*. 2016Dec.34 (3):165-172. Doi: 10.5534/wjmh.2016.34.3.165
- [3] Brunner RJ, Demeter JH, Sindhwani P. Review of guidelines for the evaluation and treatment of leukocytospermia in male infertility. *World J Mens Health*. 2019May.37 (2):128–137. Doi: 10.5534/wjmh.180078
- [4] Eldamnhoury EM, Elatrash GA, Rashwan HM, El-Sakka AI. Association between leukocytospermia and semen interleukin-6 and tumor necrosis factor-alpha in infertile men. *Andrology*. 2018 Sep.6 (5):775–780. Doi: 10.1111/andr.12513
- [5] Bhushan S, Schuppe HC, Tchatalbachev S, Fijak M, Weidner W, Chakraborty T, Meinhardt A, Testicular innate immune defense against bacteria. *Mol Cell Endocrinol*. 2009 Jul;306 (1–2):37–44. Doi: 10.1016/j.mce.2008.10.017
- [6] Guan R, Wang Q, Sundberg EJ, Mariuzza RA. Crystal structure of human peptidoglycan recognition protein S (PGRP-S) at 1.70 Å resolution. *J Mol Biol*. 2005 Apr;347 (4):683–691. Doi: 10.1016/j.jmb.2005.01.070
- [7] Wang M, Liu L-H, Wang S, Li X, Lu X, Gupta D, Dziarski R, Human Peptidoglycan Recognition Proteins Require Zinc to Kill Both

Gram-positive and Gram-Negative Bacteria and Are Synergistic with Antibacterial Peptides. *J Immunol.* 2007 Mar;178 (5):3116–25.

Doi: 10.4049/jimmunol.178.5.3116

- [8] Yang J. Human Peptidoglycan Recognition Protein 1 in Innate Immunity – A Review. *Pak J Zool.* 2019;51 (3) 801-1202.

Doi: <http://dx.doi.org/10.17582/journal.pjz/2019.51.3.rev1>

- [9] Dziarski R, Gupta D. Mammalian peptidoglycan recognition proteins (PGRPs) in innate immunity. *Innate Immun.* 2010 Jun;16 (3):168–174.

Doi: 10.1177/1753425910366059

- [10] Edition F. Examination and processing of human semen. Vol. Edition, V, World Health Organization. 2010. 286 p.

- [11] Sandoval JS, Raburn D, Muasher S. Leukocytospermia: Overview of diagnosis, implications, and management of a controversial finding. *Middle East Fertil Soc J.* 2013 Sep;18 (3):129–34.

Doi.org/10.1016/j.mefs.2013.02.004

- [12] Luo Q, Yao F, Zhang L, Li X, Qing C, Su R, Guo Y, Huang Z, Li J. Elevated expression of Pglyrp1 in peripheral blood mononuclear cells and PGLYRP-1 in serum from systemic lupus erythematosus patients. *Int J Clin Exp Med.* 2019 Apr;12 (4):3398–408.

- [13] De Marzi MC, Todone M, Ganem MB, Wang Q, Mariuzza RA, Fernández MM, Malchoidi EL. Peptidoglycan recognition protein-peptidoglycan complexes increase monocyte/macrophage activation and enhance the inflammatory response. *Immunology.* 2015 Jul;145 (3):429–42.

- [14] Alzaidi, Z., Yildiz, Ş.M., Saatçi, Ç. et al. The effect of cytokine leukemia-inhibitory factor (LIF) and interleukin-11 (IL-11) gene expression on the primary infertility related to polycystic ovary syndrome, Tubal factor, and Unexplained infertility in Turkish women. *Egypt J Med Hum Genet* 22, 85 (2021). <https://doi.org/10.1186/s43042-021-00201-9>