Accepted: 16 June 2016

DOI: 10.1111/and.12685

ORIGINAL ARTICLE

WILEY ANDROLOGIA

Inflammatory and anti-inflammatory cytokines in the seminal plasma of infertile men suffering from varicocele

M. Zeinali[1] | A. Hadian Amree[2] | H. Khorramdelazad[3] | H. Karami[4] | M. Abedinzadeh[3,4]

[1]Social Determinants of Health Research Center, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran

[2]Hemoglobinopathy Institute, Thalassemia Research Center, Mazandaran University of Medical Science, Sari, Iran

[3]Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

[4]Department of Urology, Shahid Rahnemoon Hospital, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran

Correspondence
Mehdi Abedinzadeh, Department of Urology, Shahid Rahnemoon Hospital, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran.
Email: abedinoro@yahoo.com

Summary

Varicocele is one of causes of the declined sperm quality and low sperm production, which can lead to infertility in males. There are several experimental and epidemiological findings which support the idea that inflammatory mechanisms play an essential role in varicocele pathogenesis. Besides, in this pathological state, interleukin-37 (IL-37) as an anti-inflammatory cytokine is able to bind interleukin-18-binding protein (IL-18BP), and subsequently binds IL-18 receptor β, inhibiting the pro-inflammatory activity of IL-18. To explore the interaction between IL-37 and IL-18 in infertility, we measured the amount of these cytokines in the seminal fluid of infertile men affected by varicocele. The seminal plasma levels of IL-37 and IL-18 were measured in 75 infertile men with varicocele and 75 healthy fertile controls (age range, 30–48 years) using enzyme-linked immunosorbent assay. The seminal levels of IL-37 and IL-18 were significantly increased in infertile men with varicocele when compared to fertile controls ($p < .0001$). Because of the essential role(s) of cytokines in inflammatory response of cell systems, it could be possible that sperm motility is reduced following increased IL-18, activated neutrophils and reactive oxygen species in semen of infertile patients with varicocele. Moreover, the results of this study indicated that interaction between IL-37 and IL-18Rβ can lead to reduced inflammatory responses. It seems that IL-37 might be a potential biomarker and therapeutic target for male infertility.

KEYWORDS

IL-18, IL-37, inflammation, male infertility, varicocele

1 | INTRODUCTION

Male genital tract inflammation is one of the most common causes of male infertility (Kopa, Wenzel, Papp, & Haidl, 2005). In infertile males, inflammation is often clinically silent and could be caused by infection and other pathological factors such as varicocele (Nallella et al., 2004). Moreover, an increased number of WBCs, including macrophages and neutrophils, are reported in semen samples of men affected by either genital tract infection or infertility (Bagheri, Hassanshahi, Zeinali, Abedinzadeh, & Khorramdelazad, 2016), which disrupt the fertilising ability of spermatozoon (Lobascio et al., 2015; Niederberger, 2012). During genital tract inflammation or cellular immunity against microbial antigens, activated leucocytes in human seminal fluid are able to release elastase (PMN elastase), cathepsin G, collagenase, reactive oxygen species (ROS), inflammatory and anti-inflammatory cytokines (Makker, Agarwal, & Sharma, 2009). Some investigations have suggested that varicocele-related infertility is also associated with cytokines (Fraczek & Kurpisz, 2015; Hakimi et al., 2014; Matalliotakis et al., 2002; Sahin et al., 2006). Additionally, various immunocompetent cells are capable to release cytokines in the male urogenital tract (Kocak, Yenisey, Dündar, Okyay, & Serter, 2002). These molecules play an important role in cell signalling and perform broad pleomorphic activities (Cho, Esteves, & Agarwal, 2016). Furthermore, cytokines may be mediators of oxidative stress and have the potential to alter redox equilibrium (Hagan et al., 2015; Havrylyuk,

Chopyak, Boyko, Kril, & Kurpisz, 2015) and they are also able to influence sperm function and fertility (Aitken, Baker, & Nixon, 2015; Fraczek, Szumala-Kakol, Dworacki, Sanocka, & Kurpisz, 2013). As an anti-inflammatory cytokine, IL-37 is associated with plasma cells and this new member of the IL-1 family is constitutively expressed in the cytoplasm of peripheral blood mononuclear cells (PBMCs) and monocytes (Boraschi et al., 2011). Upregulated expression of IL-37 has been found in several chronic inflammatory and autoimmune diseases such as *Mycobacterium avium* infections, atherosclerotic plaques, psoriatic plaques, Crohn's disease and lupus (Boraschi et al., 2011). On the other hand, recent data indicate a role for IL-18 in the pathogenesis of several inflammatory diseases (Dinarello, Novick, Kim, & Kaplanski, 2013). IL-18 is able to activate neutrophils, which are involved in the pathogenesis of infertility and varicocele (Leung et al., 2001). To suppress pro-inflammatory activity of IL-18, IL-37 binds to IL-18BP and subsequently binds to IL-18Rβ. IL-37 also binds to the IL-18R α-chain, but the affinity of this cytokine is much lower than IL-18 (Nold et al., 2010). It seems that the major role of IL-37 secreted from macrophages in semen fluid is to facilitate a negative feedback mechanism to suppress excessive inflammatory responses that is helpful in the sperm motility (Tete et al., 2012).

Regarding the role of inflammatory responses, activation of neutrophils by IL-18 and also presence of macrophage in semen of infertile men with varicocele, the aim of this study was to examine IL-37 and IL-18 seminal levels in fertile donors and infertile patients with varicocele and to explore the therapeutic potency of IL-37 in this disease.

2 | PATIENTS AND METHODS

Seventy-five patients, aged 30–48 years, suffered from varicocele were enrolled in present experimental research programme. All patients were clinically examined, and the diagnosis of varicocele was confirmed by physical examination and colour Doppler ultrasonography. Varicocele grade ranged from II to III. All patients were affected by primary infertility for at least 2 years, and any other cause of infertility was excluded. Exclusion criteria included urogenital infection, sexually transmitted diseases, hypogonadism, genetic defects, and occupational exposure to spermatogenetic-toxic chemicals, chemotherapy or radiotherapy treatment, vasectomy, and retrograde ejaculation. Semen analysis was performed for each separate patient and control specimen. All participating subjects gave an informed consent according to guidelines of the Helsinki Declaration.

2.1 | Semen analysis

Seminal fluid was analysed at 1 hr after collection, and semen parameters, including ejaculate volume, sperm count, per cent motility and sperm morphology, were assessed according to WHO classification (World Health Organization, Department of Reproductive Health & Research, 2010). Specimens with greater than 1 million leucocytes/ml (or >5/HPF) were considered as pyospermia. A single well-trained and experienced technician performed all semen examinations.

2.2 | Cytokines detections

We utilised enzyme-linked immunosorbent assay (ELISA) kits (Adipogen, San Diego, USA) to detect and quantify IL-37 and (eBioscience, San Diego, USA) for IL-18 in aliquots of frozen–thawed seminal plasma. The ELISA was conducted according to manufacturer's guidelines, with a sensitivity of 10 and 9.2 pg/ml respectively.

TABLE 1 Demographic data and comparison of semen characteristics, between fertile donors and men with varicocele (mean ± SEM and $p < 0.05$ considered statistically significant)

	Normal donors ($n = 75$)	Infertile men with varicocele ($n = 75$)	p value
Age (years)	35.1 ± 1.3	37.4 ± 2.1	.01
Duration of infertility (year)	–	3.75 ± 2.7	.04
Sperm concentration (10^6/ml)	71.6 ± 18.4	30.9 ± 28.4	.003
Sperm motility (%)	59.2 ± 19.42	36.2 ± 32.5	.001
WBC (HPF)	1.8 ± 0.32	5.68 ± 0.65	<.0001

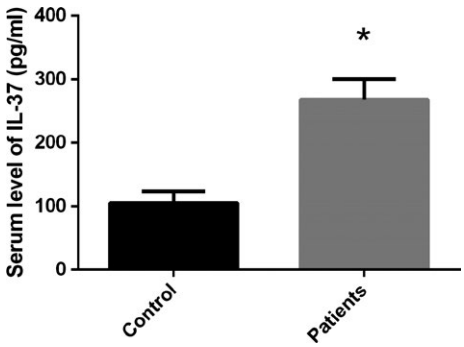

FIGURE 1 IL-37 levels in seminal plasma of fertile donors (control) and infertile men with varicocele (patients). Results are expressed as mean ± SEM of three experiments. *Statistically significant difference between control and patients ($p < 0.05$)

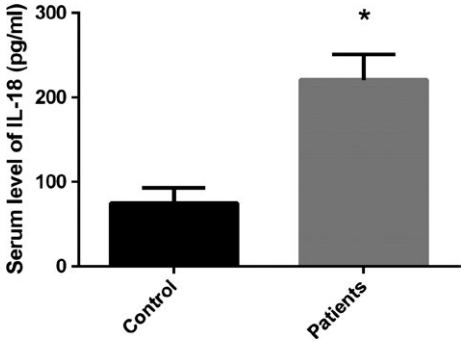

FIGURE 2 IL-18 levels in seminal plasma of fertile donors (control) and infertile men with varicocele (patients). Results are expressed as mean ± SEM of three experiments. *Statistically significant difference between control and patients ($p < .05$)

2.3 | Statistical analysis

To statistically analysis the data, the SPSS package version 18 was employed. The significance of the differences within the individual groups of the tested population was evaluated with the parametric *t* test and nonparametric Mann–Whitney *U* test. A *p* value of less than .05 was considered significant.

3 | RESULTS

The mean age was similar between men with varicocele (37.4 ± 2.1 years) and healthy donors (35.1 ± 1.3 years). Table 1 details the seminal characteristics in the fertile donors and infertile men with varicocele. The median sperm concentration and sperm motility in the fertile donors were significantly greater than in the infertile patients with varicocele ($p < .001$ and $p < .01$ respectively). Moreover, the seminal levels of IL-37 were 265.46 ± 34.02 and 109.63 ± 18.95 in infertile men with varicocele and healthy controls, respectively, showing statistically significant difference between the two groups ($p < .0001$; Fig. 1). It was also observed that the seminal IL-18 levels were 209.5 ± 10.93 and 73 ± 17.06 pg/ml in the patients and controls respectively ($p < .0001$; Fig. 2).

4 | DISCUSSION

It has been widely accepted and established that during the inflammatory responses, a broad spectrum of inflammatory mediators, including cytokines, is released from inflamed tissues, organs and migrated immune cells (Elenkov, Iezzoni, Daly, Harris, & Chrousos, 2005). Additionally, neutrophils and macrophages are the main leucocytes found in semen which can be detrimental to spermatozoon through ROS production and apoptosis induction (Tremellen & Tunc, 2010). However, semen of fertile men normally contains these immune cells that do not appear to affect sperm function. Besides, it has been suggested that small number of neutrophils may have advantageous effects through removal of abnormal and degenerative spermatozoa (Agarwal, Virk, Ong, & du Plessis, 2014). Furthermore, ROS provided by leucocytes or granulocytes have damaging effects on human spermatozoa, causing a marked loss of sperm motility and morphology and thus reducing hyperactivation and oocyte penetration (Koppers, Mitchell, Wang, Lin, & Aitken, 2011). It has been well evidenced and theorised that increased levels of ROS result in oxidative stress, which can seriously damage sperm function, motility, fertilising potency and the integrity of sperm genome (Crippa, Magli, Ferraretti, Pipitò, & Pescatori, 2015). The present study was designed to investigate seminal levels of IL-37 and IL-18 in infertile males with varicocele. To the best of our knowledge, this is the first report of an association between seminal IL-37 and IL-18 levels and male infertility. Accordingly, in this case–control study, we showed the elevated seminal IL-37 levels, also known as IL-1F7, as a member of anti-inflammatory cytokines derived from macrophages. Moreover, our findings showed that semen pattern of IL-18 increased significantly in infertile men compared to fertile donors. Bagheri et al. reported the elevated level of other pro-inflammatory molecules such as S100A12 in semen of infertile men with varicocele (Bagheri et al., 2016). In another study, Hakimi et al. (2014) showed that the semen level of IL-10 as an anti-inflammatory cytokine was significantly increased and this led to reduced immune responses against pathogen in *Chlamydia trachomatis*-infected patients. In this research programme, increased semen levels of IL-18 in infertile men may reveal that this member of inflammatory cytokines is a cause of accumulation of activated neutrophils (Netea et al., 2000) in the lower part of genital tract, which may be associated with reduction of sperm motility in these patients.

Taken together, regarding our findings and studies cited above, it could be concluded that several cell types in the male reproductive system, including migrated and resident immune cells, are involved in development of immunological responses in infertility. Activation of neutrophils by IL-18 may also play a role in the pathogenesis of infertility. On the other hand, IL-37 secreted from resident macrophages can regulate and reduce inflammatory responses. Our results may reemphasise that these cytokines could be considered as potential novel biomarkers and therapeutic targets in male infertility. Furthermore, regarding the important role(s) of cytokines in the pathogenesis of male infertility, we suggest evaluating expression of other members of IL-1 family and their respective receptors on immune cells in semen of infertile male suffering from varicocele.

ACKNOWLEDGEMENTS

Authors of current article take this opportunity to appreciate all of patient who warmly co-operated in this research programs. This project was financially supported by a grant from Rafsanjan University of Medical Sciences.

CONFLICT OF INTEREST

None of the authors of this study declared conflict of interest.

REFERENCES

Agarwal, A., Virk, G., Ong, C., & du Plessis, S. S. (2014). Effect of oxidative stress on male reproduction. *The World Journal of Men's Health*, *32*, 1–17.

Aitken, R. J., Baker, M. A., & Nixon, B. (2015). Are sperm capacitation and apoptosis the opposite ends of a continuum driven by oxidative stress? *Asian Journal of Andrology*, *17*, 633–639.

Bagheri, V., Hassanshahi, G., Zeinali, M., Abedinzadeh, M., & Khorramdelazad, H. (2016). Elevated levels of S100A12 in the seminal plasma of infertile men with varicocele. *International Urology and Nephrology*, *48*, 343–347.

Boraschi, D., Lucchesi, D., Hainzl, S., Leitner, M., Maier, E., Mangelberger, D., … Posselt, G. (2011). IL-37: A new anti-inflammatory cytokine of the IL-1 family. *European Cytokine Network*, *22*, 127–147.

Cho, C. L., Esteves, S. C., & Agarwal, A. (2016). Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. *Asian Journal of Andrology*, *18*, 186–193.

Crippa, A., Magli, M., Ferraretti, A., Pipitò, A., & Pescatori, E. (2015). Oxidative stress evaluation in sperm samples from fertile and infertile men. *Andrology S1*, *2*, 2167–0250.1000.

Dinarello, C. A., Novick, D., Kim, S., & Kaplanski, G. (2013). Interleukin-18 and IL-18 binding protein. *Frontiers in Immunology*, *4*, 289–294.

Elenkov, I., Iezzoni, D., Daly, A., Harris, A., & Chrousos, G. (2005). Cytokine dysregulation, inflammation and well-being. *NeuroImmunoModulation*, *12*, 255–269.

Fraczek, M., & Kurpisz, M. (2015). Cytokines in the male reproductive tract and their role in infertility disorders. *Journal of Reproductive Immunology*, *108*, 98–104.

Fraczek, M., Szumala-Kakol, A., Dworacki, G., Sanocka, D., & Kurpisz, M. (2013). In vitro reconstruction of inflammatory reaction in human semen: Effect on sperm DNA fragmentation. *Journal of Reproductive Immunology*, *100*, 76–85.

Hagan, S., Khurana, N., Chandra, S., Abdel-Mageed, A., Mondal, D., Hellstrom, W., & Sikka, S. (2015). Differential expression of novel biomarkers (TLR-2, TLR-4, COX-2, and Nrf-2) of inflammation and oxidative stress in semen of leukocytospermia patients. *Andrology*, *3*, 848–855.

Hakimi, H., Akhondi, M. M., Sadeghi, M. R., Chamani, L., Arababadi, M. K., Ahmadabadi, B. N., … Fathollahi, M. S. (2014). Seminal levels of IL-10, IL-12, and IL-17 in men with asymptomatic *Chlamydia* infection. *Inflammation*, *37*, 122–126.

Havrylyuk, A., Chopyak, V., Boyko, Y., Kril, I., & Kurpisz, M. (2015). Cytokines in the blood and semen of infertile patients. *Central-European Journal of Immunology*, *40*, 337–344.

Kocak, I., Yenisey, C., Dündar, M., Okyay, P., & Serter, M. (2002). Relationship between seminal plasma interleukin-6 and tumor necrosis factor α levels with semen parameters in fertile and infertile men. *Urological Research*, *30*, 263–267.

Kopa, Z., Wenzel, J., Papp, G., & Haidl, G. (2005). Role of granulocyte elastase and interleukin-6 in the diagnosis of male genital tract inflammation. *Andrologia*, *37*, 188–194.

Koppers, A. J., Mitchell, L. A., Wang, P., Lin, M., & Aitken, R. J. (2011). Phosphoinositide 3-kinase signalling pathway involvement in a truncated apoptotic cascade associated with motility loss and oxidative DNA damage in human spermatozoa. *Biochemical Journal*, *436*, 687–698.

Leung, B. P., Culshaw, S., Gracie, J. A., Hunter, D., Canetti, C. A., Campbell, C., … McInnes, I. B. (2001). A role for IL-18 in neutrophil activation. *The Journal of Immunology*, *167*, 2879–2886.

Lobascio, A., De Felici, M., Anibaldi, M., Greco, P., Minasi, M., & Greco, E. (2015). Involvement of seminal leukocytes, reactive oxygen species, and sperm mitochondrial membrane potential in the DNA damage of the human spermatozoa. *Andrology*, *3*, 265–270.

Makker, K., Agarwal, A., & Sharma, R. (2009). Oxidative stress & male infertility. *Indian Journal of Medical Research*, *129*, 357–367.

Matalliotakis, I., Arici, A., Goumenou, A., Koumantakis, G., Selam, B., Matalliotakis, G., & Koumantakis, E. (2002). Distinct expression pattern of cytokines in semen of men with genital infection and oligo-teratoasthenozoospermia. *American Journal of Reproductive Immunology*, *48*, 170–175.

Nallella, K. P., Allamaneni, S. S., Pasqualotto, F. F., Sharma, R. K., Thomas, A. J., & Agarwal, A. (2004). Relationship of interleukin-6 with semen characteristics and oxidative stress in patients with varicocele. *Urology*, *64*, 1010–1013.

Netea, M. G., Fantuzzi, G., Kullberg, B. J., Stuyt, R. J., Pulido, E. J., McIntyre, R. C., … Dinarello, C. A. (2000). Neutralization of IL-18 reduces neutrophil tissue accumulation and protects mice against lethal *Escherichia coli* and *Salmonella typhimurium* endotoxemia. *The Journal of Immunology*, *164*, 2644–2649.

Niederberger, C. (2012). Re: Seminal leukocytes are good samaritans for spermatozoa. *Journal of Urology*, *188*, 1315–1319.

Nold, M. F., Nold-Petry, C. A., Zepp, J. A., Palmer, B. E., Bufler, P., & Dinarello, C. A. (2010). IL-37 is a fundamental inhibitor of innate immunity. *Nature Immunology*, *11*, 1014–1022.

Sahin, Z., Celik-Ozenci, C., Akkoyunlu, G., Korgun, E. T., Acar, N., Erdogru, T., … Ustunel, I. (2006). Increased expression of interleukin-1α and interleukin-1β is associated with experimental varicocele. *Fertility and Sterility*, *85*, 1265–1275.

Tete, S., Tripodi, D., Rosati, M., Conti, F., Maccauro, G., Saggini, A., … Toniato, E. (2012). IL-37 (IL-1F7) the newest anti-inflammatory cytokine which suppresses immune responses and inflammation. *International Journal of Immunopathology and Pharmacology*, *25*, 31–38.

Tremellen, K., & Tunc, O. (2010). Macrophage activity in semen is significantly correlated with sperm quality in infertile men. *International Journal of Andrology*, *33*, 823–831.