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Original Article

Interleukin-6 in Seminal Plasmas of Azoospermic and Severe Oligo-Astheno-Teratozoospermic Patients

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Abstract

Background: The IL-6 levels in seminal plasma have an important impact on sperm quality. This study aimed to evaluate the correlation between IL-6 levels in seminal plasma and sperm parameters for Moroccan population.

Methods: This is a case control study. Semen samples were obtained from patients who consulted the Medical Analysis Laboratory and Reproductive Biology Labomac, Casablanca, Morocco from Apr 2022 to Jun 2023, among them 50 presenting azoospermia, 25 severe oligo-astheno-teratozoospermia and 25 normozoospermia.

Results: Significant differences regarding age of azoospermic patients (P=0.008) and BMI for OATS and azoospermic (P=0.032, P=0.047 respectively), also for physical activity azoospermic P<0.01 and P=0.02 for OATS patients same difference for micronutrient intake especially for zinc. Sperm parameters present also statistically significant in terms of motility, concentration, and morphology (P < 0.05). Semen interleukin-6 levels showed a significant difference for azoospermic patients (P=0.045) also for OATS (P=0.001). On the other hand, leucoscreen analysis revealed a significantly higher mean leukocytes concentration in azoospermic and OATS patients (P=0.004 and P<0.001 respectively).

Conclusion: The seminal plasma IL-6 assay could be used as a sensitive biomarker of silent infection or inflammation of the male genital tract.

Keywords: Male infertility; Interleukin-6; Seminal plasma; Azoospermia; Ligo-astheno-teratozoospermia

Introduction

Cytokines play an important role in intercellular communication (1). In addition to their role in immune regulation, there is evidence that some of these peptides are directly involved in the

regulation of testicular function and may also be potent modulators of testicular steroid release (2). Human sperm contains a range of cytokines, but their impact on sperm quality and function remains controversial (3). Inflammatory cytokines are produced by white blood cells (primarily macrophages) in response to foreign antigens, pathogens and in the context of chronic inflammation (4).

These cytokines are regulatory produced and secreted by leukocytes and other cells and they have been implicated as growth and differentiation factors (5). The seminal plasma contains several cytokines and chemokines which are normally present in the male genital tract. They can also stimulate sperm peroxidation and increased reactive oxygen species (ROS) (6). The high level of cytokines, especially IL-6 causes a decrease in sperm motility (7). Moreover, there is a positive correlation between IL-6 contains in seminal plasma and sperm parameters (8).

The male and female reproductive tracts contain numerous immunoreactive cells can modulate the immune response also affect tissues outside the immune system (9,10). IL-6 is considered a pro-inflammatory cytokine produced by many different cells, such as Leydig and Sertoli cells. However, the association between IL-6, sperm parameters and leukocytospermia has been discussed (11) but to yet established for Moroccan population. In this study, we aimed to determine the relationship between interleukin-6 levels in seminal plasma, leukocyte concentration, and abnormal sperm parameters.

According to the definition given by the WHO laboratory manual for the examination and processing of human semen, severe oligo-astheno-teratozoospermia (OATS) is characterized by the presence of total number of spermatozoa below than normal in the ejaculate (oligozoospermia), progressive motile (PR) below the lower reference limit (Asthenozoospermia), morphologically normal spermatozoa lower than normal (Teratozoospermia) (12).

Methods

Samples and patient's selection criteria

This case-control study was conducted on patients who consulted the medical analysis laboratory and reproductive biology, Labomac from Apr 2022 to Jun 2023. Overall, 170 men aged between 27 and 50 yr old from subfertile couples including patients diagnosed with severe oligo-astheno-teratozoospermia (OATS) (concentrations $<16\times10^6$ ml; progressive motility <30%; morphology <4%), others are azoospermic patients and the rest are normozoospermic with normal-appearing sperm. Overall, 170 patients were assessed for eligibility, 100 patients met the selection criteria and were analyzed (Fig. 1).

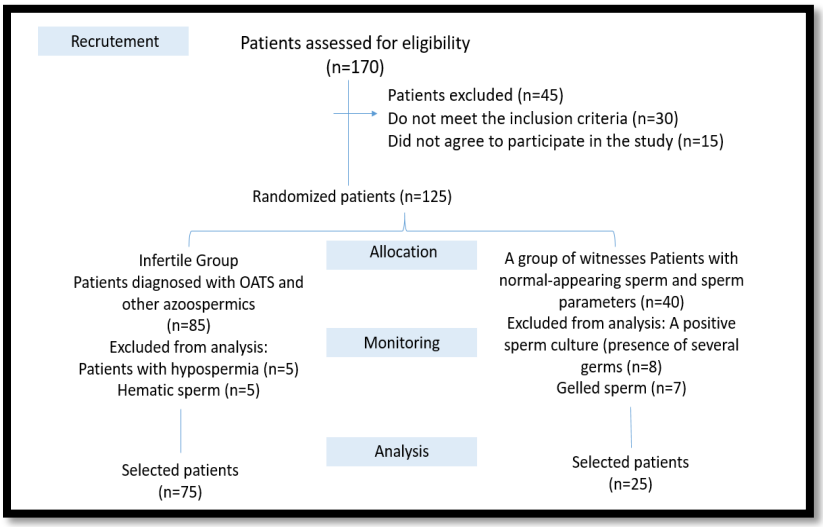

Fig. 1: Presentation of the criteria for choosing the study population (n=100)

The following possible confounding variables are excluded: Infertile patients receiving hormonal treatment or other medications such as antibiotics, anti-inflammatory medications such as non-steroidal anti-inflammatory drugs (NSAIDs), patients using participants using antioxidant supplements, omega-3, or severe inflammatory diseases of the reproductive system such as urethritis, orchitis, epididymitis or prostatitis also a history of chemotherapy, radiotherapy, chronic diseases. All participants with excessive alcohol consumption or high tobacco use. Individuals exposed to environmental toxins or chemicals. Participants with high levels of psychological stress or psychiatric disorders.

BMI, physical activity and micronutrient intake assessment

To have more information about participants, three questionnaires were completed for each patient. The first questionnaire provided the different socio-demographic characteristics and anthropometric data. Anthropometric assessment mainly includes height and weight measurements. BMI was calculated as weight (Kg) divided, height (Kg/m^2). Patients with a BMI≥ 25 kg/m^2 were considered as overweight.

The second questionnaire contains dietary information collected using a semi-quantitative food frequency questionnaire (FFQ) with 116 food items. Participants were asked to indicate the serving the frequency of each of the foods and drinks included in the FFQ. The questionnaire included 9 options for frequency of intake, ranging from <1 time per month to ≥6 times per day. Nutrient intakes were estimated by summing the nutrient contribution of all food items in the questionnaire (13). Then average daily energy and nutrient intakes were calculated using NUTRITIONIST IV software, version 3.5.2, N-Squared [NZ] Ltd., Palmerston North, New Zealand.

The third questionnaire is about physical activity assessment. Physical activity (PA) and sedentary behavior were estimated using the International Physical Activity Questionnaire (IPAQ) (14). Our primary exposure of interest was total hours of metabolic equivalent (MET) physical activity per week. METs for each level of physical activity were calculated (Walking = 3.3 METs, Moderate PA = 4 METs and Vigorous PA = 8 METs). According to these scores, participants were divided into three groups depending on the intensity of PA as described in the IPAQ: Low (<599 MET-min/ week), moderate (600–2999 MET-min/week), high (3000–5999 MET-min/week) (15).

Semen analysis

The semen samples were collected from patients by masturbation after 3-5 days of abstinence. After liquefaction at 35°± 2°C, microscopic analysis was performed in accordance with the standards and guidelines of WHO 2021. We determined the volume, pH, count, progressive mobility, morphology, vitality (12).

Determination of leukocytes concentration

Peroxidase staining is a reliable method for determining leukocytes in semen by the Leucoscreen kit (FertiPro, Beernam, Belgium). First, all round cells were counted on a Makler chamber, and the concentration of round cells was determined. A threshold of 1 million/ml is a WHO reference to indicate the presence or absence of leukospermia.

Preparation of seminal plasmas

After sperm parameters analysis, 1 ml of sperm was centrifuged at 1500 rpm for 15 min. Seminal plasma was collected and stored at -20° until further use.

Quantitative detection of human IL-6 in semen plasma

IL-6 concentration was determined in all seminal plasma samples using the cobas® 6000 e 601 Electro Chemiluminescence Analyzer Series module using an ELISA principle immunochemical provided by the Elecsys® IL-6 kit (Roche Diagnostics, Penzberg, Germany). Tests were carried out according to the manufacturer's in-

structions. The analytical sensitivity of the test was 1.5 pg/mL and a value up to 7 pg/mL is considered normal.

To avoid any potential bias when carrying out this study, certain conditions were also taken into consideration. The assay difficulties related to poorly liquefied samples, the presence of debris, or a gelled semen sample. In addition, a poor calibration of the machine or the expiration of the dosing kit date. Other measures have been taken to prevent improper storage of samples or long of storage. Finally, the ratio between freezing and thawing was considered to minimize any impact on the assay results.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics ver. 27.0.1.0 (IBM Corp., Armonk, NY, USA). The data are presented as means mean ± standard deviation (SD), for age, semen parameters, IL-6 levels, and leukocytes concentration, BMI, physical activity and micronutrient intake. A one-way ANOVA was used to compare these variables across different study groups. The assumptions of normal distribution and homogeneity of variances were confirmed, with normality verified using Shapiro-Wilk test and homogeneity of variances assessed using Levene's test. When significant differences were found, post-hoc analyses, specifically the Tukey test, was conducted to identify which groups differed from each other. A *P*-value less than 0.05 was considered statistically significant.

Ethical Considerations

This study was reviewed and approved by the Ethics Committee of Mohammed VI University of Sciences and Health (UM6SS) (Casablanca, Morocco) (No. CE/UM6SS/09/23). All methods performed were in accordance with relevant guidelines of the Declaration of Helsinki as revised in 2013. The informed consent form was obtained from each participant before the initiation of the study.

Results

Patient's characteristics

The age of the patients participating in this study ranged between 27 and 50 yr with a mean of 41.11 ± 6.322 yr. The mean age values for azoospermics and OATS were 44.45 ± 5.888 yr and 36.94 ± 5.994 yr, respectively. There was a statistically significant age difference for azoospermics (*P*=0.008) unlike OATS (*P*=0.101) (Table 1). Duration of infertility for healthy men 2.38 ± 1.371 yr unlike the infertile population studied: 8.72 ± 4.748 yr (*P*<0.001) for azoospermia and 5.06 ± 2.886 yr (*P*=0.002) for OATS. The mean body mass index (BMI) of patients with OATS is 24.2±2.102 Kg /m^2 and azoospermia 28.3±2.154 Kg /m^2 presenting a statistical significance difference: *P*=0.032; *P*=0.047 respectively indicated in Table 1.

Table 1: Baseline characteristics of the study population (N=100), including age and duration of infertility

Patients criteria	Populations	Median ± SD	P-value
Patient age (yr)	Azoospermia	44.45 ± 5.888	0.008*
	OATS	36.94 ± 5.994	0.101
	Normospermic	39.96 ± 5.504	
Duration of infertility (years)	Azoospermia	8.72 ± 4.748	<0.001*
	OATS	5.06 ± 2.886	0.002*
	Normospermic	2.38 ± 1.371	
BMI (Kg /m^2)	Azoo	28.3±2.154	0.032*
	OATS	24.2±2.102	0.047*
	Normozoospermic	23.6±1.687	

Descriptive indicators of study values are presented as mean ± SD. OATS; Severe oligo-astheno-teratozoospermia; Normozoospermia constitute the control group; * shows a significant difference

Table 2 shows different physical activity levels for patients. The azoospermic participants performed a mean value of 755 MET-min/week for low physical activity levels. Notably no patient in this category have reached high physical activity indicating a sedentary lifestyle (P<0.01) same for patients presenting OATS there is also a statistically significant for physical activity (P=0.02).

Table 3 presents micronutrient intake for participants. There is not statistically significant for vitamins intake including vitamin A, E, C and Folate (P>0.05), same for beta carotene and selenium for azoospermics and OATS. Therefore, there is a statistically significant difference in Zinc for OATS patients and azoospermia (P=0.043 and P=0.02 respectively).

Table 2: Physical activity (PA) levels in azoospermic patients and OATS compared to control group (n=100)

Variable	PA levels (METs-min/week)			
Category of patients	Low PA	Moderate PA	High PA	*P*-value
Azoospermia (n=50)	755±98	300±67	0	<0.001*
OATS (n=25)	600±120	735±89	2000±500	0.02*
Normozoospermia (control group) (n=25)	450±75	2750±310	4570±420	

Descriptive indicators of study values are presented as mean ± SD. OATS; Severe oligo-astheno-teratozoospermia; Normozoospermia constitute the control group; * shows a significant difference

Table 3: Comparison of micronutrient intake between infertile patients and control subjects

Micronutrient intake	Populations	Median ± sd	P-value
Vitamin a (µg)	Azoospermic	978±34.398	0.08
	Oats	1345 ± 752	0.2
	Normozoospermic	1279± 639	
Vitamin e (mg)	Azoospermic	9.34±1.670	0.06
	Oats	12.12 ± 2.443	0.056
	Normozoospermic	14.25 ± 3.005	
Vitamin c (mg)	Azoospermic	56.2±19.665	0.07
	Oats	61.25 ± 18.432	0.3
	Normozoospermic	70.15 ± 7.687	
Folate (µg)	Azoospermic	440.6±76.7	0.07
	Oats	565.2±69.7	0.1
	Normozoospermic	670.2 ± 72.8	
Beta-carotene (µg)	Azoospermia	789.630±567	0.058
	Oats	7234±342	0.6
	Normozoospermic	8456.7±976	
Sélénium (µg)	Azoospermia	78±6.453	0.3
	Oats	57.6±39.237	0.1
	Normozoospermic	90±30.562	
Zinc (mg)	Azoospermia	8.23±3.564	0.02*
	Oats	4.32±2.452	0.043*
	Normozoospermic	12±1.76	

*Indicates a statistical significant difference

Semen parameters and IL-6 levels among studied groups

Azoospermics present a significant difference of *P*=0.029 for sperm count. OATS patients presented a significant difference affecting all parameters (Sperm count: *P*<0.001; Progressive Motility: *P*=0.001; Morphology: *P*=0.002 and vitality: *P*=0.031). The data demonstrated a significant difference in leukocytes concentration for azoospermics and OATS: *P*=0.004 and *P*<0.001 respectively. Therefore, IL-6 expression showed a significant difference between azoospermic patients (*P*=0.045) and OATS (*P*=0.001) (Table 4).

Table 4: Presentation of all sperm parameters in fertile men (control group) and infertile men (patients with azoospermia and severe oligo-astheno-teratozoospermia) and IL-6 levels of all populations studied (n=100)

Semen parameters	Populations	Median ± sd	P-value
Sperm concentration (10^6/ml)	Azoospermic	0	0.029*
	Oats	1.19 ± 1.743	<0.001*
	Normozoospermic	60.18 ± 31.538	
Progressive motility (%)	Azoospermic		
	Oats	25.12 ± 15.443	0.001*
	Normozoospermic	43.25 ± 13.005	
Vitality (%)	Azoospermic		
	Oats	61.25 ± 19.523	0.031
	Normozoospermic	73.25 ± 8.331	
Morphology (%)	Azoospermic		
	Oats	4.44 ± 1.209	0.002*
	Normozoospermic	9.00 ± 5.164	
Leukocyte concentration (10^6/ml)	Azoospermia	1.12	0.004*
	Oats	0.73	<0.001*
	Normozoospermic	0.34	
Il-6 levels (pg/ml)	Azoospermia	350.64	0.045*
	Oats	39.57	0.001*
	Normozoospermic	1.56	

Descriptive indicators of study values are presented as mean ± SD. All sperm parameters are presented in this table along with interleukin-6 levels for all participating populations. OATS; Severe oligo-astheno-teratozoospermia; Normozoospermia constitute the control group; * Indicates a statistically significant result (*P*<0.05)

Statistical analysis showed a significantly high mean value in patients with azoospermia of 350.64 pg/ml followed by a mean level of 39.57 pg/ml for OATS unlike finally normozoospermics 1.56 pg/ml.

Leukocytospermia and IL-6 levels

Leucoscreen analysis was able to demonstrate a significant increase in the average concentration of leukocytes for azoospermics 1.12×10^6/ml lower for OATS: 0.73×10^6/ml, unlike the control samples 0.34×10^6/ml.

Discussion

The involvement of immunity in the etiology of male infertility remains today to be far from being explained (16). Several different factors appear to play a crucial role in infertility which appears to be inflammation of the genitourinary tract (17). The release of pro-inflammatory cytokines is a natural process of inflammation (18). Several studies have shown the role of IL-6 in spermatogenesis (19), which is mainly produced by Sertoli cells through follicle-stimulating hormone (20). IL-6 has negative effects on the male reproductive system, causing pro-inflammatory stress with reduction in testosterone levels (21).

In this study, we targeted a population with azoospermia and others with abnormalities in concentration, progressive motility, and morphology of spermatozoa (OATS).

Data showed a mean value of 350.64pg/ml for azoospermic patients (P=0.045) and 39.57pg/ml for OATS patients (P<0.001). Camejo et al reported a higher concentration of IL-6 in the seminal plasma of infertile men compared to fertile men (22). On the other hand, IL-6 levels were simultaneously linked to an abnormal spermogram (OATS).IL-6 acts by binding to its receptor, mainly detected at the intermediate part, while activating different signaling pathways, including growth and differentiation including spermatogenesis (11).

The altered sperm quality in this case is a consequence of low expression of the IL-6 receptor (IL-6R) (23). Another IL-6/IL-6R pathway may produce the opposite effect by causing cell growth arrest and induction of apoptosis, which would alter sperm parameters (24, 25).

Seminal plasma is generally composed of energetic substrates for sperm in the form of simple sugars, antioxidant agents to protect and prevent possible damage to sperm, as well as minerals and salts (26, 28). It is also composed of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, TNFα, IFNγ and cytokines with anti-inflammatory properties including IL-10, transforming growth factor beta (TGF-β) andprostaglandin E2. Therefore, an optimal inflammatory response is important for normal fertility and pregnancy (29). IL-6 can then regulate uterine receptivity to embryo implantation and induce immune tolerance at the implantation site. Therefore, its significant increase can produce an opposite effect leading to repeated early abortions site (29, 30).

IL-6 expression was accompanied in many cases by leukospermia, which explains the presence of chronic inflammation of the urogenital tract since pro-inflammatory cytokines are a consequence of an immune response. The increase in free radicals is explained by several studies and therefore a low level of antioxidant capacity (31). The inflammatory process within the male genitourinary tract may also reduce the fertilizing potential of mature sperm (32, 33).

Male infertility may also be linked to lifestyle. In this case-control study, physical activity in infertile men with sperm abnormalities demonstrated a significant difference (azoospermia P=0.032 and OATS P=0.047). Moderate to vigorous physical activity was associated with higher sperm concentration (34). Reduced physical activity leads to greater sedentary behavior in infertile men (35). Specific types of physical activity affect sperm quality parameters differently (36). Infertile men present an overexpressed IL-6 presenting also an abnormal BMI≥25kg/m^2 which explains the relationship between overweight and inflammatory profile (37).

Hofny et al studied the impact of high BMI with abnormal sperm parameters especially sperm concentration and motility (38). We also analysed the impact of micronutrient intake on sperm quality, there is not a statistical significant for vitamins (A, E, C) same for folate, except for zinc for patients with OATS and azoospemria (P=0.043; P=0.02 respectively). Zinc is a mineral essential for normal functioning of male reproductive system. Many studies have demonstrated a positive correlation between seminal plasma zinc concentrations with sperm parameters (39

Age is the most critical parameter of male fertility (40, 41). Advanced paternal age leads to genetic modifications, increased cells renewal of testicular tissue, thus alterations of the spermatozoa (42, 44). The absence of natural antioxidant defense mechanisms following a vitamin and mineral deficiency is correlated with release of these cytokines in men over than 40 yr old (45). All these factors explain the long duration of infertility for this category of infertile patients. IL-6 can therefore be considered as diagnostic biomarker of male infertility.

This study had certain limitations, only a semen sample was taken from each participant, we knew that collecting multiple semen samples over 1-2 wk was distinct but this may reduce the number of participants and thus decrease the power of this study. We assessed nutrient intake and sperm

quality. However, answers on an FFQ may not reflect the concentration of nutrients in the blood or seminal plasma. Measuring relevant antioxidants in seminal plasma may be preferable. However, a single measurement may not capture nutrient exposures during the entire period of spermatogenesis, and it can show other factors like variation in absorption. The strength of the present study was the control for potential confounding factors like age, BMI, micronutrient intake, physical activity and semen leukocyte concentration. The study has also focused on a specific group of patients (azoospermia and OATS) contributing to valuable information of male infertility and provides a basis for future research.

Conclusion

Measurement of cytokines, including IL-6 in seminal plasma, could be a sensitive marker of early infection or inflammation of the male genitourinary tract. IL-6 could be considered as an important biomarker parameter of genitourinary tract inflammation in routine semen analysis, which could be a crucial step to analyze a cause of male infertility, since the role and function of leukocytes in sperm, as well as their clinically relevant pathological threshold. Additional studies are needed to verify the role of oxidative stress produced by leukocytes on sperm function. Additionally, studies on the long-term clinical effects of interleukin-6 will be helpful in developing better prevention and treatment strategies.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that they have no conflict of interest.

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