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Evaluation the level of antisperm antibodies, some cytokines and zinc for oligozoospermia, asthenozoospermia patients compared with normozoospermia

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Abstract---Evaluation of the level of anti-sperm antibodies, some cytokines and zinc for oligozoospermia patients, asthenozoospermia patients and normozoospermia patients. Semen samples were collected from asthenozoospermic, oligozoospermic, and normozoospermic men who attended the fertility center. The collected samples of normozoospermia patients were (15), Asthenozoospermia patients were (15), and Oligozoospermia patients were (13). Seminal plasma for all study groups was evaluated for interleukin-1, interleukin-6, and zinc levels. The result showed there were statistically significant differences ($p < 0.05$) in sperm concentration, progressive motility, and liquefaction time in the asthenospermia and oligospermia groups compared with the normospermia group. This study found that antisperm antibody, interleukin 1, and interleukin 6 were higher in the oligozoospermia and asthenozoospermia groups than in the normozoospermia group. The levels of antisperm antibodies and interleukins increase, while zinc levels decrease in the seminal plasma of patients with oligospermia, as well as those with asthenospermia, compared to patients with normospermia.

Keywords---antisperm, antibodies, oligozoospermia, asthenozoospermia, normozoospermia.

Introduction

Infertility is a disease that means can't occur pregnant after 12 months of regular sexual activity without protection (Borght and Wyns, 2018). A reduction in male

fertility potential may result from congenital or acquired conditions including urogenital abnormalities, varicocele, infections of the genital tract, genetic abnormalities, endocrine disturbances, testicular failure, cancer, systemic diseases, and exposure to gonad toxic factors and immunologic problems (Hamada et al., 2011). An immunological cause may be present in 5–15% of male infertility problems, including infections, cryptorchidism, primary testicular failure, testicular trauma, epididymitis, varicocele, idiopathic infertility, and epididymitis. As a result, males with primary or secondary infertility have ASA (Jewad et al., 2019). The presence of antisperm antibodies in serum is one of the potential immunologic causes of infertility. ASA is present in both primary and secondary infertile males (Jewad et al., 2019). Cytokines are a component of the male reproductive system's autocrine/paracrine network. Also, Fraczek and Kurpisz (2015) found that immune responses caused by cytokines seem to be both protective and/or risk factors for male fertility. The IL-1 gene family contains genes with crucial roles in spermatogenesis (Rozwadowska et al, 2005). Interleukin (IL)-6 regulates spermatogonial proliferation, germ cell differentiation, and Sertoli cell steroidogenesis and protein production (Martnez et al., 2010). Zinc is an important trace mineral for optimal male reproductive function (Zhao et al, 2016). Zinc is one of the necessary trace elements needed for proper male reproductive physiology and plays a crucial function in spermatogenesis (Kumar and Singh, 2016).

Materials and Methods

Participants

This study was conducted in the laboratory of advanced research of the Department of Laboratory Analysis/Faculty of Science/University of Kufa and in the laboratory of the Fertility Center in AL-Saader Medical City during the period from October 2021 to December 2021. Semen samples were collected from Asthenozoospermic, Oligozoospermic, and Normozoospermic who attended the fertility center. The collected samples of normozoospermia patients were (15), Asthenozoospermia patients were (15) and Oligozoospermia patients were (13). The age of infertile patients was between (20-55) years. The wife's age was between (12-38).antisperm antibody, interlukine-1, interleukin-6, and zinc levels were evaluated in seminal plasma for all study groups.

Study Design

The study was designed according to the following steps:

- Semen samples were examined microscopically and macroscopically.
- The samples were divided into oligozoospermia, asthenozoospermia, and normozoospermia as a control group according to the quid lines of World Health Organization WHO 2010
- The semen samples were placed in a centrifuge to separate the seminal plasma from the sperm pellet.
- The seminal plasma was placed in an Eppendorf tube and kept in the refrigerator at a temperature of -20 °C until the appropriate time for examining the studied immunological tests.

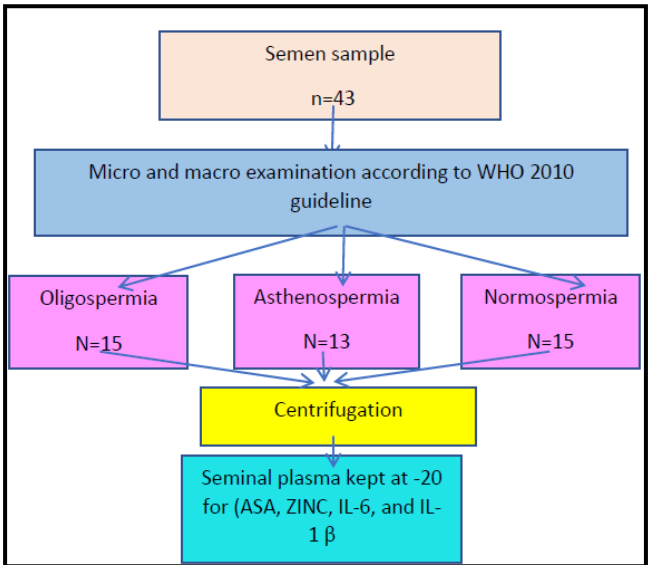

Figure 1. The proposal design of the study

Semen Collection and Analysis

After (2–7) days of sexual abstinence, seminal samples were obtained directly in a dry, clean, and sterile disposable container by masturbation in a room next to the laboratory for seminal fluid analysis. The container was labeled with the patient's name, age, and sexual abstinence at the time of sample collection. The collected specimens were placed at 37°C for 30 minutes in an incubator to allow liquefaction. The liquefied specimens were mixed carefully for a few seconds, and then the specimens were examined under a microscope. Analysis and classification of infertile patients were performed according to WHO (2010) utilized to estimate the results of seminal fluid analysis.

Macroscopically examination

- **Volume:** The volume of the seminal fluid is determined by aspirating the sample from the specimen container using a pipette or syringe. The usual volume of seminal fluid is between 1.5 and 6 ml; a sample is considered hypo-volumic if it is less than 1.5 ml and hyper-volumic if it is greater than 6 ml (WHO, 2010).
- **Liquefaction time:** At room temperature, the full sample typically liquefies in 15 minutes; however, in exceptional cases, it may take up to 60 minutes or longer. This should be noted if complete liquefaction does not occur within 60 minutes. Normal samples of liquefied semen may contain jelly-like granules that do not liquefy; these granules appear to have no clinical importance. However, the presence of mucus filaments may impede semen analysis.
- **Round Cells concentration:** The concentration of pus cells were measured by determined ten random fields and multiplying by 10^{16}, the normal concentration of round cells is < 1 per ml.

Leukocytes concentration

Leukocytes were counted using the Leucoscreen method, Briefly, one drop of semen was mixed with one drop of working solution (Leucoscreen stain and hydrogen peroxide), covered with a cover slip for 2 min, and then the result was read at a Magnification of ×400. Peroxidase-positive cells stained yellow to brown, whereas other cells stained pink (Mahran, and Saleh., 2014).

Calculate the proportion of peroxidase-positive cells as follows

Proportion positive round cells
Number of positive round cells divides by (Number of positive round cells + Number of negative round cells).

Than calculate the concentration of peroxidase-positive white blood cells in the semen sample as follows

Concentration (mill/mL) = Proportion positive round cells x total concentration of round cells

Biochemical analysis

Seminal plasma ASA and cytokine (IL-1 and IL-6) levels were determined using an immunological method (Enzyme-Linked-Immuno-Sorbent-Assay), which employs the quantitative sandwich enzyme immunoassay technique, and zinc levels in seminal plasma were determined using a flame atomic absorption device.

Statistical Analysis

Data of the present study was analyzed by using SPSS Statistics Version 23.0 (IBM SPSS Statistics for Windows, Version 23.0. IBM Corp). Comparison of differences between continuous variables was done by using the Paired t-Test when compared two means and One Way ANOVA test to compare three variables; Multiple Pairwise Comparisons were performed by using least significant differences (L.S.D). Chi squared test was performed to analyze categorized data; Pearson's correlation analysis was used to determine the relationship between variables. P values ≤ 0.05 and ≤ 0.01 considered as significant and highly significant (Abdulla et al., 2022; Alhasnawi and Aljanaby, 2022; Hadi and Aljanaby, 2022).

Results

Semen parameters in studied groups for infertile men

In table 1 showed there were statistically significant differences ($p < 0.05$) in sperm concentration, progressive motility, and liquefaction time in the asthenospermia and oligospermia groups compared with the normospermia group. While there was no statistically significant difference in normal sperm morphology, semen volume and leucocytes in the two groups that were compared with the control group (normozoospermia).

Table 1
Semen parameters of normozoospermic, oligozooospermic and asthenozoospermic of infertile men

Semen parameters	Normozoospermia N=15	Oligozoospermia N=15	Asthenozoospermia N=13	P-value
Sperm concentration (Million/ml)	64.50+ 25.193a	5.67+ 4.431b	38.38+ 22.593a	<0.01**
Progressive motility (%)	55.33+ 14.573a	22.53+ 16.501b	16.77+ 9.382b	<0.01**
Normal sperm morphology (%)	40.33+ 14.201	41.00+ 17.57	43.00+ 8.972	0.878 ns
Liquefaction time (Min)	33.54+2.222b	35.73+1.710a	36.07+2.344a	0.006**
Semen volume (ml)	2.767+ 1.099	2.840+ 0.966	2.554+ 1.259	0.783ns
Leucocytes (Million/ml)	1.00+	1.47+1.457	1.31+1.109	0.476 ns

**=significant differences at P≤0.01, ns= Non significant differences at P≤0.05, Means with the same small letters in each row have no-significant differences in respect to (L.S.D) Multiple Comparison. By using One Way ANOVA test.

Antispam antibody, cytokines and in seminal plasma for infertile men

This study found an increase in antisperm antibody, interleukin 1 and interleukin 6 in the oligozoospermia and asthenozoospermia groups compared to the normozoospermia group. The results of table 2 indicated there were no statistically significant differences in antisperm antibody, intrleukin1B (IL-1), and interleukin (IL-6) in the asthenospermia and oligospermia groups compared with normozoospermia.

Table 2
Interleukin 6 in the oligozoospermia and asthenozoospermia groups compared to the normozoospermia group

Antisperm antibody ,cytokines and zinc levels	Normozoospermia N=15	Oligozoospermia N=15	Asthenozoospermia N=13	P-value
Antisperm antibody (np/ml)	541.67+ 144.064	609.13+ 161.248	569.77+ 105.072	0.425ns
Interleukin 1 β (IL-β) Pg/ml	644.80+ 158.246	637.07+ 138.085	691.31+ 196.344	0.651ns
Interleukin 6 (IL-β) Pg/ml	18.139+ 19.604	47.624+ 55.825	29.773+ 24.066	0.107ns

Ns= Non significant differences at P≤0.05, by using One Way ANOVA test.

Discussion

The current study found a decrease in sperm concentration in oligozoospermia and asthenozoospermia compared with the normozoospermia group. Balkan et al. (2008) found that this could be due to problems with chromosomes, gene regulation, the environment, infections and the immune system, and endocrine dysfunction. Oligozoospermia, defined as less than 15 10^6 sperm/mL of seminal ejaculate, and asthenozoospermia, defined as less than 40% motility (WHO 2010). The findings of current study showed a significant increase in liquefaction time in the oligospermia and asthenospermia groups compared with the normospermia group. The abnormal group had significantly greater median pH and liquefaction time than the normal group (demirkol et al., 2021). The current study showed a significant increase in antisperm antibody levels, interlukine1 β and interleukin 6 in infertile men. Asthenospermia patients compared with the normospermia group.

This might be because the presence of ASA in sperm considerably decreased the sperm concentration and motility (A+B) of the infertile ASA-positive male (Cui et al, 2015). Our results agreed with the study that showed the correlation between leukocytospermia and decreased sperm count, decreasing motility, and further elevation of seminal IL-6 and TNF was supported by Eldamnhoury et al. (2018). According to a different study (Aghazarian et al., 2013), upregulation of seminal plasma concentrations of various cytokines, including interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor-alpha (TNF-), has been related to poor semen quality. Another study (Haervig et al., 2018) found that men with male factor infertility had a 17 percent higher mean IL-6 level than men who did not have male factor infertility. Our results agreed with the study that showed in a patient with azoospermia and oligozoospermia; demonstrate the effect of ASA on sperm parameters by comparing ASA concentration to those of a control group (Al-marzoqi et al, 2015). Many earlier studies have demonstrated that ASA has a role in inducing infertility.

Our work confirms these findings by demonstrating a significant increase in the concentration of serum and seminal ASA in the infertile group compared to the fertile (control) group (ali, 2011). The results of the current study also found a significant decrease in zinc level in Asthenospermia and Oligospermia patients compared with the normospermia group. This might be because Zinc (Zn) is an important trace element for healthy male spermatogenesis and plays a key role in antioxidant defense enzymes (Camejo et al., 2011). Zinc is linked to the quality of sperm. Our results agreed with the study that shows Men with normal fertility have higher seminal zinc levels than men with infertility, and seminal zinc levels are closely associated with total sperm count (Camejo et al., 2011).

Conclusions

The levels of antisperm antibodies and interleukins increase, while zinc levels decrease in the seminal plasma of patients with oligospermia, as well as those with asthenospermia, compared to patients with normospermia.

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