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Evaluation of the Concentration of Anti-Spermatozoa Antibodies in Seminal Plasma of Normozoosperms and Azoosperms of Patients at the Pasteur Institute of Cote d'Ivoire

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Abstract

Under normal circumstances, spermatozoa are protected from the immune system by the blood-testis barrier. The breakdown of this barrier is the origin of the synthesis of antisperm antibodies (ASA). The presence of sperm agglutinates in semen is characteristic of ASA. But is the presence of agglutinates in semen necessarily linked to the level of ASA in semen? The objective of this study was to assess the concentration of anti-sperm antibodies (ASA) in normozoosperms and infertile men with azoospermia. The biological material consisted of samples of human sperms: 30 samples with azoospermia and 32 with normozoospermia. The ASA assay was performed in seminal plasma using the DRG® Sperm Antibody ELISA (seminal plasma) kit (EIA-4249). The reading was carried out using a microplate reader at 450 nm. Data analysis was performed using Graph Pad Prism 7 software. The results obtained showed that the difference in ASA concentration between these two categories of sperm was not significant, with an average ASA level of 31.54 ± 2.45 U/mL in azoospermic ejaculate and 27.63 ± 1.51 U/mL in normozoosperms. Statistical analysis showed higher ASA concentrations in azoosperms with 6.67% of these declared positive. The ASA positivity rate made it possible to distinguish secretory azoospermias from obstructive ones. Also, the presence of ASA is not necessarily linked to the presence of agglutinates in the semen.

Keywords

Anti-Sperm Antibodies, Azoospermia, Normozoospermia, Male Infertility

1. Introduction

Anti-sperm antibodies (ASA) are glycoproteins [1]. They are present in human body fluids, mainly in the form of IgG and IgA (rarely IgM) [1]. They are responsible for about 9% to 12% of infertility [2]. The prevalence of anti-sperm antibodies in men ranges from 2.8% to 26% [3]. ASA are characterized by the plurality of their effects on spermatic functions [4] [5]. Also, their negative impact has been demonstrated at several stages of fertilization [5]. The action of these antibodies on human fertility involves two quite different mechanisms. On one hand, there may be a direct effect of the antibodies on the spermatozoa, thus disrupting their motility and their fertilizing power. On the other hand, there may be a disturbance of spermatogenesis as a result of an autoimmune-type reaction. This can be the cause of oligospermia or azoospermia [6] [7]. The ASA can serve as biochemical markers of infertility [3] [8]. The level of ASA in azoosperms makes it possible to distinguish obstructive azoospermias from those which are secretory. Tuech [9] asserted that the presence of agglutinates in semen should immediately lead to the search for ASA. The other sperm abnormalities (asthenospermia, necrozoospermia, leukospermia, oligozoospermia) that may be observed are not specific to the presence of ASA and do not in themselves justify their investigation. So, we asked ourselves if the presence of agglutinates in semen is necessarily linked to the level of ASA in semen. The objective of this study was to assess the concentration of ASA in normozoosperms and infertile men with azoospermia.

2. Material and Methods

2.1. Material

The study was carried out at the Reproductive Biology Unit (UBR) of the Department of Clinical and Fundamental Biochemistry of the Pasteur Institute of Cote d'Ivoire (IPCI). The biological material consisted of samples of human sperms: 30 azoosperms and 32 normozoosperms. The samples were taken from subjects who came to the reproductive biology laboratory for a spermiological check-up during the sample collection period from Jun to December 2018. The collection of sperm was carried out with the consent of the patients for the use of their sample for research purposes, and the authorization of the National Commission of Ethics and Research of Côte d'Ivoire (CNER-CI); Ordinance No. 36/MLS/CNER/TB.

2.2. Methods

The selection criteria for the collection of semen were as follows: have regular intercourse (at least two intercourses per week); observe 3 days of abstinence from

sexual intercourse before the semen collection. The non-inclusion criteria concerned all men who did not meet the above-defined inclusion criteria. For the study, sperms with abnormalities other than azoospermia of the spermogram were excluded.

2.3. Performing the Spermogram

The spermogram was performed according to the World Health Organization [10] standards. The semen was collected by masturbation after three days of abstinence from sexual intercourse. After collection, the semen samples were liquefied at a temperature of 37˚C in an oven for one hour, then used for the performance of the spermogram. The macroscopic examination of the semen made it possible to determine the color, volume, pH and viscosity of the samples. The microscopic parameters, namely the count, the mobility, the vitality and the percentage of spermatozoa of normal morphology were evaluated using the SQA-Vision machine.

2.4. Preservation of Seminal Plasma

The sperm samples were centrifuged at 3000 RPM for 15 minutes. Then, the seminal plasma was collected and stored at −20˚C.

2.5. Determination of ASA in Seminal Plasma

Determination of ASA concentrations were determined using Immunological method Enzyme-linked immunosorbent assay (ELISA) with the DRG® Sperm Antibody ELISA (seminal plasma) kit (EIA-4249). The kit contains all reagents and standards for calibration and control of test results. One solution for the positive control (70 - 120 U/mL) and one for the negative control (0 U/mL). The calibration solutions were 31 U/mL, 62 U/mL, 125 U/mL and 250 U/mL. The preparation of the sperm for the determination of ASA was made following the instruction contained in the notice leaflet of the DRG® Sperm Antibody ELISA (seminal plasma) kit (EIA-4249). All reagents were placed at room temperature before use. The wash solution was diluted 1/10 with distilled water. Then, the seminal plasma was diluted 1/100 with the dilution buffer. 50 µL of each standard were placed in the wells of the microplate. Then, 50 µL of diluted seminal plasma was placed in each of these wells. These were then covered with an adhesive film and incubated for 60 min at 37˚C. Next, the microplate was shaken thoroughly to remove its contents, then rinsed three times with 200 µL of diluted wash solution. Residual water was removed by tapping the microplate on absorbent paper. A volume of 50 µL of enzyme conjugate was placed in each well. The microplate was covered with adhesive paper and incubated for 60 min at 37˚C. After this incubation time, the microplate was shaken vigorously to remove its contents, then rinsed 5 times with 200 µL of diluted washing solution. Residual water from the wells was removed by tapping the microplate on absorbent paper or a rag. The microplate was read at 450 nm within 10 minutes after stopping the reaction using a microplate reader (Microread 1000 ELISA Plate Analyzer).

3. Statistical Analysis

Graph Pad Prism 7 software (Graph Pad Software Inc., La Jolla, Calif., USA) and Excel were used to perform the statistical analyses. Results are reported as mean ± standard deviation. Comparisons between ASA concentrations of azoospermic and normozoospermic seminal plasma were made using Mann-Whitney tests for unmatched independent samples. A 95% confidence interval was used. A p value < 0.05 was considered statistically significant.

4. Results

The mean age of the normozoosperms was 40.06 ± 1.18 years with a maximum of 53 years and a minimum of 27 years. That of azoosperms was 39.2 ± 1.8 years with a maximum of 57 years and a minimum of 28 years (Figure 1).

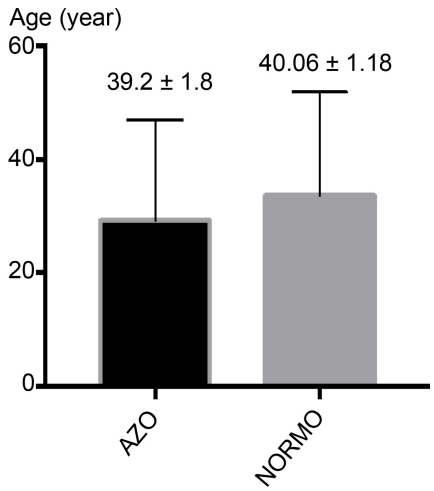

Figure 1. Average ages of normozoosperms and azoospermes (AZO: Azoosperms; NORMO: Normosperms).

The mean pH value of the sperm of the normozoosperms was 7.68 ± 0.04 and that of the volume was 2.88 ± 0.18 mL. The average sperm count was 60.47.106 sperm/mL, with an average of typical forms 6.15% ± 0.28%. In azoosperms, the semen volume was substantially identical to that of normozoosperms with a value of 2.47 ± 0.23 mL. The average pH for the azoosperms was 7.88 ± 0.07 (Table 1).

Concentration of Antisperm Antibodies

The assay of the antisperm antibodies in the seminal plasma of the azoosperms gave an average concentration of 31.54 ± 2.45 U/mL which was higher than that of the normozoosperms which gave 27.63 ± 1.51 U/mL (Figure 2). The p-value which was 0.30 is greater than the threshold set at 0.05. Thus, the difference between the ASA concentration of azoosperms and normosperms is not significant. Among the azoosperms, two showed a pathological ASA concentration level (Figure 3).

Table 1. Different parameters of the spermogram.

		Azoosperms (n = 30)	Normozoosperms (n = 32)
Age (year)	Mean	39.2 ± 1.8	40.06 ± 1.18
	Minimum	28	27
	Maximum	57	53
pH	Mean	7.88 ± 0.07	7.68 ± 0.04
	Minimum	7.5	7.5
	Maximum	9	8
Volume (mL)	Mean	2.47 ± 0.23	2.88 ± 0.18
	Minimum	0.3	1.5
	Maximum	5	5.6
Sperm count (10^6 spz/mL)	Mean	-	60.47 ± 7.427
	Minimum	-	15
	Maximum	-	168
Typical shapes (%)	Mean	-	6.15 ± 0.28
	Minimum	-	4
	Maximum	-	10

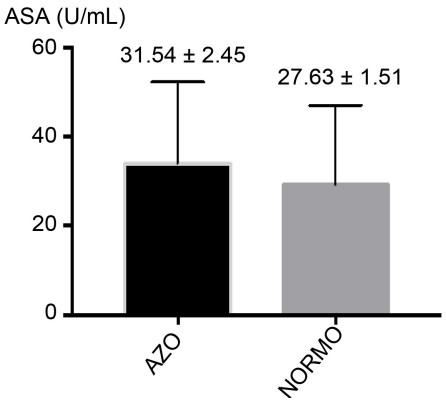

Figure 2. Concentrations of anti-sperm antibodies in azoospermia (AZO) and normozoospermia (NORMO).

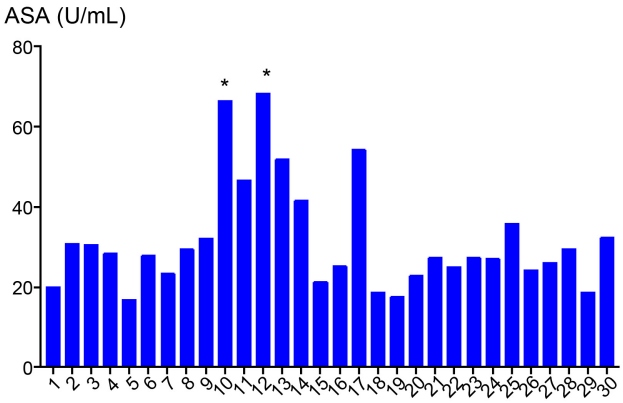

Figure 3. Concentrations of ASA in azoospermic seminal plasma. *Azoospermia positive to ASA.

5. Discussion

Normozoospermia and azoospermia have been defined using standards set by WHO [10]. The WHO standards provide an interpretive framework for each sperm parameter. These standards allow a standardization of the results of the evaluation of male fertility and lead to the identification of the various pathologies of the sperm [10]. The spermogram can be used to define the various abnormalities of the sperm, but its usefulness is limited in the context of the diagnosis of idiopathic infertility. Thus, additional diagnostic examinations of male infertility provided an answer to the causes of idiopathic infertility [11].

In this study, the presence of ASA was identified by ELISA in azoosperms and normosperms. This study showed that both categories of semen contained ASA but at lower concentration. The presence of ASA in seminal plasma is associated with their presence in blood serum [9]. Indeed, immunoglobulins G (IgG) in semen originate from the blood while immunoglobulins A (IgA) are produced locally [9]. The Mann-Whitney test did not show significant differences between ASA concentrations in normozoosperms and azoosperms. In contrast, it showed a high mean concentration of ASA in azoosperms compared to normozoosperms. The results obtained a similar to those of Patricia *et al.* [12] who found an increase in the concentration of antisperm antibodies in the semen of infertile men.

During their formation process, the germ cells are physiologically protected from exposure to the immune system. Therefore, an autoimmune reaction against spermatozoa can occur after a lesion in the genital tract exposes spermatozoa to the immune system. Several factors such as testicular trauma, testicular cancer, undescended testis, urogenital inflammatory conditions, obstruction, or varicocele have been associated with the presence of ASA [13]-[22]. So, the presence of ASA could therefore provide information on the effectiveness of spermatogenesis in cases of azoospermia.

The level of ASA in normozoosperms has not reached the pathological threshold. While in azoosperms, 6.67% of the sperm tested positive to ASA. The level of ASA in azoospermic sperm distinguishes the two types of azoospermia. Thus, the presence of ASA led Lee *et al.* [8] to propose the assay of serum antibodies as a diagnostic test for obstructive azoospermia. The presence of ASA in seminal plasma is therefore highly predictive of obstructive azoospermia. Based on this work, 6.67% of azoospermic sperms present with obstructive azoospermia.

Therefore, seminal plasma ASA could be used for non-invasive predictive method to confirm obstructive azoospermia [8]. Which could contribute to improved success in TESE-ICSI (testicular sperm extraction associated with intracytoplasmic sperm injection) to attain pregnancy [23].

However, in some cases, the cause of antibodies remains idiopathic [24]. So, the underlying mechanisms linking ASA presence must be explore for the management of immunological male infertility.

This study should be carried out on a much larger scale, in correlation with the

patients' infectious history. Similarly, by combining certain serum and hormonal data, we could obtain a solid database for a better understanding of the value of ASAs in seminal plasma.

6. Conclusion

Evaluation of antisperm antibodies in seminal plasma of azoosperms and normozoosperms showed that the level of ASA concentration in seminal plasma of azoosperms and normozoosperms was not significantly different. The presence or absence of spermatozoa in seminal plasma does not influence the level of ASA. It is the same for agglutinates. As the presence of ASA in seminal plasma is linked to effective spermatogenesis, its exploration opens up the possibility of non-invasive methods for diagnosing obstructive azoospermia.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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