Scientific Research Publishing

Advances in Reproductive Sciences, 2025, 13(1), 63-69
https://www.scirp.org/journal/arsci
ISSN Online: 2330-0752
ISSN Print: 2330-0744

Place of Seminal Biochemical Markers in the Etiological Diagnosis of Azoospermia in Cotonou in 2023

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How to cite this paper: Azonbakin, S., Houndetoungan, D., Madohonan, Y., Sossa, J., Bi, E.G., N'bouke, E., Soglo, G., Adjagba, M., Dossou, J.P. and Laleye, A. (2025) Place of Seminal Biochemical Markers in the Etiological Diagnosis of Azoospermia in Cotonou in 2023. *Advances in Reproductive Sciences*, **13**, 63-69.
https://doi.org/10.4236/arsci.2025.131006

Received: January 12, 2025
Accepted: February 25, 2025
Published: February 28, 2025

Abstract

Azoospermia, defined as a complete absence of spermatozoa in the ejaculate, is a relatively common condition among infertile men. Establishing the etiological diagnosis can be challenging and often requires various investigations. This cross-sectional study, conducted in Cotonou among 35 azoospermic men, aimed to assess the role of biochemical analysis of seminal fluid in determining the etiological diagnosis. The results revealed significant heterogeneity in biochemical profiles. Most of the patients had normal levels of alpha-glucosidase, fructose, and zinc, indicating normal accessory gland function and suggesting non-obstructive azoospermia. However, a significant subgroup exhibited abnormalities in these markers, suggesting a possible obstructive azoospermia. The heterogeneity in biochemical profiles observed in this study highlights the need for further studies to better characterize azoospermia in Benin.

Keywords

Azoospermia, Seminal Fluid, Biochemical Markers, Benin

1. Introduction

According to World Health Organization (WHO), couple infertility is defined as the inability of a couple to conceive after at least 12 months of regular, complete, and frequent sexual intercourse without the use of any contraceptive methods [1].

It is a major global public health issue, affecting approximately 17.5% of the world's adult population, which is equivalent to one in six people [1]. This problem affects about 15% of the population in Sub-Saharan Africa [2] [3]. Male infertility which accounts for 30% to 50% of couple infertility cases [4], is sometimes linked to azoospermia, an abnormal condition characterized by the complete absence of spermatozoa in the ejaculate [5]. This condition is increasingly common, with a prevalence of 16% in Morocco and 18.5% in Benin [6] [7]. Azospermia is detected through spermogram, a standard test used in the exploration of male infertility [5]. However, this test alone is insufficient to determine the exact cause, as it is limited to a quantitative and morphological assessment of spermatozoa. Azoospermia is a common medical condition typically identified through a spermogram and accounts for 20% of cases in Benin, Guinea, and Senegal [8] [9]. Couple infertility raises many concerns for patients, and the diagnosis of azoospermia is often perceived by men as a devastating reality [7].

Therefore, a more in-depth diagnostic approach is necessary to establish an accurate etiological diagnosis. Hormonal analysis, particularly measuring FSH, LH, testosterone, and prolactin levels, is commonly performed [5]. However, this test does not always clearly identify the cause of azoospermia, which is crucial for effective management of the condition. Hormonal testing is often not affordable for all patients. In this context, biochemical analysis of seminal plasma, which provides complementary information to the spermogram, is increasingly used to guide etiological diagnosis and tailor therapeutic management [2] [4]. Indeed, the biochemical analysis of seminal plasma reveals variations in secretions and enables the establishment of a biochemical and functional map of the male genital system, especially when investigating azoospermia, oligospermia, or asthenospermia detected during a spermogram. Nevertheless, in Chicago, data on the biochemical analysis of seminal fluid remain limited, despite the high prevalence of azoospermia. In this context, the present study aimed to evaluate the role of this analysis in the etiological exploration of azoospermia in Cotonou.

2. Methods

This was a cross-sectional study, with both descriptive and analytical objectives, conducted from March to November 2023, at the Laboratory of Histology, Reproductive Biology, Cytogenetics and Medical Genetics of the Faculty of Health Sciences of the University of Abomey-Calavi (FSS-UAC). It focused specifically on the biochemical analysis of seminal plasma in 35 men suffering from azoospermia. Participants had given their informed consent to participate in this research. The study variables included age, occupation, pH, sperm volume, and concentrations of α-glucosidase, fructose and zinc, measured by spectrophotometry. Sperm volume was measured using a syringe graduated in milliliters, while pH was assessed by placing a drop of homogenized sperm onto a Merck® brand pH paper strip. After a few seconds, the observed color was compared to the standard range. Biochemical markers were investigated by spectrophotometry

using a DiaSource® Elisa Reader with the EpiScreen Plus™ reagent from Laboratoire FertiPro® for *α*-glucosidase, a microplate reader with the Fructose test® reagent from Laboratoire FertiPro® for fructose and a UV-Visible spectrophotometer (Evolution 60S) with the Seminal Plasma Zinc Detection Kit 5-Br-PAPS (5-bromo-1,3-phenylarsazonic acid) reagent from Zhuhai Cariad Technology. Table 1 summarizes the principles of biochemical parameter assays. These analyses were performed on seminal plasma following the manufacturer's recommendations. The data collected were enterd using KoboCollect software, then statistically analyzed using Epi-info and SPSS software. The results of this study were displayed in tables and graphs, created using the Microsoft Office Suite. To assess the associations between the dependent variable (etiological diagnosis) and the independent variables (age, seminal parameters), Pearson's and Student's Chi-square tests were used, with a significance threshold set at 5%. Only participants who provided written consent were included, which justifies the small size of our sample. Due to the financial cost of hormone testing and imaging procedures, not all patients underwent these assessments. Authorizations were obtained from the managers of the various laboratories prior to sample collection. Ethical approval was obtained from the research ethics committee (CERISBA) under number N˚179 of 10/30/23.

Table 1. Principles of biochemical parameter assays.

Parameters	Principles of assay	Normal values
α-glucosidase	PNPG + alphaglucosidase → alpha-D-glucopyranoside + PNP (yellow)	≥20 mUI/ejaculate
Fructose	Reaction, in the presence of HCl, with indole (complex formation)	≥2.4 mg/ejaculate
Zinc	Reaction with 2-(5-Bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl)aminophenol]	≥2.4 µmol/ejaculate

3. Results

The study involved 35 azoospermic patients aged between 28 and 58 years, with a mean age of 39.22 ± 2.68 years. The majority of patients (54.28%) were between 30 and 40 years old. Patients older than 55 years old were few, comprising only 3.04% of the patients. Twenty-two patients (57.58%) had primary infertility, while 13 patients (42.42%) had secondary infertility. These patients had varying medical and surgical histories, summarized in Table 2.

A normal ejaculate volume was observed in the majority of patients (69.70%). Hypospermia (an ejaculate volume less than 1.5 ml) was observed in 27.27% of cases, while only 1.37% had hyperspermia (ejaculate volume greater than 6 ml).

Table 3 summarizes the results of the biochemical assay. It covers three major biochemical markers: alpha-glucosidase, fructose, and zinc. For each marker, results are presented according to reference values established as normal or low.

The results of biochemical assays, as a function of pH, did not show any

significant association (Table 4). A decrease in *α*-glucosidase, fructose, and zinc was observed in patients with hypospermia. Imaging and hormone tests were not performed in all patients. Therefore, these data were not included in the present study.

Table 2. Medical history of patients with azoospermia.

	Count	Percentage (%)
Medical history		
None	24	68.57
Varicocele	03	8.57
Testicular atrophy	02	5.71
Hypertension	01	2.86
Diabate	01	2.86
Testicular subtorsion	01	2.86
Hydrocele repair	01	2.86
Mumps	01	2.86
Surgical history		
Surgery/inguinoscrotal hern	02	5.71
Testicular biopsy	01	2.86

Table 3. Results of biochemical parameter assays of seminal plasma.

Biochemical parameters	Count	Percentage (%)
Alpha glucosidase		
Normal (≥ 20 mUI/ejaculate)	25	71.43
Low (< 20 mUI/ejaculate)	10	28.57
Total	35	100
Fructose		
Normal (≥ 2.4 mg/ejaculate)	27	77.14
Low (< 2.4 mg/ejaculate)	08	22.86
Total	35	100
Zinc		
Normal (≥2.4 µmol/ejaculate)	32	91.43
Low (<2.4 µmol/ejaculate)	03	08.57
Total	35	100

Table 4. Variation of biochemical parameters according to pH and semen volume.

Parameters	pH		p-value	Volume			p-value
	Normal (n = 4)	Acide (n = 31)		Hypospermia (n = 9)	Normal (n = 25)	Hyperspermia (n = 1)	
Alpha glucosidase	27.4 ± 23.59	52.27 ± 21.81	0.42	13.21 ± 9.97	64.65 ± 25.34	-	<0.0001
Fructose	11.24 ± 10.35	10.60 ± 5.78	0.896	1.41 ± 1.07	13.73 ± 3.09	-	<0.0001
Zinc	8.98 ± 4.89	12.43 ± 3.45	0.48	6.08 ± 3	12.95 ± 2.88	-	<0.0001

4. Discussion

The spermogram allows the evaluation of spermatic functions in men, but does not always clearly identify the main cause of abnormal conditions, especially in the case of azoospermia. Hence, complementary analyses are crucial in the case of azoospermia, including biochemical analysis of seminal plasma. This study investigated, for the first time in Benin, the place of biochemical markers in the etiological diagnosis of azoospermia. Due to the financial cost, hormone dosage and ultrasound data are not available for all patients. Therefore, they were not included in this work.

The mean age of patients was 39.22 years (±2.87 years), with a range of 28 to 58 years. It should be noted that the majority of participants (54.28%) were between 30 to 40 years old. This age range is almost consistent with findings from the literature [10] [11]. Indeed, a study conducted in Burkina Faso by Sakande *et al.* (2012) reported a mean age of 36 years in azoospermic patients, with 55% of them aged between 30 and 40 years [10]. Similarly, in Cotonou, Hounnasso *et al.* (2013) found the average age to be 37 years, with 57.3% of patients falling within the 31-40 age group [11]. These data suggest that the average age at diagnosis of azoospermia is generally around forty, with the majority of cases occurring in the 30 to 40 age group. Furthermore, taking a detailed patient history is crucial for identifying potential risk factors and guiding further investigations. Notably, a significant majority (68.57%) reported no history directly linked to azoospermia. This finding highlights the etiological complexity of azoospermia and suggests the presence of idiopathic forms, as also indicated by data from the literature [12]. However, a significant percentage of patients had a history of varicocele (8.57%), inguino-scrotal hernia repair (5.76%), and testicular biopsy (2.86%). These conditions are recognized risk factors for male infertility, as they can impair spermatogenesis and contribute to azoospermia [12].

The biochemical analysis of seminal fluid markers in azoospermic patients revealed interesting results. Indeed, the majority of patients had normal levels of alpha-glucosidase (71.43%), fructose (77%) and zinc (91%). These results, correlated respectively with epididymal, seminal vesicular and prostatic function, suggest that these organs are generally functioning normally. They strongly indicate that the azoospermia observed in the majority of patients is likely due to alterations in spermatogenesis or dysfunctions of the hypothalamic-pituitary axis. This

points towards a secretory rather than obstructive etiology of azoospermia, in line with findings from the scientific literature [12]. Although the majority of patients had normal biochemical results, a significant subgroup had decreased levels. These biochemical abnormalities, in correlation with accessory gland dysfunction, could be the cause of severe alteration of spermatic parameters, indicating a possible obstructive mechanism [13] [14]. If data from hormonal and imaging explorations had been available, they would have helped us to better interpret the results of the biochemical parameters and draw better conclusions. Therefore, this study warrants continuation.

The small sample size (n = 35) is an important limitation of this study. Indeed, this small sample size may limit the generalizability of the results and reduce the statistical power of the analysis. Furthermore, the absence of a control group (fertile men) is another limitation, as direct comparison would allow for better interpretation of the observed biochemical abnormalities. Nevertheless, this study produced meaningful results.

5. Conclusion

This study highlights the value of biochemical analysis of seminal fluid in exploring azoospermia. It underscores the heterogeneity of biochemical profiles and the need for a personalized diagnostic approach, including detailed anamnesis and additional analyses. However, the small sample size limits the generalizability of the results. Larger-scale studies, incorporating a control group and a more in-depth analysis of genetic and environmental factors, are needed to confirm these findings and better characterize the various subtypes of azoospermia in Benin.

Acknowledgements

To Clémence Gouton for reading and correction of the English.

Conflicts of Interest

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

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