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Original Research Article

Evaluation of minerals in the seminal plasma of azoospermic semen

Marie-Florence N'Guessan[1,2,*], Yapi Guillaume Yaye[4], Founzégué Amadou Coulibaly[1,2], Eric Ayekoue[1,2], Konan Sylvère N' Zi[1,2], Noël Coulibaly[3], Gervais Mélaine M'Boh[2], Souleymane Méité[2], Yapi Félix Houphouët[1] and Allico Joseph Djaman[1,2]

[1]*Laboratoire de Pharmacodynamie biochimique de l'Unité de Formation et de Recherche Biosciences de l'Université Félix Houphouët-Boigny, Abidjan (Côte d'Ivoire), 22 Bp 522 Abidjan 22*
[2]*Département de Biochimie clinique et fondamentale de l'Institut Pasteur de Côte d'Ivoire (IPCI) 01Bp490 Abidjan 01*
[3]*Service d'Urologie du Centre Hospitalier Universitaire de Treichville, Abidjan (Côte d'Ivoire) 01Bp V 3 Abidjan 01*
[4]*Unité Pédagogique Biochimie-Microbiologie de l'Unité de Formation et de Recherche Agroforesterie, Université Jean Lorougnon Guédé de Daloa (Côte d'Ivoire), Bp 150 Daloa.*

***Correspondence Info:**
Marie-Florence N'Guessan,
Laboratoire de Pharmacodynamie biochimique de l'Unité de Formation et de Recherche Biosciences de l'Université Félix Houphouët Boigny-Abidjan (Côte d'Ivoire)
E-mail: miflo2@yahoo.fr

Abstract

Background: Seminal plasma is largely variety of compounds which reflect the functioning of the male genital tract. However, only citrate, zinc, fructose, alpha-glucosidase, and L-carnitine based on their concentration level are used to explore azoospermia state. Other constituents of seminal plasma should also be the subject of a study for the identification of new specific markers azoospermia. Several studies have reported that the minerals involved in maintenance and function of male fertility.
Aim: Assay of sodium, potassium, calcium, magnesium, phosphorus, chlorine and iron is designed to identify new biochemical markers of azoospermia.
Material and Methods: Analysis of seminal plasma was carried out with 30 samples of normozoospermic semen and 30 samples of azoospermic semen. The assay of calcium, phosphorus, magnesium and iron was done using the automated Cobas® c311 de Roche/Hitachi. The evaluation of the concentration level of chlorine, sodium and potassium in the seminal plasma was performed using the automated Roche® 9180 Electrolyte Analyser. The data collected were analyzed using the software Graph Pad Prism 5. *P*- values less than 0.05 was considered the level of significance.
Results: The results of this study showed that the correlation test performed between concentrations level of minerals in the seminal plasma of normozoospermic and azoospermic semen showed no significant difference statistically with $P > 0.05$.
Conclusion: Absence of spermatozoa in the semen does not affect her osmolality. Thus sodium, potassium, calcium, magnesium, phosphorus, chlorine and iron have no diagnostic value for the male reproductive system disorders, including azoospermia.
Keywords: Azoospermia, Normozoospermia, Seminal plasma, Mineral elements

1. Introduction

Azoospermia is a complete absence of sperm in the semen. Its investigation is performed through the clinical examination, additional tests of biology and medical imaging [1]. Seminal biochemistry is part of biological exploration tests of azoospermia. It determines the concentration of biochemical markers in seminal plasma to specify the level and the topography of an obstruction in azoospermia. It provides information only on type of azoospermia : secretory or excretory [2]. The seminal plasma is an important indicator of semen quality and monitoring of male infertility [2]. It contains variety of compounds that reflect the functional state of the male genital tract. However only citrate, zinc, fructose, alpha-glucosidase and L-carnitine based on their concentration level used to study the functional state of the accessory glands. They are called the seminal plasma markers [3]. The other compounds of the seminal plasma should also be investigated in order to identify new specific markers of azoospermia.

Minerals are essential for the performance of vital biological functions [4]. They are divided into two groups: macronutrients and trace elements. In the cell, they are involved not only in the stable of osmotic pressure but also as the catalyst of several biochemical reactions [5]. Several studies done have helped to identify them in seminal plasma

[6-8]. They intervene in the maintenance of male fertility [9]. Semen quality is correlated to the concentration level of some of minerals and their presence reflects satisfactorily the functional state of accessory gland of the male genital tract [5, 10-13]. They are used in treatment and prevention of infertility [9]. They are essential in the regulation of the acrosome reaction, spermatozoa capacitation and spermatozoa motility [14, 15]. They may serve as tools to support the assessment of male infertility [16].

In view of their involvement in maintaining male fertility, mineral elements such as sodium, potassium, calcium, magnesium, phosphorus, chlorine and iron present in seminal plasma may be a subject to an investigation to identify new biochemical markers of azoospermia exploration. Analysis of these minerals in the seminal plasma of azoospermics and normozoospermics has for objective to determine their concentration levels on one hand and to define the relationship between minerals and azoospermia on the other hand.

2. Material and methods

The study was conducted at the Pasteur Institute of Côte d'Ivoire (IPCI). The semen samples were collected with the consent of patients in accordance to the standards of the National Ethics and Research Commission of Côte d'Ivoire (NIRB-CI); Order No. 36 / MLS / NIRB / TB.

Sixty samples of semen normozoospermic and azoospermic of men were selected for this study. Normozoospermics semen samples were considered the control group.

2.1 Inclusion criteria: Only men with azoospermia and normozoospermia after realization of their spermogram and respecting conditions semen collections were included in this study.

2.2 Exclusion criteria: The exclusions criteria were rejecting all people who have not observed the conditions for spermogram realization and those with leucospermia and hyperviscosity.

2.3 Sample Analysis

2.3.1 Realization of spermogram: The semen collection was obtained through masturbation after three days of abstinence. The semen analysis was performed according to the standards of the World Health Organization (WHO) [17]. After collection, semen samples were liquefied at a temperature of 37^0 C in an incubator for one hour and then used for analysis. Macroscopic examination of the semen was used to determine the color, volume, pH and viscosity of samples. Concentration, motility, morphology of spermatozoa was assessed by microscopic examination.

For the biochemical analysis of semen collected, samples were centrifuged at 3000 tr/mn for 10 minutes and seminal plasma was collected and stored at -8°C until the day of the analysis.

2.3.2 Dosage of chlorine, sodium and potassium: It was made from the dilution of 100 µl of seminal plasma to 1 / 5th. Different assays have been made from the method of ion-selective electrode using the analyzer Roche® 9180 Electrolyte Analyser.

2.3.3 The dosage of calcium, inorganic phosphate, magnesium and iron: It was made by the Cobas® c311 de Roche/Hitachi analyser which is a selective multi-parameter analyzer for the analysis of classical biochemistry and immunoassay in homogeneous phase. The dosage of ions calcium is made by the method of Schwarzenbach with o-cresolphthalein complexon. The method used for the determination of inorganic phosphorus has been the one phosphomolybdate direct according to Daly and Ertingshausen. The dosage of iron was produced from the method of Guanidine / FerroZine. For magnesium assay, the colorimetric method using Chlorophosphonazo III was used.

2.4 Statistical Evaluation

The obtained data was analyzed by using software Graph Pad Prism 5.0. The results are summarized as arithmetic mean values and standard deviation (SD). The differences between the mean values of minerals in seminal plasma of azoospermics and normozoospermics were analyzed for statistical significance by student T-test (Nonparametric tests) and Mann–Whitney U test. Probability level values at $P < 0.05$ were regarded as significant.

3. Results

The study included 30 azoospermics and 30 normozoospermics according to WHO criteria. The average age of azoospermics group was 39 (range 23-45) years. While the control group (normozoospermics) had an average age of 39 years with a 26-45 year. Data collected from spermogram of azoospermics and control groups (normozoospermics) are shown in (Table 1). The average values of ion concentration Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}, PO_4^{3-} and Fe^{2+} in both groups were not statistically significant (Table 2).

Table 1: Azoospermics and normozoospermics semen characteristics

Semen characteristics	Normozoospermic	Azoospermic
N	30	30
Volume (ml)	3.42±0.24	2.96±0.25
pH	7.58±0.05	7.61±0.04
Concentration (10^6/ml)	68.88±6.74	00±00
Mobility 1H (a+b) (%)	47.20±1.53	00±00
Mobility 4H (a+b) (%)	31.20±1.48	00±00
Morphology (%)	23.52±1.82	00±00

Table 2: The average concentration of minerals in the seminal plasma of azoospermics and normozoospermics

Minerals (mg/L)	Normospermics	Azoospermics	*P*-value	significance
Na^+	192.8 ± 28.20	189.0 ± 11.66	0.627	NS
K^+	26.63 ± 8.182	28.41 ± 7.33	0.609	NS
Cl^-	138.3 ± 9.705	130.8 ± 13.74	0.136	NS
Ca^{2+}	194.8 ± 77.21	213.2 ± 49.23	0.588	NS
PO_4^{3-}	439.1 ± 58.38	417.1 ± 67.71	0.353	NS
Mg^{2+}	35.90 ± 19.22	44.27 ± 19.34	0.210	NS
Fe^{2+}	5.663 ± 3.40	5.397 ± 2.47	0.877	NS

$_*p<0.05$ significant
*NS Not significant

4. Discussion

In this study, the comparison of minerals concentration in seminal plasma of normozoospermics and azoospermics semen was established.

Sodium is the major extracellular cation. In semen, it takes part in exchange between seminal plasma and sperm. It is essential to the intracellular and extracellular pH balance [18, 19]. The average sodium concentration in the seminal plasma of azoospermics, compared to that of normozoospermics is not statistically significant. The sodium cannot be used as a biochemical marker in differentiating azoospermic semen, from those normozoospermic in azoospermia diagnostic. These results are different from those obtained by Sakandé *et al* [6]. They reported a significant difference between the sodium concentration in the seminal plasma of normospermics and those of pathogenic state. For Ruskova *et al* [20], sodium has a limited diagnostic value for male reproductive system disorders. Nevertheless there is a significant correlation between semen characteristics and the sodium concentration in seminal plasma [21].

The osmolality of the extracellular environment has an essential role in regulating spermatozoa metabolism. The extracellular medium is rich in sodium and chlorine while the intracellular medium is rich in potassium and phosphorus. Sodium exchanges between the cell and the external environment are made through protein structures. The sodium enters in the cell through voltage-gated channels and receptor-dependent, exchangers, cotransporter; it goes out through the Na+/K+-ATPase pump [19,22].

Ion channels of the plasma membrane are the heart of control of spermatozoa function [23]. Indeed, the ion flux through the proteins channel allows a transfer of information between the spermatozoa and their environment [22,23]. Under normal physiological conditions, the spermatozoa membrane potential and intracellular pH are values such that the ion channels remain active at a minimal level. They are potentially activated only in the presence of agonists or physiological stimuli [24]. These different channels are used to maintain constant ion concentration in the extracellular and intracellular environment.

The comparison of potassium concentration in the seminal plasma of normospermics and azoospermics is not significant. Potassium cannot be used as a biochemical marker of differentiation between them as Ruskova *et al.* [20] confirms. Nevertheless, it appears that sodium / potassium ratio in the seminal plasma is important for spermatozoa motility and function [8]. Potassium is the major intracellular cation. It plays a key role in cellular repolarization. The subsequent depolarization at the entrance of sodium and calcium into the cell is followed by repolarization occurring by the potassium efflux. The return of the cell to the initial equilibrium state is provided by the Na^+/K^+-ATPase membrane [25].

The evaluation of the chlorine concentration in the seminal plasma of azoospermics and normozoospermics indicates that it cannot be a differentiating factor. The role of the chloride ion is relatively unknown although it is the main anion balancing the sodium and potassium cations in the extracellular medium [26]. Concerning spermatozoa, chlorine is involved in cellular homeostasis process, spermatogenesis and capacitation [27]. There are proteins involved in the transportation of chloride ions across the plasma membrane [27].

For Briggiler-Marín *et al.* [14], the level of the calcium concentration in seminal plasma is essential because it plays an important role in spermatozoa mobility and capacitation. The results of our studies show that the average concentration of calcium in the seminal plasma of the azoospermics and normozoospermics showed no significant difference. Calcium cannot then act as a biochemical marker for the differentiation of azoospermic and normozoospermic semen as confirmed by the work of Wong *et al.* [5]. The presence or absence of spermatozoa in the semen does not influence the calcium concentration in seminal plasma. Contrary to the study conducted by Sundaram *et al.* [28] who reported that the difference in calcium concentration in seminal plasma of infertile and fertile men has a low level of significance. In semen calcium can be found in three forms: free, complexes or bound to a protein. Only a small portion, from 2% to 4% of calcium in the semen is in ionised form

[29]. Its concentration is higher in spermatozoa than in the seminal plasma. The transport of calcium across the plasma membrane is done by the CatSper channel, in association with other ion channels, Slo3 K^+channel, Na^+/ H^+, Na^+ / Ca^{2+} exchanger [22,30]. These channels provide the regulation of cellular homeostasis. The entry of calcium by cation channels (CatSper) is essential for many important physiologically process, such as hyperactivation, chemotaxis and acrosome reaction [22].

Phosphorus is produced mainly in the epididymis [31]. It is involved in the regulation of male fertility [32]. The results of this study showed that the average concentration of phosphorus in the seminal plasma of normozoospermics and azoospermics could not to serve as biochemical marker for the exploration of azoospermia. Adamopoulos and Deliyiannis [11] showed in their study that the concentration of inorganic phosphate was higher in semen asthenozoospermic but lower in those azoospermic and normozoospermic. Phosphate is one of the majority anions of the cell where it ensures the electrical neutrality by combining with potassium.

Magnesium is present at a high concentration in semen [7]. It acts as a cofactor for several enzymatic reactions involving energy metabolism (ATP) and nucleic acid synthesis [33]. It plays an important role in spermatogenesis, particularly in spermatozoa motility. The magnesium concentration presents no significant difference in the seminal plasma of normozoospermics and azoospermics as confirmed by the study of Sundaram *et al* [28]. Umeyama *et al* [7] reveal from their work that the magnesium concentration in seminal plasma is almost identical between fertile and infertile men. There is a concentration gradient between the content of free magnesium in the extracellular medium and that of the intracellular medium and an electrical gradient which tend to bring the magnesium in the cell. There are magnesium pumps such as those of calcium and sodium that take out magnesium of the cell and which keep cellular homeostasis [34].

The average concentration of iron in seminal plasma of semen normozoospermics is slightly higher than that of azoospermic semen; however it does not present significant difference. Marzec-Wróblewska *et al.* [35] reported that there is no significant correlation between the iron concentration in the semen of fertile and infertile men. Iron plays an important role in male fertility and spermatogenesis [36]. Iron's ability to serve as both electron donor and acceptor makes it irreplaceable metal for various metabolic and physiological pathways. Indeed, it is a component of many enzymes and metalloprotein. Only a small fraction of iron is in free form, any excess free iron is toxic to the body. In the semen we note the presence of specialized proteins that regulate homeostasis. They are involved in the transportation of iron and its regulation metabolism [37].

5. Conclusion

Mineral components such as sodium, potassium, calcium, magnesium, phosphorus, chlorine, and iron, while playing an important role in functioning and maintenance of male fertility, have no diagnostic value for disorders of the male reproductive system, particularly azoospermia. The presence or absence of spermatozoa in the semen does not influence its osmolality. This study highlighted the role of the plasma membrane in maintaining cellular homeostasis.

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