Lysosomal Enzymatic Activities in Seminal Plasma of Iraqi Patients with Male Infertility

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ABSTRACT:

BACKGROUND:

Infertility is defined as the inability to conceive after one year of unprotected sexual intercourse. Some cases of male infertility are due to a specific cause whereas others are due to less obvious etiologies and results in less drastic changes in semen parameters. Arylsulfatase A, arylsulfatase B and alkaline proteinase have not been studied adequately in relation to male infertility.

OBJECTIVES:

To study the association between seminal plasma arylsulfatase A, arylsulfatase B and alkaline proteinase enzyme activities and semen parameters in different Iraqi male infertile patients.

PATIENTS AND METHODS:

Sixty patients with male infertility were included in this study. The male patients were categorized according to their seminal fluid parameters to oligospermia (n=32), azoospermia (n=22) and asthenospermia (n=6). All results obtained were compared with age – matched healthy controls group consisting of 39 subjects. The experiments were carried out in the laboratories of the physiological chemistry department at the college of Medicine / Al – Nahrain University during the period from November 2004 to July 2006.

RESULTS:

arylsulfatase A and arylsulfatase B were significantly decreased in all infertility groups. Seminal plasma arylsulfatase A decreased 73.55% in oligospermia group, 75.14% in azoospermia group and 73.87% in asthenospermia group in relation to the normal fertile group. Seminal plasma arylsulfatase B decreased 72.78% in oligospermic patients, 22.34% in azoospermic patients and 44.82% in asthenospermic patients compared with the normal fertile group. On the contrary, seminal plasma alkaline proteinase increased 1.55, 1.86 and 2.11 fold in oligospermic, azoospermic and asthenospermic groups respectively from normal group.

CONCLUSION:

It is possible to use seminal plasma arylsulfatase A, arylsulfatase B and alkaline proteinase as fertility markers in the different male infertility types.

KEY WORDS: Male infertility, Oligospermia, Azoosperemia, Asthenospermia, Arylsulfatase, Alkaline proteinase.

INTRODUCTION :

Infertility is defined as the failure of a couple to achieve a pregnancy after at least one year of frequent unprotected intercourse. If a pregnancy has not occurred after 3 years, infertility most likely persists without medical treatment [(1)].

Studies in the United States and Europe showed a 1 – year prevalence of infertility in 15 % of couples. As shown in multicenter studies, 30 to 35 % of subfertility can be attributed to predominantly female factors, 25 to 30 % to male factors, and 25 to 30 % to problems in both partners; in the remaining cases, no cause can be identified [(1,2)].

Studies on the enzymatic status of spermatozoa are specialized and are limited to a few enzymes. The fertilizing capacity of a sperm sample is usually estimated by the concentration of spermatozoa and their motility and morphology. There are, however, some samples which while having normal

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spermiograms are infertile. It would thus appear that the classical, biological criteria of fertility are sometimes insufficient and might be overcome by some biochemical criteria. The enzymatic profile of spermatozoa should constitute a good indication of functional metabolic activity. The enzymes present in seminal fluid are shown to be derived from secretions of seminiferous tubules, spermatozoa, epididymis, seminal vesicles and prostate gland [3].

Seminal plasma possesses considerable proteolytic activity which is implicated in the degradation of seminal proteins to proteoses and free amino acids [4-7]. Several proteolytic agents secreted by the male accessory glands also participate in the two important processes which spontaneously occur in the ejaculated semen viz. semen coagulation and liquefaction [7-10]. The enzymatic equipment of spermatozoa is similar to that of seminal plasma [11]. A close relationship exists between the enzymatic equipment of spermatozoa and their fertilizing capacity.

Lysosomal hydrolases are considered to be important constituents of human seminal fluids. An extraordinary similarity between the enzymatic activities of the spermatozoa and those of the seminal plasma suggests that these enzymes need not all be of acrosomal origin. So much so, that the semen from azoospermic also shows lysosomal hydrolase activity. Considering the fact that the acrosome is a specialized lysosome [12], some hydrolases are also found to be present on the acrosome. A number of the hydrolases originate from accessory glands.

Male infertility is generally diagnosed in terms of the concentration, motility and morphology of spermatozoa. Often, these parameters do not truthfully reflect the fertilizing capacity of the samples. For instance, some samples exhibiting normal spermiograms are often seen to be infertile. Therefore, there have been increasing efforts to device suitable biochemical parameters, which could serve as effective markers for infertility [7]. There is a constant need to identify cellular markers of sperm quality so that in addition to their diagnostic value, they may facilitate the identification of specific deficiencies of sperm – function [11].Lysosomal arylsulfatase A and arylsulfatase B comprise a group of homologous but functionally distinct enzymes. ASA (EC 3.1.6.8) catalyzes the desulfation of the sphingolipid 3-*O*-sulfogalactosylceramide. ASA is synthesized as a 62-kDa polypeptide and bears three *N*-linked oligosaccharide side chains at asparagines 158, 184, and 350. Two of these oligosaccharide side chains (at Asn-158 and Asn-350) are accessible by the phosphotransferase, whereas the side chain at Asn-184 is not phosphorylated [13,14].

Arylsulfatase B (EC 3.1.6.12) (*N*-acetylgalactosaminidase-4-sulfatase/ASB) removes the sulfate from position 4 of *N*-acetylgalactosamine sugar residues at the non-reducing terminus of dermatan and chondroitin-4-sulfate. It is synthesized as a 64-kDa precursor, which is proteolytically processed to yield the mature 47-kDa enzyme. ASB bears five *N*-linked oligosaccharide side chains, but their degree of phosphorylation has not yet been determined [14].

ASA and ASB are highly homologous enzymes. Comparison of amino acid sequence reveals 29% identity of residues. Both enzymes also share a similar three-dimensional structure. Both consist of a central β – pleated sheet of ten strands, which is decorated by helices on both sides. They also share an intramolecular salt bridge and a more peripheral four-stranded β – pleated sheet. Therefore, they may also share a conserved phosphotransferase recognition domain [14]. Two soluble arylsulphatases (arylsulphatase A& B) are known to occur in human tissues. They are readily separated from a third insoluble enzyme (arylsulphatase C), from which they are easily distinguished by virtue of different substrate specificities and different response to certain inhibitors such as phosphate ions [15,16].

Binding of sperm to the zona pellucida (ZP) is the first step of gamete interaction that leads to fertilization. In mice, ZP3 and ZP2 sulfoglycoproteins are the primary and secondary receptors for acrosome intact and acrosome reacted sperm, respectively. Both ZP3 and ZP2 are highly glycosylated, with evidence of sulfation on their saccharide moieties. Specifically, oligosaccharides of ZP3 are significant for initial sperm binding [17].

Alkaline proteinase is a serine endopeptidase with a broad spectrum of action [7,18]. In addition to cleavage of peptide bonds, it is able to catalyze peptide amide hydrolysis. A special feature of alkaline proteinase is its ability to digest native proteins, thereby inactivating enzymes such as DNase and RNase without recourse to a denaturation process [18].

The presence of proteinases and peptidases in human seminal plasma is known since long. One of the

suggested roles of these enzymes is their fibrinolytic activity responsible for the liquefaction of ejaculated semen. These enzymes may also be important in sperm transport. Proteolytic enzymes have been found to dissolve the zona pellucida of mammalian egg and high semen viscosity associated with low proteinase activity [19].

MATERIALS AND METHODS:

PATIENTS:

Sixty patients with male infertility were recruited al – Kadhimiya teaching hospital – Baghdad and included in this study. (November 2004 – July 2006), the patients were invited to participate in this study and diagnosed according to a protocol described by WHO (WHO laboratory manual, 1992). Sixty patients (92.3%) were enrolled. Of the five patients (7.7%) did not enroll for one or more of the exclusion criteria and for refusing to participate in the study.

METHODS:

Semen samples were obtained by masturbation and samples were collected in a disposable sterile container, the patients and normal volunteers were asked to abstain from sexual activity for at least 72 hours to enhance optimal quality and quantity of semen. The freshly ejaculated samples were allowed to liquefy for 30 minutes at room temperature before evaluation for sperm characteristics according to guidelines laid down by World Health Organization (1992)[20]. The seminal plasma and sperm extract samples were prepared according to a modified procedure of previous studies [21-23]. Briefly, liquefied semen was centrifuged at 600 x g for 20 minutes at 4ºC. The supernatant seminal plasma was stored at - 20ºC until assay.

Arylsulfatases A and B were assayed according to the procedure of Baum et al. (1959)[24] with some modifications. The modifications consisted of omitting the dialysis step and shortening the incubation time. In brief, the activity of arylsulfatase A was determined by adding 0.3 mL of seminal plasma sample to 0.3 mL of (10mM p-nitrocatechol sulfate, dipotassium salt, 0.50 mM sodium pyrophosphate, 1.7 M sodium chloride in 0.50 M sodium acetate buffer ,pH 5.0) reagent . The tubes were incubated at 37ºC for 30 minutes. The reaction was terminated with 0.3mL of 1.0 M sodium hydroxide. The absorbances of liberated 4 – nitrocatechol were measured at 515nm [25,26]. Arylsulfatase B activity was estimated by adding 0.3 mL of seminal plasma sample in each of two sets of assay tubes containing 0.3mL of (50mM p-nitrocatechol sulfate, dipotassium salt, 10mM barium acetate in 0.50M sodium acetate buffer, pH 6.0) reagent incubated at 37ºC. The reaction in one set was stopped after 10 minutes and that in the other set after 30 minutes by adding 0.3 mL of 1.0 M sodium hydroxide. The absorbances of the liberated 4 – nitrocatechol were measured at 515nm[25,26].The assay procedure of alkaline proteinase was carried out according to previous investigators with some modifications regarding to substrate type and incubation period. Alkaline proteinase hydrolyzes hemoglobin denatured with urea, and liberates Folin positive amino acids and peptides, which are determined as tyrosine equivalents [7,18,25,27].

One unit releases one micromole of Folin positive amino acid in 10 minutes at 37ºC, pH 7.5 using denatured hemoglobin as substrate [7,18,25,27].

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences computer program (SPSS for windows, release 10.0, SPSS Inc., Chicago, IL, USA). All values were given as mean with corresponding standard deviation.

RESULTS:

The characteristics of subjects participated in this study with their seminal fluid parameters were listed in table 1. It was found that there were no significant differences ($P \leq 0.05$) between the age values of different patient groups and control group. ANOVA analyses revealed the presence of high significant differences in the sperm counts and progressive motility percents between infertile groups and the controlgroup.

Table 1 : Characteristics of patients and controls with their seminal fluid parameters

Variable	Azoospermia (n=22)	Oligospermia (n= 32)	Asthenospermia (n=6)	Control (n=39)	ANOVA
Age(year)	32.09±4.51	30.90±3.52	35.00±3.84	31.87±3.76	0.122
Seminal fluid volume (mL)	3.75±1.05	3.04±1.23	3.66±1.40	3.46±1.72	0.308
Sperm count (million/mL)	-	8.44±5.13(***)	53.04±11.10(***)	87.34±38.72	0.000
Progressive motility (%)	-	60.89±9.40	38.22±14.64(**)	64.06±8.90	0.000
Morphology (%)	-	75.34±8.25	75.38±7.48	78.29±7.84	0.279

- All values are expressed as mean ± SD
- "n" indicates the number of cases
- "**" indicates significant difference from the control group at p level ≤ 0.01
- "***" indicates significant difference from the control group at p level ≤ 0.005

Table 2 summarizes the enzymatic activities of some important lysosomal enzymes measured in this study. Arylsulfatase A and arylsulfatase B activities were measured concurrently in the seminal plasma of infertile and fertile subjects, all values showed significant (P≤ 0.001) decrease in the infertile patients compared with control group while seminal plasma alkaline proteinase increased significantly (P≤ 0.001) in all infertility groups compared with those of controls.

Table 2: Lysosomal enzymatic activities in male infertility patients and controls

Enzyme	Azoospermia (n=22)	Oligospermia (n=32)	Asthenospermia (n=6)	Control (n=39)	ANOVA
Arylsulfatase A					
Seminal plasma (nmole/min/mL)	0.235±0.023(***)	0.250±0.046(***)	0.247±0.045(***)	0.945±0.21	0.000
Arylsulfatase B					
Seminal plasma (nmole/min/mL)	7.942±1.211(***)	2.784±0.396(***)	5.643±1.323(***)	10.226±2.27	0.000
Alkaline proteinase					
Seminal plasma (µmole/min/mL)	1.615±0.182(***)	1.347±0.253(***)	1.831±0.120(***)	0.868±0.150	0.000

- All enzymatic activities are expressed as mean±SD.
- *** indicates the degree of significance between control and infertility groups at p level values of ≤ 0.001.

DISCUSSION :

Since semen is a mixture of complex functional proteins, alkaline proteinase which can mediate protein degradation could play a vital role in determining the semen quality [19]. Allison and Hartree (1970) have analyzed the role of lysosomal enzymes in the acrosome and their possible role in the fertilization from rat spermatozoa [12]. Arylsulfatase A, a mucopolysaccharidase was the most affected. This enzyme is involved in the stability and permeability of plasma membrane of spermatozoa of boars [27]. It was known to play a significant role of arylsulfatase in sperm capacitation, acrosome reaction and gamete interactions [27]. In the present study, seminal plasma arylsulfatase A showed significantly low levels (73.55%) of oligospermia, (75.14%) of azoospermia and

(73.87%) of asthenospermia as compared to the normal fertile group. Whereas, seminal plasma arylsulfatase B decreased (72.78%) in oligospermic patients, (22.34%) in azoospermic patients and (44.82%) in asthenospermic patients of the normal activity. These ratios indicate that arylsulfatase A and arylsulfatase B could well serve as biochemical markers for male infertility. The decrease in the arylsulfatase A and arylsulfatase B activities found in different types of male infertility may be attributed to either a decrease in the de novo synthesis of lysosomal enzymes or the presence of endogenous inhibitors. Unlike arylsulfatase A and arylsulfatase B, alkaline proteinase showed a distinctly different pattern of response to sperm density. Alkaline proteinase increased 1.55, 1.86 and 2.11 fold in oligospermic, azoospermic and asthenospermic respectively as compared to normal samples. The different response of alkaline proteinase from other lysosomal enzymes may suggest a non – lysosomal location of this enzyme. The induction of acrosome reaction is found to be implicated by arylsulfatases, the level of which was found to be low in all abnormal categories. Whereas, reverse situation exists in the levels of alkaline proteinase, therefore, the ratio of the arylsulfatase A/ alkaline proteinase levels were calculated. The ratio was found to be significantly lower in abnormal groups, 0.18 in oligospermia, 0.145 in azoospermia and the ratio in asthenospermia was 0.13 while the control group ratio was 1.08.

CONCLUSION:

From this study, it is concluded that calculation of the arylsulfatase A / alkaline proteinase ratio will be a good marker of infertility status.

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