ACROSIN ACTIVITY IN SPERMATOZOA OF INFERTILE NIGERIAN MALES

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ABSTRACT

The objective of this study was to evaluate acrosin activity in spermatozoa of infertile Nigerian men and to compare with those of the fertile men. The acrosin activity was evaluated using the Acroscreen reagent kit. The acrosin activity of the fertile men was 40.7 ± 5.2 mIU/ 10^6 sperm (range 30.1 - 51.3) and those of the infertile men was 22.4 ± 8.33 mIU/ 10^6 sperm (range 5.7 - 39.1). The difference in the mean was statistically significant ($P < 0.001$). There was a subpopulation of the infertile men who had their acrosin activity within normal range of the fertile men, 32.9 ± 1.57 mIU/ 10^6 sperm. But the difference in the mean was statistically significant ($P < 0.001$). Acrosin activity decreased with increased morphological changes in the spermatozoa. It is concluded that acrosin activity in the infertile Nigerian men is significantly lower than that in the fertile men. Acrosin activity may also be affected by morphological changes in the spermatozoa

KEY WORDS

Acrosin activity, spermatozoa, male infertility.

INTRODUCTION

Recent studies have demonstrated a high rate of infertility in many countries in sub Saharan Africa. The problems of infertility are shared between both males and female sexes. But male factors may contribute up to 50% of the causes of infertility in couples (1, 2). Despite the usefulness of semen analysis, it does not entirely predict fertility. Persistent azoospermia is not consistent with paternity. However, if the semen analysis in the male partner of an infertile couple produce results similar to those of fertile men, the issue becomes complex. The conventional sperm analysis are confined to sperm density, morphology, motility and other physical characteristics. Unfortunately, none of these physical indices appear to accurately predict fertility potential of spermatozoa (3). It has been suggested that the level of acrosin activity of sperm may be a useful determinant of sperm fertility potential (4). Sperm acrosin is a serine protease with enzyme activity similar to that of trypsin existing in acrosomes. It is an important proteolytic enzyme that is capable of hydrolyzing the zona pellucida of oocyte and plays a vital role in the

process of fertilization (5, 6). Low levels of acrosin appear to be associated with subfertility and infertility (7). The acrosin activity is independent of the standard semen parameters such as sperm density, percent motility and morphology (8).

The objective of this study was to determine the levels of acrosin activity in infertile men who are attending the fertility clinic of Aminu Kano Teaching Hospital, Kano, Northern Nigeria, and to compare them with those of the fertile men.

MATERIALS AND METHODS

The simplified method for measuring acrosin activity in spermatozoa as modified by Deutsch *et al.* (3) was used. The reagent kit Acroscreen, supplied by Bioscreen, Inc, USA, a photometric enzyme method was used for the determination.

One hundred infertile males aged 24-54 years (mean 29.5) and twenty fertile men who have fathered two or more children aged 27-50 years (mean 30 years) were recruited for the study. The fertile men served as controls. They had their routine semen analysis within normal range. The study group comprised of 75 oligospermic men and 25 normospermic but infertile men (unexplained infertility) who had lived with their wives for a minimum of one year without bearing children. Semen specimens were obtained as fresh ejaculate by masturbation after 3-5 days of abstinence. Semen samples were liquefied at 37°C in a water bath before analysis. After liquefaction, sperm density and other physical indices were evaluated.

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100 I aliquot of the semen was diluted with the kit's wash to obtain a count of between 3-5 million cells/ml. The kit protocol was followed for assaying the semen specimens as directed by the supplier. Students t -test for paired parametric means was used to compare the mean acrosin activity of spermatozoa of patient and control subjects.

RESULTS

The results are summarized in Tables 1, 2 and 3. The mean of the acrosin activity in semen samples of the control subjects when compared with the patients, was significantly different ($P<0.001$). The range of the control subjects was 30.1 - 51.3 mIU/ 10^6 sperm while that of the patients was 5.7 -39.1 mIU/ 10^6 sperm. Whereas the least acrosin activity in the control subjects was 30 mIU/ 10^6 sperm, the least in the patients was 8 mIU/ 10^6 sperm. Table 2 shows acrosin activity in the spermatozoa of infertile men. Seventy five subjects had low acrosin activity with a mean of 18.9mIU/ 10^6 sperm (range 5.8 -32.0) while twenty five normospermic but infertile men had mean acrosin activity of 32.9 mIU/ 10^6 sperm (range 29.5 - 36.0). The difference in the mean when compared with fertile men was statistically significant ($p<0.001$). Table 3 shows that acrosin activity decreased with increased sperm cell morphological abnormalities which was statistically significant ($p<0.001$).

DISCUSSION

The results showed that acrosin activity in spermatozoa of men with proven fertility were higher than those of the infertile men, however, there is a

subpopulation of normospermic but infertile men who had acrosin activity within the normal limit of the control subjects. Although these patients were investigated for infertility their mean sperm density was within normal limit. This result is similar to that of Chaudhury *et al.* (9) who reported that acrosin system was adversely affected in unexplained infertile subjects. It was concluded that acrosin activity evaluation in this group of patients can be used as a sensitive biochemical marker.

Available evidence in Africa suggest that the increased incidence of infertility is due to a high prevalence of sexually transmitted diseases. The low acrosin activity observed in oligospermic men may be due to oxidative damage induced by oxygen derived oxidants associated with white blood cell infiltration (10).

This result also shows that there is a relationship between acrosin activity and fertility potential of spermatozoa. Human spermatozoa requires capacitation to become competent for fertilization. Acrosin (EC 3.4.21.10) is a sperm acrosomal protease that plays an important role in this process. Acrosin is involved in the acrosome reaction, that is the binding of spermatozoa to the zona pellucida and /or the penetration of spermatozoa through the zona pellucida (10). The acrosin reaction occurs in correct calcium environment. Hyper-motility is required to provide the thrust necessary to penetrate cumulus oophorus and the zona. The acrosomal membrane of the sperm head has been postulated to have specific molecules for attachment to the zona before penetration of the egg. The acrosome contains the enzyme acrosin which is a trypsin- like serine protease that hydrolyzes the

Table 1. Acrosin activity of spermatozoa of infertile and control subjects (means $\pm$ SD)

Subjects	Age (mean)	Sperm density ($\times 10^6$ cell/ml)	Acrosin activity mIU/ 10^6 sperm	Range
Infertile males n=100	29.8	16.7	22.43 $\pm$ 8.33**	5.7-39.1
Fertile males n=20	30.0	42.6	40.7 $\pm$ 5.3	30.1-51.3

** $P<0.001$

Table 2. Acrosin activity of spermatozoa of infertile men

Subjects	Age (mean)	Sperm density ($\times 10^6$ cell/ml)	Acrosin activity mIU/ 10^6 sperm	Range
Oligospermia I (n=75)	28.7	9.3	18.9 $\pm$ 6.55**	5.8-32.0
Normospermic but infertile men (n=25)	30.9	22.6	32.9 $\pm$ 1.57	29.5-36.0

** $p<0.001$

Table 3. Relationship between acrosin activity and morphological changes in spermatozoa

Percentage morpho-logical abnormalities	Number (n)	Acrosin activity mIU/10 ⁶ sperm	p- value
Less than 30%	25	32.9±1.57	
Greater than 30%	75	18.9±6.55**	< 0.001

**p<0.001

egg membrane to enable the spermatozoa to navigate the interstices of the corona radiata (10, 11 12).

The mechanisms of capacitation and fertilization mediated by acrosin activity have been discussed (12). Because the sperm cell membrane molecules necessary for attachment to the zona must be retained, the outer membrane must remain intact after the release of the enzymes. The spermatozoa joins with the egg by membrane fusion of a mid portion membrane, the equatorial region of the sperm head. The acrosome reaction creates structural alteration required for the various constraints to be overcome. The result of the acrosin activity in this study are comparable with those of other authors (7, 8). In the acrosin activity assay proacrosin is activated to its active form, acrosin by incubation at pH 8.0 so that total acrosin activity is measured (5, 6). The acrosin activity was higher in spermatozoa with morphological abnormalities of less than 30% than in those with morphological abnormalities of greater 30% (Table 3). There was a significant negative correlation between acrosin activity and morphological abnormalities. The results show that acrosin, a delicate enzyme can be disturbed under various conditions such as sperm malformation and local inflammation with leucocyte infiltration (14, 15).

It is concluded that acrosin activity was adversely affected in oligospermic and in unexplained infertile men. Its activity was also affected by changes in sperm cell morphology.

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