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Effect of Genital Tract Infection on Alpha Glucosidase Enzyme in Semen of Infertile Male Patients

Manar Maged Ahmed Mohammed Shiha*, Samir Mohamed Elhanbly, Ahmed Fathy State
Department of Dermatology, Andrology and STDs, Faculty of Medicine, Mansoura University, Egypt
***Corresponding author:** Manar Maged Ahmed Mohammed, **Mobile:** (+20) 01095697097, **E-Mail:** manormaged123@gmail.com

ABSTRACT

Background: Certain biological substances could be thought of as reliable predictors of male infertility. An epididymal pathology might be evaluated with the use of the glucosidase determination. Reduced levels of glucosidase in semen have been linked to impaired sperm maturation in the epididymis and may possibly be connected to epididymitis and genital tract inflammation.

Objective: This study aimed to assess how genital tract infections affected the amount of alpha glucosidase in infertile men's semen.

Patients and methods: Thirty-one infertile male patients with pyospermia were included in this prospective study. Patients were recruited from the Outpatient Clinic of Andrology unit in Dermatology and Andrology & STDs Department, Mansoura University Hospital.

Results: The α-glucosidase level before treatment among all studied cases was assayed in infected seminal fluid. The mean level was 7.2 ng/mL. It increased significantly after treatment to 15.8 ng/mL, $p<0.001$. Receiver operating characteristic (ROC) curve of α-glucosidase was conducted for prediction of presence of bacterial infection in seminal fluid. The α-glucosidase level showed high accuracy AUC (AUC=0.918). The best cut off value was 13.1 ng/mL, sensitivity was 78.5%, specificity was 78%, PPV was 70.1%, NPV was 95.2%, and accuracy was 87.1%. No significant difference was found regarding the baseline α-glucosidase level according to type of infertility or urinary symptoms among all studied cases ($p>0.05$ for each). No significant difference was found regarding the baseline α-glucosidase level according to organism type among all studied cases ($p>0.05$).

Conclusion: Alpha-glucosidase levels are dramatically reduced in male reproductive tract infections, indicating a problem with the epididymis's secretory ability.

Keywords: Genital tract infection, NAG, Infertility means.

INTRODUCTION

Around 15% of couples have infertility, which is defined as the failure to develop a clinical pregnancy following 12 months of consistent unprotected sexual activity [1]. In 20% of instances, the male factor is the primary cause of infertility, and it plays some role in the remaining 50%. There are several aetiologies for male factor infertility in around 15% of instances, infectious diseases are to blame [2].

The primary enzyme responsible for hydrolyzing the carbohydrate alpha-glucosidase is alpha-glucosidase. It is connected to various metabolic processes, such as the processing of glycoproteins and glycolipids as well as the intestinal digestion of carbohydrates [3]. Neutral alpha-glucosidase (NAG), a particular epididymal enzyme marker, is mostly released by epididymal epithelial cells in seminal plasma [4]. Its activity is regarded as a trustworthy indicator for assessing epididymal patency and function [5]. Alpha-glucosidase, fructose, and zinc levels in seminal plasma are all considerably lower in male reproductive tract infections, indicating a possible impairment of the epididymis, seminal vesicles, and prostate's secretory function [6].

The epididymis is crucial for the development of spermatozoa, their acquisition of progressive motility, and their capacity for fertilisation [7]. Neutral alpha-glucosidase (NAG), a particular epididymal enzyme marker, is mostly released by epididymal epithelial cells in seminal plasma [4]. Its activity is regarded as a trustworthy indicator for assessing epididymal patency and function [5]. Traditional sperm parameters [8], seminal ATP levels, and spermatozoa's ability to fertilise [9], as well as reactive oxygen species (ROS) levels and peroxidase-positive white blood cell concentrations, which both negatively influence fertilisation, are negatively connected with NAG activity [10].

The current study's objective was to assess how genital tract infections affected the amount of alpha glucosidase in infertile men's semen.

PATIENTS AND METHODS

This prospective research comprised 31 pyospermic male patients who were infertile. The Mansoura University Hospital's Dermatology and Andrology & STDs Department outpatient clinic was used to recruit patients. It was conducted over a period of 12 months from June 2020 to June 2021.

Inclusion criteria: Men who are infertile and have pyospermia, which is determined by perioxiadase stain (greater than a million PMN leukocytes in 1 mL of semen) [11]. Age ranged from 21 to 45.

Exclusion criteria: Short-term infertility (less than a year). Varicocele. Obstructive azoospermia. Cigarette smoking and drug abuser. Genetic diseases. Hypogonadism. Hyperprolactinemia. Systemic

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diseases. Patients had taken drugs affecting fertilization within last 3 months (chemotherapy, antiandrogen and antiepileptic drugs)

All patients were subjected to:
1. Full history taking: Age, employment, and residence. Fertility history. A history of illnesses that could have a negative impact on fertility. A history of other issues that could have a negative impact on fertility. Sexual background.
2. General physical examination: Signs of hypogonadism. Gynecomastia and galactorrhea. Anosmia, cleft palate, recurrent chest infection.
3. Genital examination: Penis: Scarring, hypospadias, etc. **Testis:** Location, size, and regularity. **Epididymis:** Thickened, tender, or cystic head, body, and tail. **Vas deferens:** If perceptible or not, and whether or not it is beaded. Scrotal enlargement. **Varicocele:** Varicocele was examined in both upright and supine positions, during both silent breathing and the Valsalva manoeuvre, with the two sides being compared to look for aberrant visible or palpable veins inside the spermatic cord and surrounding the testis.
4. Laboratory investigations:
Semen collection and processing: After 3-5 days of sexual abstinence, 25 semen samples were taken from the infertile patients. The patients were instructed to pee, then wash and dry their glans penis using ordinary water and soap. In a secluded room next to the lab, the samples were acquired by masturbation and ejaculated into sterile containers. The patients were given specific instructions on how to keep their fingers and penises away from inner containers. The lab received the semen samples immediately and with the appropriate labelling (full name, age, serial number of the patient, date and time of collection).

Computer assisted semen analysis: As soon as the semen samples were liquefied, they were inspected. According to **WHO** [11] recommendations, the ejaculate's volume, PH, concentration, shape, motility, and pyospermia were assessed.

Culturing of semen samples: Within an hour following semen collocation, each sample was inoculated on one of three agar medium plates (nutrient agar, MacConkey agar, and blood agar) and incubated aerobically at 37°C for at least 48 hours. Less than 10,000 colony forming units per milliliter (CFU/ml) was considered significant bacterial growth. According to Bergey's textbook of systematic bacteriology, the typical microbiological methods for identifying bacterial isolates included analysing colony characteristics, staining responses, and biochemical assays [12].

Alpha glucosidase measurement: Alpha glucosidase was tested in semen using perioxidase stain to identify pyospermia. **[Human α -Glucosidase (a-Glu) ELISA** kits Cat. No 201-12-0858, USA].

Ethical approval: This experiment was ethically approved by Mansoura University's IRB No.: MS.21.02.1394). After being fully informed, all participants provided written consent. The study was conducted out in line with the Helsinki Declaration.

Statistical analysis
The obtained data was inspected, coded, tabulated, and entered into a computer using a social science statistical tool. After the data were submitted, the type of data obtained for each parameter was appropriately analysed. The Shapiro-Wilk test was used to determine if the data distribution was normal. Data that were not parametric were deemed significant. Using parametric assumptions, the mean and standard deviation of numerical data (SD) were computed. Also, the amount and frequency of non-numerical data. The Student T-Test was performed to determine the statistical significance of the difference in means between the two research groups. In a one-way comparison, ANOVA was employed to compare the means of more than two groups. Comparing qualitative variables was done using the chi-square and Fisher's exact test. The paired sample t-test was used to evaluate how parameters vary over time.

The receiver operating characteristic (ROC) curve was used for measuring the sensitivity and specificity of quantitative diagnostic tests that split patients into two groups. The optimal cut-off point was discovered to be the one that maximised the AUC value. According to AUC, a test with a greater than 0.9 area has high accuracy, a test with a 0.7 to 0.9 area has moderate accuracy, a test with a 0.5 area has low accuracy, and a 0.5 result is chance. Correlation analysis to determine the degree to which two quantitative variables are connected. The correlation coefficient determines the size and direction of the linear relationship between two variables. Column chart was used to create visual presentations of means. They were displayed in vertical columns. Error bars are simply representations of the standard deviations. Bar chart was used to create visual presentations of percentages. They were displayed in horizontal columns. A circular graph's data was represented using a pie chart. The pie slices display the data's relative sizes. $P \leq 0.05$ was regarded as significant.

RESULTS
The current study was conducted on 31 infertile male patients. Table (1) showed that the mean age of the studied group was 33.6, and ranged from 25 to 42 years. Among studied cases, 19.4% were doctors, 19.4% were nurses, 22.6% were students, 19.4% were carpenters, and 19.4% were farmers. Residence was rural in 41.9% and urban in 58.1%.

Table (1): Age of the studied cases

			n=(31)	
Age (years)		**Mean ± SD**	33.6 ± 4.7	
		range	25-42	
Occupation	**Doctor**	**N, %**	6	19.4
	Nurse	**N, %**	6	19.4
	Student	**N, %**	7	22.6
	Carpenter	**N, %**	6	19.4
	Farmer	**N, %**	6	19.4
Residence	**Rural**	**N, %**	13	41.9
	Urban	**N, %**	18	58.1

SD, standard deviation

Among all studied cases, 74.2% responded to antimicrobial therapy, while 25.8% did not respond to antimicrobial therapy, with significant differences between both groups (p=0.007) (Table 2).

Table (2): Response to antimicrobial therapy

	Responders	Non responders	p
All cases	23 (74.2%)	8 (25.8%)	**0.007**

The α-glucosidase level before treatment among all studied cases was assayed in infected seminal fluid. The mean level was 7.2 ng/mL. It increased significantly after treatment to 15.8 ng/mL, p<0.001 (Table 3).

Table (3): The α-glucosidase level among all studied cases before and after antimicrobial therapy

	Before treatment		After treatment		p
	mean	**± SD**	**mean**	**± SD**	
α-glucosidase (ng/mL)	7.2	± 1.3	15.8	± 2.2	**<0.001**

SD, standard deviation.

The α-glucosidase level before treatment did not differ significantly between those who achieved later on response and those who did not (p>0.05). While after treatment, the α-glucosidase level was significantly higher in responders than non-responders (p=0.002) (Table 4).

Table (4): Comparison of the α-glucosidase level among responders and non-responders to antimicrobial treatment

		All cases	Responders	Non responders	p
		n=31	**n=23**	**n=8**	
		Mean ± SD	**Mean ± SD**	**Mean ± SD**	
α-glucosidase (ng/mL)	**Before treatment**	7.2 ± 1.3	6.9 ± 1.3	8.3 ± 2.5	0.168
	After treatment	15.8 ± 2.2	17.6 ± 3.5	10.9 ± 2.1	**0.002**

SD, standard deviation.

The level of the α-glucosidase increased significantly after treatment in cases who responded (p<0.001), but not in those who did not respond to antimicrobial therapy (p>0.05) (Table 5).

Table (5): Comparison of the α-glucosidase level among responders and non-responders to antimicrobial treatment before versus after treatment

	α-glucosidase (ng/mL)		
	Before treatment	**After treatment**	**p**
	Mean ± SD	**Mean ± SD**	
Responders	6.9±1.3	17.6±3.5	**<0.001**
Non responders	8.3±2.5	10.9±2.1	0.208

Receiver operating characteristic (ROC) curve of α-glucosidase was conducted for prediction of presence of bacterial infection in seminal fluid. The α-glucosidase level showed high accuracy AUC (AUC=0.918). The best cut off value was 13.1 ng/mL, sensitivity was 78.5%, specificity was 78%, PPV was 70.1%, NPV was 95.2%, and accuracy was 87.1% (Table 6 & figure 1).

Table (6): Validity of α-glucosidase for prediction of presence of bacterial infection in seminal fluid

	α-glucosidase
AUC	0.918
Cut off (ng/mL)	13.1
Sensitivity (%)	87.5
Specificity (%)	87
PPV (%)	70.1
NPV (%)	95.2
Accuracy (%)	87.1

AUC, area under ROC, ROC, receiver operating curve; PPV, positive predictive value; NPV, negative predictive value.

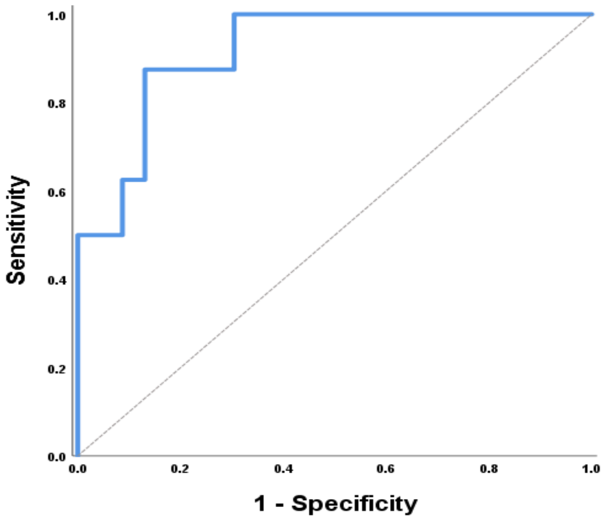

Figure (1): ROC curve of α-glucosidase for prediction of presence of bacterial infection in seminal fluid

No significant difference was found regarding the baseline α-glucosidase level according to type of infertility or urinary symptoms among all studied cases (p>0.05 for each) (Table 7).

Table (7): Comparison of the baseline α-glucosidase level according to other parameters among all studied cases.

Variable		**Cases (N=31**		**p**
		α-glucosidase		
		mean	**±SD**	
Infertility	**Primary**	7.5	±2.5	0.478
	Secondary	6.8	±2.2	
	Present	6.1	±1	
Urinary symptoms	**Absent**	6.7	±2.3	0.156
	Present	8.0	±2.7	

No significant difference was found regarding the baseline α-glucosidase level according to organism type among all studied cases (p>0.05) (Table 8).

Table (8): Comparison of the baseline α-glucosidase level according to organism type among all studied cases.

	Cases		**p**
	N=31		
	α-glucosidase		
	mean	**±SD**	
Staphylococcus aureus	7.6	±2.5	0.646
E. coli	6.8	±2.2	
Klebsiella spp.	6.2	±2	
Streptococci spp.	5.3	-	
Pseudomonas spp.	4.4	-	

The α-glucosidase level showed no significant correlations with age, sperm count, and pus cells among studied cases (Table 9).

Table (9): Correlation of α-glucosidase with age, sperm count, and pus cells among studied cases

	α-glucosidase	
	r	p
Age	-0.312	0.087
Sperm count	0.028	0.882
Pus cells	-0.071	0.705

r, correlation coefficient

Non responders were subjected to second microbiologic culture, which revealed Staph aureus in 87.5%, and Kleibsiella in 12.5% (Table 10).

Table (10): The results of second microbiologic culture of non-responders.

Variable		n=(8)
Second cultures	Staph aureus	7 (87.5%)
	Kleibsiella spp.	1 (12.5%)

Antimicrobial sensitivity tests were performed on non-responders. Linzolid, Aztreonam, and Cefepime were the most responsive antibiotics. Tetracycline, gentamycin, ciprofloxacin, levofloxacin, and cephalexin were the most resistant antibiotics. Table (11) showed other antimicrobial sensitivity patterns.

Table (11): Antimicrobial susceptibility pattern of all studied samples among second cultures of non-responders.

	Culture Growth N=8					
	Sensitive		Intermediate		Resistant	
	N	%	N	%	N	%
Ciprofloxacin	1	12.5%	0	0%	7	87.5%
Levofloxacin	1	12.5%	0	0%	7	87.5%
Amoxicillin/clavulanic acid	1	12.5%	1	12.5%	6	75.0%
Ampicillin/sulbactam	1	12.5%	1	12.5%	6	75.0%
Tetracycline	0	0%	0	0%	8	100.0%
Gentamycin	1	12.5%	0	0%	7	87.5%
Piperacillin/tazobactam	2	25.0%	0	0%	6	75.0%
Cefotaxime	2	25.0%	1	12.5%	5	62.5%
Amikacin	2	25.0%	0	0%	6	75.0%
Cefoperazone/sulbactam	2	25.0%	1	12.5%	5	62.5%
Cefoprazone	1	12.5%	1	12.5%	6	75.0%
Ceftriaxone	1	12.5%	1	12.5%	6	75.0%
Cephalexin	1	12.5%	0	0%	7	87.5%
Cefuroxime	1	12.5%	1	12.5%	6	75.0%
Penicillin	1	12.5%	1	12.5%	6	75.0%
Erythromycin	5	12.5%	0	0%	3	37.5%
Norfloxacin	6	12.5%	0	0%	2	25.0%
Vancomycin	6	0%	1	12.5%	1	12.5%
Clindamycin	5	62.5%	0	0.0%	3	37.5%
Cefepime	4	75.0%	1	12.5%	3	37.5%
Aztreonam	7	75.0%	0	0%	1	12.5%
Imipenem	8	62.5%	0	0%	0	0%
Meropenim	8	50.0%	0	0%	0	0%
Linzolid	8	87.5%	0	0%	0	0%

DISCUSSION

To our knowledge, no prior research has been done to determine the impact of genital tract infections on the alpha glucosidase enzyme in the male infertility patient's semen. The patients in the current research ranged in age from 25 to 42 years, with a mean age of 33.6 ± 4.7 years. Primary infertility affected 21 patients (67.7%) men, whereas subsequent infertility affected 10 patients (32.3%) males. **Elgozali *et al.*** [13] investigated a group of patients with infertility and pyospermia and discovered that their age range was 22-45 years. This is similar to the finding about the mean age. Nonetheless, there was a little discrepancy in the age distribution between **Abdulla** [14] and **Bhatt *et al.*** [12]**,** 31- 40 years and 25-50 years respectively. According to our findings, **Sonbol and Elhanbly** [15] observed that 17 instances (56.7%) of infertility were of the primary type, while the remaining 13 cases (43.3%) were of the secondary type. Similar findings were made by **Abbas** [16], who discovered that primary infertility affected 56.3% of pyospermic individuals whereas secondary infertility affected 43.8%. **Elgozali *et al.*** [13] discovered, in contrast to our findings, that only 5 men (10%) were secondary infertile out of a sample of 50 infertile males with pyospermia, with 45 men (90%) being main infertile.

In this study, semen cultures from the patients under investigation revealed the development of the following positive isolates: Staph aureus growth was found in 74.2% of cases, E coli growth in 12.9%, Kleibsiella growth in 6.5%, Streptococci growth in 3.1%, and pseudomonas growth in 3.2%. According to this finding, Staphylococcus aureus, Streptococcus saprophyticus, and Escherichia coli had the greatest occurrence rates, with rates of 28.3%, 19.6%, and 13.0%, respectively, in **Isaiah *et al.*** [17] investigation on 140 patients with pyospermia. Next, at 10.8% for each, were Proteus mirablis, Kleibsiella pneumonia, and Proteus vulgaris. At 5%, Pseudomonas aeruginosa. **Sonbol and Elhanbly** [15] also found positive staphylococci (80%), Escherichia coli (6.7%), Kleibsiella pneumoniae (6.7%), streptococci (3.3%), and pseudomonas aeruginosa (3.3%), and their findings are comparable to our one. Similar to this, **Amadi *et al.*** [18] investigation found that staphylococcal aureus infections were prevalent in seminal fluid at a rate of 62.5%. Also, it is comparable to **Nasrallah *et al.*** [19], who discovered that Staph. Aureus (46.2%) was the most prevalent isolated bacterium. **Abdulla** [14] examined infertile individuals with pyospermia and discovered that enterococcus faecalis (30%), coagulase-positive staphylococci (20%), Escherichia coli (13.3%), Pseudomonas aeruginosa (10%), and Kleibsiella pneumoniae (10%) had substantial increases in the number of positive isolates. According to several research, staphylococcus aureus infection is a minor source of contamination [20]. The distinction between infection, contamination, and colonisation of the genital system is not made with sufficient clarity in the WHO definition of seminal tract infection. Gram-positive skin bacteria, such as commensal staphylococcal, streptococcal, and diphteroidal species contaminate the semen that travels through the vaginal canal. Staphylococcus aureus is widely acknowledged to be pathogenic and requires rapid treatment [21].

Male fertility may be negatively impacted by infection in a variety of ways, including spermatogenesis impairment, autoimmune mechanism induction, obstruction of the ejaculatory ducts, dysfunction of the ejaculated sperms, decrease in spermatozoa motility, and induction of reactive oxygen species (ROS) [22].

Antimicrobial sensitivity tests were done for all bacterial growths. The antibiotics Amoxicillin/clavulanic acid and Piperacillin/tazobactam were the most sensitive. Whereas ciprofloxacin, Tetracycline, Gentamycin, Cephalexin, Levofloxacin, Ampicillin/Sulbactam, Cefotaxime, Cefoprazone, and Ceftriaxone were the antibiotics with the highest rates of resistance. Among all studied cases, 74.2% responded to antimicrobial therapy, while 25.8% did not respond to antimicrobial therapy, with significant differences between both groups (p=0.007). Similarly, **Sonbol and Elhanbly** [15] found that Rifampicin, Cefoperazone/sulbactam, Amoxicillin/clavulanic acid, and Piperacillin/tazobactam were the four antibiotics that were the most sensitive. Whereas Levofloxacin, Cephalexin, and Ciprofloxacin were the most resistant drugs. Bacteria were reported to be extremely susceptible to the antibiotics Piperacillin/tazobactam, Imipenem, Meropenem, Gentamicin, Doxycycline, Amikacin, and Nitrofurantoin by **Nasrallah *et al.*** [19]. According to another study, Nitrofurantoin (91.5% and 71.7%, respectively) was the most effective antibiotic against both gram-positive and gram-negative organisms, followed by Ampicillin/sulbactam (73.9% and 58.9%), Levofloxacin (56.5% and 71.7%, respectively), and Gentamicin (56.5% and 53.8%, respectively) [12]**. Isaiah *et al.*** [17] discovered that Imipenem and Vancomycin were effective against Staphylococcus aureus and Staphylococcus saprophyticus. While staph aureus was known to be resistant to Ampicillin, Penicillin, and Chloramphenicol. **Uneke and Ugwuoru** [23] found that it was more responsive to Nitrofurantoin and Perfloxacin. In addition, whereas Streptococci were resistant to Ampicillin and Penicillin, they were susceptible to Cotrimoxazole and Tetracycline. Another research found that while staph aureus was resistant to Cephalexin and Cotrimoxazole, it was more responsive to the antibiotics Azithromycin, Ofloxacin, and Sparfloxacin [13]. Staph aureus was found by **Abdulla** [14] to be responsive to Ciprofloxacin and Cephaloridine but resistant to Penicillin.

In the instant study**,** the α-glucosidase level before treatment among all studied cases was assayed in infected seminal fluid. The mean level was decreased to 7.2 ng/mL. It was increased significantly after treatment to 15.8 ng/mL (p<0.001). Similarly, in a prior studies by **Schuppe *et al.*** [24] and **Haidl *et al.*** [25], they showed a reduction in the concentration of α-glucosidase level in patients with genital tract infection. Indeed, **Marconi *et al.*** [6] showed that the concentrations of sperm, zinc, fructose, and alpha-glucosidase were all considerably lower in the infection group when compared to the control group. Although **Cooper *et al.*** [26] discovered a substantial drop in alpha-glucosidase levels in patients with acute epididymitis, prior studies showed that the effect on the epididymis' secretory function in patients with chronic epididymitis is not significant [27]. **Qiu *et al.*** [28] study, compared to subfertile and infertile males, normal men had much greater levels of NAG. Also, compared to other infertile males, infertile men had greater levels of NAG. According to these findings, typical semen has a high amount of NAG, and lower levels of NAG may suggest lower-quality semen. The findings also showed that individuals with normozoospermia had levels of NAG that were considerably greater than those with teratozoospermia, asthenospermia, severe oligozoospermia, asthenoteratozoospermia, oligoasthenospermia, oligoasthenoteratozoospermia, and azoospermia. Patients with azoospermia had considerably lower levels of NAG than those with teratozoospermia, asthenospermia, asthenoteratozoospermia, oligoasthenospermia, and oligoasthenoteratozoospermia. In addition, NAG levels were considerably greater in individuals with teratozoospermia and oligoasthenospermia than they were in those with severe oligozoospermia.

Their results demonstrated that NAG was substantially correlated with semen concentration, motility, and morphology, which is in agreement with research by **Levrant *et al.*** [29] and **Said *et al.*** [30], but not the **Pea *et al.*** [31] study. As a result, the NAG level may vary between countries. The findings also indicated that individuals with azoospermia had the lowest levels of NAG, whilst normal men had the greatest levels, indicating that NAG might be used as a marker to distinguish between normal, subfertile, and infertile men. Asthenospermia, severe oligozoospermia, asthenoteratozoospermia, oligoasthenospermia, and oligoasthenoteratozoospermia could all be distinguished using NAG levels [28].

In the present study, the level of the α-glucosidase increased significantly after treatment in cases who responded (p<0.001), but not in those who did not respond to antimicrobial therapy (p>0.05).

CONCLUSION

- Alpha-glucosidase levels are markedly reduced in male reproductive tract infections, which suggests that the epididymis's secretory ability is impaired.
- Alpha-glucosidase levels in males were considerably higher following antibiotic therapy than they were before to any genital infection therapies.

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