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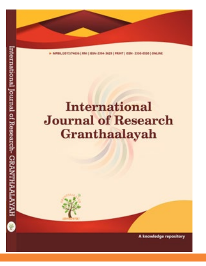

Original Article

INFLUENCE OF BACTERIOSPERMY ON THE LEVEL OF APOPTOSIS OF EJACULATED SPERMATOZOODS

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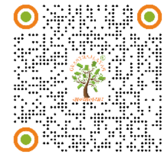

ABSTRACT

Numerous microorganisms may be involved in the pathogenesis of diseases of the male reproductive system. Bacterial metabolism can alter the biochemical properties of spermatozoa, thereby compromising their survival. The objective of this study was to comparatively examine the effect of different types of bacteriospermia on the apoptosis of ejaculated human spermatozoa. The study group consisted of 20 healthy, fertile, normozoospermic volunteers aged 20 to 35 years and 62 patients with different types of bacteriospermia. All samples tested underwent bacteriological examination within 3 hours of collection, in accordance with WHO recommendations. The apoptosis status in each group was determined by flow cytometry. Bacteriospermia was detected in 47.56% of the subjects examined. Escherichia coli (14.63% of the total number of cultures) was the most frequently isolated bacterium, followed by Klebsiella pneumoniae (10.98%) and Acinetobacter s (7.31%). Gram-positive bacteria (Lactobacillus s, Staphylococcus haemolyticus) accounted for 6.1% of the spermatozoa, while Gram-negative bacteria (E. coli, Acinetobacter s, Bacteroides ureolyticus, K. pneumoniae) were the most frequently found. They induced significantly less sperm apoptosis than Gram-negative bacteria. The mean total apoptosis rate of sperm isolated from semen containing Gram-positive bacteria is 17.94 ± 1.64%, compared to 33.997 ± 1.91% for those isolated from semen containing Gram-negative bacteria. Sperm microbiota-induced apoptosis may be a mechanism involved in the development of male infertility. The development of new biomarkers for male infertility is crucial for improving the diagnosis and prognosis of this condition.

Keywords: Bacteriospermia, Male Infertility, Apoptosis, Sperm

INTRODUCTION

Since ejaculate is a mixture of secretions obtained from the urogenital tract and male accessory glands, seminal fluid culture reveals the presence of microbes in any part of the seminal tract De Francesco et al. (2011). In recent years, increasing attention has been paid to urogenital tract infections; many microorganisms may be involved in the pathogenesis of diseases of the male reproductive system Shash et al. (2023). Bacterial infiltration of the male reproductive system triggers a local immune response, which is usually accompanied by the release of cytokines and leukocytospermia Frączek and Kurpisz (2015),Agarwal et al.

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(2018),Ventimiglia et al.(2020), which is often associated with a decrease in male reproductive capacity. Finally, there is an opinion that bacterial metabolism can alter the biochemical or physicochemical characteristics of seminal plasma or the medium used for sperm processing, which may compromise sperm survival both in vivo and in vitro Heidari Pebdeni et al. (2022). There is growing evidence that certain bacterial species contribute to deterioration of sperm quality by directly reducing sperm viability and motility, altering sperm morphology, and indirectly affecting sperm quality through oxidative stress and immune or autoimmune reactions Domes et al. (2012). However, the most important mechanism leading to the death of ejaculated sperm during urogenital tract inflammation/infection is related to apoptosis.

Microorganisms have been detected in semen Frączek and Kurpisz (2015) with varying effects on the reproductive tract and sperm quality. However, no extensive studies have been conducted linking the type of bacteriospermia and the level of apoptosis in ejaculated sperm.

The aim of the work: to conduct a comparative study of the influence of different types of bacteriospermia on the apoptosis of ejaculated human spermatozoa.: to conduct a comparative study of the influence of different types of bacteriospermia on the apoptosis of ejaculated human spermatozoa.

MATERIAL AND METHODS

The study group consisted of 20 healthy, fertile, normozoospermic volunteers aged 20 to 35 years, recruited from the Astrakhan Center for Family Health and Reproduction, and 62 patients with various types of bacteriospermia, treated at urology hospitals in Astrakhan and Akhtubinsk. All patients provided written consent to participate in the study. Semen samples were obtained by masturbation after 3–5 days of sexual abstinence. After liquefaction (30 minutes at room temperature), the samples were subjected to routine semen analysis in accordance with recommendations published by the World Health Organization Lutsky et al. (2023). All samples were subjected to microbiological analysis. Semen samples were plated on blood agar (BA) and MacConkey agar (MCA) plates in the microbiology laboratory within 3 hours of sample collection, according to WHO recommendations, followed by aerobic incubation at 37°C for 24–48 hours. Samples with significant bacterial growth (≥ 106 CFU/ml) were further tested to the species level using biochemical identification tests. For Gram-positive bacteria: Gram stain, catalase, slide coagulase, novobiocin, bacitracin, bile esculin, and optochin; for Gram-negative bacteria: Gram stain, TSI agar (triple sugar iron), SIM test (motility, indole, sulfur), Simmons citrate, urease, and oxidase Koneman et al. (1997). Semen samples with normal semen parameters, no antisperm antibodies, and no signs of bacterial infection (peroxidase-positive leukocytes <0.2 × 10 /ml and negative bacterial culture) were selected as controls. Sperm from the collected semen samples were separated from seminal plasma by centrifugation at 600 g for 8 min. The semen pellets were washed with warm phosphate-buffered saline (PBS), pH 7.4, and adjusted to a final concentration of 4 × 10 sperm/ml PBS. Apoptosis was analyzed by flow cytometry. The ANNEXIN V-FITC APOPTOSIS DETECTION KIT I (BD Pharmingem™, USA) was used to further confirm apoptosis. Sperm suspensions (2 × 10 6 /mL) were washed once with PBS supplemented with Ca 2+ and then double-stained with annexin V-FITC and propidium iodide (PI). After 15 min of incubation on ice in the dark, the cells were diluted 1 × 1 with binding buffer consisting of 10 mM HEPES, 140 mM NaCl, and 3.3 mM CaCl2. The apoptotic status in each group was determined by flow cytometry on an Attune® NxT flow cytometer according to the manufacturer's instructions, and the data were analyzed using FlowJo software.

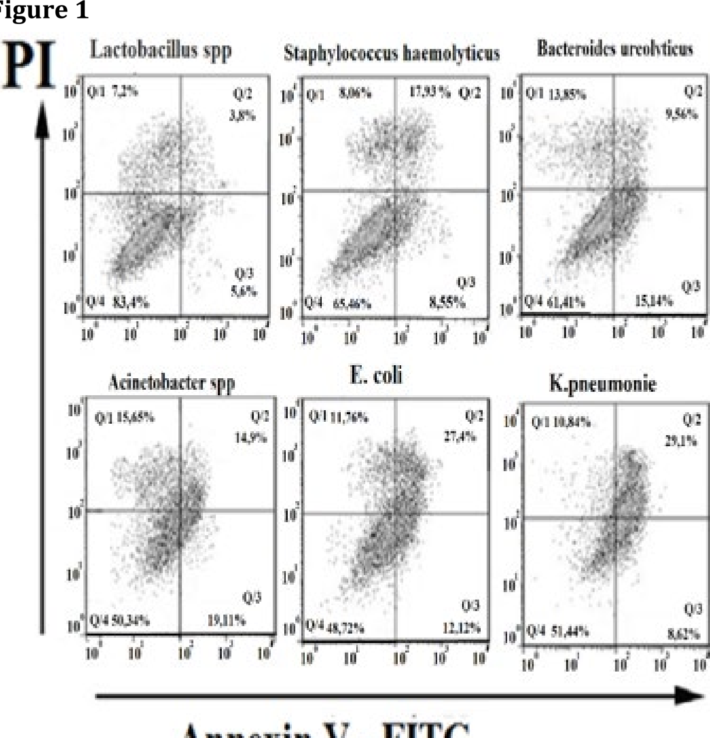
RESULTS AND DISCUSSION

Among 82 semen cultures (including controls), 47.56% had positive bacteriospermia, of which Gram-negative bacteria were isolated with a significant predominance (72.05% of all positive cultures). E. coli (14.63% of total cultures) was the most frequently isolated bacterium, followed by K. pneumoniae (10.98%) and Acinetobacter s (7.31%). Staphylococcus haemolyticus (6.1%), Bacteroides ureolyticus (4.88%), and Lactobacillus s (3.66%).

To quantify bacteriospermia-induced apoptosis in ejaculated spermatozoa, flow cytometry analysis was performed after double labeling with Annexin V-FITC/PI. Sperm were isolated from ejaculates with positive cultures for apoptosis assessment Table 1. Sperm from ejaculates with normal spermogram parameters and negative bacteriological results were used as controls. Using multiparameter analysis and simultaneous cell staining with nucleic acid dyes that do not penetrate living cells, such as propidium iodide (PI), allows differentiation between cells in the early phase of apoptosis (AnV+PI-), late apoptosis (AnV+PI+), and dead cells (AnV-PI+). The ability to simultaneously assess membrane marker expression and AnV staining is highly valuable, allowing characterization of the apoptotic cell population.

The cell profile dot plots shown were obtained in 1 of 5 independent experiments that yielded similar results. Quadrant Q1 reflects necrotic cells (AnV-PI+), quadrant Q2 reflects late apoptosis (AnV+PI+), quadrant Q3 reflects early apoptosis (AnV+PI-), and quadrant Q4 reflects the percentage of viable cells (AnV-PI-). As shown in Fig. 1, the proportion of apoptotic ejaculated spermatozoa significantly increased overall in bacteriospermia. Table 1 was compiled based on the dot graphs, which clearly reflects the dynamics of changes in the level of apoptotic spermatozoa.

Figure 1



Picture 1 Flow Cytofluorimetry of Apoptosis Induction in Ejaculated Sperm Depending on the Type and Type of Microorganisms During Bacteriospermia. Scatter Plots After Double Labeling with Annexin V-FITC and PI. The X-Axis Represents FITC Staining and the Y-Axis Represents PI (Propidium Iodide) Staining.

Externalization of phosphatidylserine or early apoptosis in the case of Lactobacillus spp does not differ significantly from the control group, but overall, the total apoptosis caused by Lactobacillus spp is twice as high as the control Table 2. It has been suggested that lactobacilli induce apoptosis due to the production of hydrogen peroxide, which causes non-selective apoptosis Krüger and Bauer (2017). A comparison of the early and late apoptosis rates for various pathogens causing bacteriospermia is also noteworthy. While Staphylococcus haemolyticus and K. pneumoniae induce minor early apoptosis, late apoptosis under the influence of these bacteria is much more pronounced Table 2. In the case of staphylococcal infection, the main proapoptotic factors are numerous toxins characteristic of staphylococci, including Staphylococcus haemolyticus. Some toxins cause membrane damage and externalization of phosphatidylserine, others activate caspases and DNA degradation, which is manifested in a positive reaction to annexin and a positive reaction to propidium iodide (Ann+PI+), called late apoptosis Zhang et al. (2017). K. pneumoniae exhibits high adhesive capacity to the surfaces of various cells, thanks to P-glycoprotein and causes Ann+PI+ apoptosis, and also promotes transcriptional expression of pro-inflammatory genes IL-6, IL-8, IL-1β and tumor necrosis factor (TNF)-α, as well as the production of IL-8, IL-1β and TNF-α, which in turn are also pro-apoptotic factors Cheng et al. (2020).

Table 1

Table 1 Bacteriospermia. Types and Types of Pathogens

pathogen	Type	Number of positive cultures	% From the total number of crops	% From the number of positive cultures
E. coli	gram negative	12	14,63%	30,77%
K. pneumoniae	gram negative	9	10,98%	23,08%
Acinetobacter s	gram negative	6	7,31%	15,38%
Staphylococcus haemolyticus	gram-positive	5	6,1%	12,82%
Bacteroides ureolyticus	gram negative	4	4,88%	10,26%
Lactobacillus s	gram-positive	3	3,66%	7,69%

Table 2

Table 2 Changes in the Level of Apoptosis of Ejaculated Spermatozoa Depending on the Type and Kind of Microorganisms in Bacteriospermia (in the Table, all Reliabilities are Calculated in Relation to the Control)

type of bacteria	Ann+PI- Early apoptosis	p	Ann+PI+ Late apoptosis	p	Total Apoptosis	p
control	3,8±0.72	-	1,9±0,21	-	4,7±0,61	-
Lactobacillus s	5,6±0,38	0.0627	3,8±0,31	0.0267	9,4±0,35	0.00023
Staphylococcus haemolyticus	8,55±0,35	0.0008	17,93±0,35	0.000027	26,48±0,62	0.000001
Bacteroides ureolyticus	15.14±0,32	0.00002	9,56±2,75	0.0274	24,74±1,07	0.000001
Acinetobacter spp	19,11±0,86	0.00003	14,9±1,9	0.000253	34,01±3,7	0.000106
E. coli	12,12±0,84	0.0003	27,4±1,8	0.000002	39,52±2,56	0.000001
K. pneumoniae	8,62±0,8	0.00288	29,1±2,85	0.00003	37,72±3,83	0.000061

Gram-negative bacteria (e.g., Escherichia coli) contain negatively charged molecules such as phosphatidylglycerol and phosphates in their cell membranes Matsumoto (2001), whereas healthy mammalian cells mainly contain phospholipids with a neutral charge, and bacterial lipids provoke the externalization of phosphatidylserine, initiating the signaling phase of apoptosis Boon and Smith (2002). Escherichia coli, a facultative anaerobe, is a major cause of urinary tract infections Beebout et al. (2022), and according to our data, bacteriospermia caused by Escherichia coli has the greatest pro-apoptotic effect on ejaculated spermatozoa. Studies have shown that in mouse cells infected with Escherichia coli (E. coli), there was an increase in the content of IL-1β, IL-6, IL-8, TNF-α, leptin and resistin, and an increase in the levels of apoptotic proteins (caspase-3, caspase-9 and bax/bcl-2) Guo et al. (2021).

Flow cytometry also allows us to assess the percentage of necrotic AnV-PI+ cells, which is particularly important for assessing the viability of ejaculated sperm. According to our data, the percentage of necrotic cells among sperm isolated from semen containing Gram-positive bacteria averaged 7.9±0.62%, while the percentage of necrotic cells in sperm isolated from semen containing Gram-negative bacteria averaged 13.025±0.84%, which is 64% higher than in Gram-positive bacteriospermia.

CONCLUSION

Thus, it is shown that semen contains unique microbial profiles that appear to be characteristic of a certain subpopulation of men. E. coli was the most frequently isolated bacterium, followed by K. pneumoniae, Staphylococcus haemolyticus, Bacteroides ureolyticus, Lactobacillus s These observations are consistent with previous studies Farahani et al. (2021). Many of the bacterial species identified in this study have a significant impact on the development of sperm apoptosis. These are primarily gram-negative bacteria E. coli and K. pneumoniae. Many types of bacteriospermia are characterized by a decrease in the functional characteristics of sperm and, as a result, subfertility and infertility Heidari Pebdeni et al. (2022). It can be speculated that microorganisms can influence the environment in which sperm mature, thereby influencing their physiology, in other words, inducing sperm apoptosis. Apoptosis induced by the sperm microbiota may be one of the mechanisms underlying male infertility. The development of new biomarkers for male infertility is crucial for improving the diagnosis and prognosis of this disease

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