The Role of Reactive Oxygen Species and Bacterial Infections in Male in Male Infertility in Al- Anbar Province / West of Iraq

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Abstract

In the male genital tract, spermatozoa and leukocytes, including neutrophils and macrophages, generate reactive oxygen species (ROS). ROS is involved in the regulation of sperm functions, such as acrosome reactions and capacitation. Infections result in excessive ROS production, resulting in a 'oxidative burst' of neutrophils/macrophages as a first-line defense mechanism. During infection, the imbalance of pro-and antioxidants favoring the former results in oxidative stress that impairs the sperm functions mentioned In addition to motility and fertilization. ROS produced during testis and epididymis infections is especially harmful to sperm due to longer contact time and lack of antioxidant protection. Only very high numbers of ROS-producing leukocytes are harmful to sperm functions in the final ejaculate.

Keywords: *Male infertility, ROS, Bacterial infection*

Introduction

Infertility affects up to 15 percent of the world 's population [(1)]. Male infertility is the cause of around 20 % of cases, but 40 percent of infertile couples will contribute [(2)]. Reactive Oxygen Species (ROS) is a molecule of oxygen which contains one or more unpaired electrons in atomic orbits. For instance, adding one electron to dioxygen (O2) forms the radical superoxide anion (O2 −), the primary form of ROS. The superoxide can be directly or indirectly catalyzed (enzymatic, metallic) converted into secondary ROS, such as radical hydroxyl (OH) [(3)]. The main ROS generated by biological machinery is superoxide [(4)]. Leukocytes (macrophages and polymorphonuclear neutrophils) have significant role in male infertility in that reactive oxygen species (ROS) are produced. At the low level, ROS plays a physiological role [(5)], but in high Levels, they induce oxidative stress, which overwhelms the Physiological processes of sperm and it cause damage. s damage was found to occur through the lipid peroxidation of the plasma membrane [(6)]. Upon gaining entry into the sperm, ROS will target the genetic materials, Destroying and inhibiting intracellular mitochondrial DNA production with ATP [(7)]. Without proper production of ATP, the functionality and sperm motility are both affected [(8)]. This can result in male infertility. Oxidative stress can also be reduce rates of success of assisted reproductive procedures such as such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [(9)]. Previously, the WHO threshold for leukocytospermia was challenged as being too high because of the detrimental effects of seminal leukocytes on sperm at low level [(10, 11)]. The number of leukocytes below 10^6 WBC/ mL (Low-Leukocytospermia) was shown to make a significant decrease of the motility and DNA integrity [(11)]. Most urogenital tract infection/ inflammation patients with infertility are asymptomatic, suggesting a high rate of chronic disease [(12, 13)]. Acute and chronic infection following male reproductive inflammation can affect the function of sperm as well as the Spermatogenetic processes which cause qualitative and quantitative changes to sperm [(14)]. With any such mechanism, bacteriospermia affects the normal process of fertility: Spermatogenesis deterioration, reduced motility of sperm , morphological alterations, altered reaction of the acrosome, anti–sperm antibodies formation, reactive oxygen species formation leading to a higher DNA fragmentation index [(15)].

Materials and Methods

Eighty seminal fluid samples of infertile males are collected, and twenty samples are collected from fertile males as control group. The semen samples was collected in sterile clean dry and leak-proof container after 2-3 days abstinence. Patients do not take prior antibiotics before culture. Patients are asked to wash their hands and external genitals before ejaculation to prevent contamination and to urinate before collecting semen.

Bacteriological study

All samples are cultured on blood agar, Mac Conkey agar and chocolate agar plates using technique for semi quantitative culture and proper incubated period. The bacterial growth were identified by conventional methods (Gram staining, colony morphology, and biochemical test). The antibiotic susceptibility test was performed by Kirby-Bauer Disk Diffusion Method as recommended by the CLSI. The antibiotic disc used for susceptibility were from bioanalyses laboratories. The antibiotic used in this study include Amikacin (30µg), Ampicillin (10µg), Ciprofloxacin (5µg), Lomefloxacin (5µg), Cefixime (5µg), Amoxicillin/clavulanic acid (30µg), Doxycycline (30µg), Azithromycin (15µg), Trimethoprim (10µg), Levofloxacin (30µg) and Meropenem (10µg). All the samples were examined by sperm analyzer type motic, for sperm parameters.

Immunological study

The semen samples are centrifuged for 7 minutes at 3000 rpm, then seminal plasma is collected in a sterile tube and frozen at-20 c ° until used to measuring the ROS levels by ELISA test.

Data Analysis

IBM© SPSS© (Statistical Package for the Social Sciences) Statistics Version 22." Analyzed the data and presented it in tables.

Results

80 Samples of semen were collected from infertile male. 60% have primary infertility, and 40% have secondary infertility. And 20 fertile males as a control group. Results of the bacterial study showed that 35% of the primary infertility group, 20% of the Secondary infertility group and 30% of the control group showed a positive culture on proper media when inoculated. This result disagrees with Hussein [16] results as he found the prevalence rate of bacterial infection in seminal fluid in infertile individuals (61.66%).

Table 1: Number of positive culture in the infertile groups.

	No. of positive culture	%
control	7(7/20)	35

Table 2: Number of positive culture in control group.

Type of infertility	No. of positive culture	%
Primary	35(35/80)	43.7
Secondary	20(20/80)	25
Total	55	68.7

***Cont...* Table 2: Number of positive culture in control group.**

Samples	E. coli	K.pneumonia	P.aeruginosa	S.aureus	S.epidermides	S.pyogens
Primary	12	8	2	3	7	3
Secondary	5	2	2	3	3	2
Total	17 (21.2%)	10 (12.5%)	4 (5%)	6 (7.5%)	10 (12.5%)	5 (6.2%)

Table 3: Bacterial types of distribution among the infertile groups (positive culture).

Table 4: Bacterial types of distribution among the control groups (positive culture).

Samples	E. coli	K.pneumonia	P.aeruginosa	S.aureus	S.epidermides	S.pyogens
Control	2	1	0	2	3	1
%	10	5	0	10	15	5

Gram-negative bacteria, such as E.coli and other Enterobacteriaceae family members, are considered to be a common cause of urinary tract infections, as well as some Gram-positive bacteria such as Staphylococcus aureus [17]. *E. coli* was isolated from 21.2% of all positive culture (table 3). The function of sperm is affected by these bacteria and their soluble factors, and the harmful effects of bacterial infection do not require sperm to come into direct contact with bacteria [18].

Table 5: Comparison of bacteriospermia and pyospermia between the control and infertile groups.

Variables	Control group (%)	Infertile group (%)	P value
Bacteriospermia	20	55	0.001 (HS)
Pyospermia	4.3	27	0.001 (HS)

The results of the comparison show a statistically significant difference between bacteriospermia and pyospermia. This result disagrees with the result of Lackner *et al*,. [19] as the percent of pyospermia in the infertile patients was 60.7% and the significant growth of pathogenic bacteria was found in a 35.7%. Also, the study shows that there is no relationship between pyospermia and bacteriospermia as some bacteriospermia may be identified as contamination from the genital tract.

Antimicrobial Sensitivity Testing

The antibiotic sensitivity test was carried out for all bacterial isolates using a disc diffusion technique.

Table 6: The pattern of antibiotic sensitivity of gram-negative bacteria isolated in this study.

	E. coli			K. pneumoniae			P. aeruginosa		
The antibiotic	**S%**	**I%**	**R%**	**S%**	**I%**	**R%**	**S%**	**I%**	**R%**
Azithromycin	73	0	27	81	0	19			
Doxycyclin	63.15	0	36.84	72.72	18.18	9.09			
Ciprofloxacin	68.42	0	31.57	90	0	10	70	0	30
Levofloxacin	90	0	10	100	0	0	80	0	20
Lomefloxacin	73	0	27	90	0	10	30	0	70
Trimethoprim	73	0	27	90	0	10			
amoxicillin/ clavulanic acid	57.89	10.52	31.57	20	0	80			
Ampicillin	40	5	55	15	0	85			
Cefixime	50	0	50	30	0	70			
Amikacin	90	0	10	75	0	25	80	0	20
Meropenem	90	0	10	75	0	25	100	0	0
Ceftriaxone									

S = Sensitive I = Intermediate R = Resistant

As showed in table (6) *E. coli* isolated in this study was found to be susceptible to amikacin, meropenem and levofloxacin with a percent of 90, and susceptible for lomefloxacin, trimethoprim and azithromycin with 73 percent for each one, and found that this bacteria resistant to cefixime with (50%), and for ampicillin with 55%. *K. pneumoniae* is found 100 % sensitive to levofloxacin followed by ciprofloxacin, lomefloxacin, and trimethoprim with a percentage of (90%) for each, and azithromycin (81%), meropenem and amikacin (75%), also, the result of this study found that this bacteria is highly resistant to ampicillin and amoxicillin/ clavulanic acid with(85%),(80%) respectively, cefixime (70%). It is possible to interpret this resistance depending on the fact that many strains of *E. Coli* acquired plasmids that give resistance to one or more types of antibiotics; therefore, antimicrobial therapy should be controlled by a sensitivity test for labrot*P. aeruginosa* is sensitive (100%) to each of amikacin, meropenem and levofloxacin. From these results, we can conclude that amikacin, meropenem and levofloxacin are the drugs of choice to treatment of Gram-negative bacteria isolated in the study.

Conclusion

Bacterial and fungal infections are significant on the speech process. In the case of bacterial infections, especially bacteria that work, a change in the pH of the medium leads to the killing of sperm and thus negatively affects fertilization. Fungal infections also produce antibodies that hinder the fertilization process

Conflict of Interest: None

Funding: self

Ethical Clearance: Not required

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