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Influence of Male Reprodactive Tract Infection on Sperm DNA Fragmentation

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Abstract
The human spermatozoon is highly susceptible to oxidative stress. Spermatozoa were the first cell type reported to show potential susceptibility to OS. A few studies demonstrated the elevation of ROS concentration in infectious diseases. Endogenous sources of reactive oxygen is peroxidase-positive leucocytes: PNL (50%-60%) and macrophages (20%-30%). An increase in proinflammatory cytokines, such as interleukin (IL)-8, and a decrease in the antioxidant superoxide dismutase (SOD) can result in a respiratory burst, production of high levels of ROS, and ultimately, OS.
We retrospectively were studed oxidative stress levels and its correlation with sperm DNA damage in 12 seminal culture positive men before and after antioxidant and antibiotic treatment.
Patients with genitourinary infection have increased sperm DNA fragmentation. The inflammatory mediators can be a direct cause of DNA fragmentation in ejaculated spermatozoa, which can ultimately lead to limited fertilizing abilities of the germ cells.This increase is proportionally greater than the influence on classical semen parameters and could result in a decreased fertility potential.Combination of antioxidant and antibiotic therapy appears to be important in providing a remedy for infection-induced high DNA fragmentation levels. In summary, there exists a significant association between sperm DNA fragmentation, oxidative stress and infection

Keywords: Oxidative stress, antibiotics, infertility.

Introduction
Male infertility is typically diagnosed upon routine semen analysis following the World Health Organisation's (WHO) semen analysis manual. Recent editions of the manual have essentially changed the diagnosis of a semen sample, prompting debate between experts as to which edition should be followed.
Deoxyribonucleic Acid (DNA) integrity analysis is proving to be a useful adjunct to semen analysis as 15% of infertile men have a normal semen analysis but they have an increased DNA fragmentation level (DFL) which has been associated with increased disease incidence in any resultant offspring. Statistics from the United States indicate that OS is one of the major causes of male infertility; that is, 30% to 40% of infertile men have elevated levels of ROS in their seminal plasma (1).Mitochondria and sperm plasma membrane are two major sites of ROS generation in sperm cells. Several study, have indicated that spermatozoa needs small amounts of ROS, to acquire the ability of fertilizing the oocytes (14, 15). It is also demonstrated that spermatozoa need a small amounts of ROS, for capacitation, hyperactivation, motility, acrosome reaction and fertilization (16, 17). The human spermatozoon is highly susceptible to oxidative stress. Spermatozoa were the first cell type reported to show potential susceptibility to OS. A few studies demonstrated the elevation of ROS concentration in infectious diseases. Mazilli *et al* demonstrated that, in patients with sperm culture-positive for aerobic bacteria, the superoxide anion production was high (18). Also, a high ROS level in chronic non-bacterial inflammation was seen (19). In some situations, the damage caused by oxidants may be repaired. Unfortunately, spermatozoa are unable to restore the damage induced by OS because they lack the necessary cytoplasmic-enzyme repair systems. This is one of the features that make spermatozoa unique in their susceptibility to oxidative insult (9). Endogenous sources of reactive oxygen is peroxidase-positive **l**eucocytes: PNL (50%-60%) and macrophages (20%-30%) (2). Peroxidative damage

directly affects the lipid Component of the membrane and also generates breaks in the chains of both nuclear and mitochondrial DNA.A large proportion of these peroxidase-positive leukocytes originate from the prostate and seminal vesicles. When these major sources of ROS are activated by various intracellular or extracellular stimuli, such as infection or inflammation, they can discharge up to 100 times more ROS than normal (3,4). An increase in proinflammatory cytokines, such as interleukin (IL)-8, and a decrease in the antioxidant superoxide dismutase (SOD) can result in a respiratory burst, production of high levels of ROS, and ultimately, OS. OS will cause sperm damage if seminal leukocyte concentrations are abnormally high as is the case in leukocytospermia (5), which the World Health Organization defines as the presence of more than one million peroxidase-positive cells per milliliter of semen (6). Various studies point to a correlation between decreased sperm function and seminal plasma with abnormally elevated levels of ROS, IL-6, IL-8, and tumor necrosis factor, all of which result in increased sperm cell membrane LPO (7, 8). As a result, we have lipid peroxidation, DNA fragmentation, and axonemal damage, denaturation of the enzymes, over generation of superoxide in the mitochondria, lower antioxidant activity and finally abnormal spermatogenesis.

Reactive oxygen species (ROS), such as the superoxide radical, hydroxyl radical and H_2O_2, pose a significant threat to cellular integrity in terms of damage to DNA, lipids, proteins and other macromolecules (10,11). ROS are generated through both endogenous and exogenous routes. Oxidative damage, produced by intracellular ROS, results in DNA base modifications, single- and double-strand breaks, and the formation of apurinic/apyrimidinic lesions, many of which are toxic and/or mutagenic (12)Therefore, not only are ROS implicated in the etiology of disease states, but the resulting DNA damage may also be a direct contributor to deleterious biological consequences. Mutagenic 8-hydroxyguanine lesions are present in elevated levels in aged and cancer cells. In addition, H_2O_2-induced oxidative DNA damage has been shown to cause microsatellite instability, which is associated with colorectal cancer (13). There is a strong relation between inflammation of male genital system and infertility (19, 20). Actually, ROS that are produced in testis infection and epididymis are dangerous for sperm, because, antioxidant protection in sperm is low and ROS can affect these cells in a long period (21, 22).

We retrospectively were studed oxidative stress levels and its correlation with sperm DNA damage in 12 seminal cultures Positive man before and after antioxidant and antibiotic treatment.

Material and methods

The sperm for routine bacteriology and susceptibility testing was collected according to appropriate protocol and cultured on 5% blood agar and Endo agar plates. After 18-24 hour incubation under 37^0C identification was done by API system (API 20E, API Staph, apiStrep, Biomrieux). Antibiotic Susceptibility Test (AST) was performed according to Kirby-Bauer method (EUCAST2017). Followimg antibiotics were tested: ampicilline/sulbactam, amoxicillin/clavulanic acid, ciprofloxacine, levofloxacine, moxifloxacine, azhithromycine, fosfomycine, nitrofurantoine, cefazoline, ceftriaxone, cefoxitine and amikacine.

Oxidative stress measurement was done by using Oxisperm kit (HT-OS20) (Halotech) wich determine excess of superoxide anions in sperm.

Fresh samples were used for DNA fragmentation test. It was assessed based on the Sperm Chromatin Dispersion (SCD) (HT-HS10, Halosperm in vitro diagnostic kit).

Results

Bacteriological analysis of semen reveal following microorganisms: Enterococcus faecalis in 5 cases(5/12 4, 41,6%), Staphylococcus aureus in 4 patients(4/12, 33,3%), Escherichia coli 2cases(2/12,16,6%), Enterobacter cloacae 1(1/12,8,3%). Both gram positive and gram negative organisms were sensitive to ampicillin-sulbactam, amoxicillin-clavulanicacidfosfomycine, nitrofutantoine, moxifloxacine and amikacin.There was a total of 56% resistance to ciprofloxacin and levofloxacin.

In 6 cases when patient had bacterial growth 10^6 CFU/ml, oxidative stress were medium(L3), in 3 cases 10^8 CFU/ml bacteria in sperm high(L4) and 3 cases 10^5 CFU/ml low-medium(L2).

DNA fragmentation test results were following:fragmented DNA more than 50% were observed in those patients who had oxidative stress L4(high), from 15% to 30% in patients with L2(low-medium) and more than 30% who had L3(medium) oxidative stress.Normal range of DNA fragmentation 0-15% were not reported.

All 12 patients received at least three weeks of antioxidant and antibacterial therapy which were initiating depend on antibiogram.

After appropriate treatment in 3 patients who had DNA fragmentation more than 50% and oxidative stress were high(L4) this parametrs were significantly reduced to 15%-30% and Low-medium(L2), in 9 patients DNA fragmentation was returned in normal range(0-15%)(Table1).

Patients Number	Bacteriology Results CFU/ ml		Oxidative stress		DNA Fragmentation %	
	Before treatement	After treatement	Before treatement	After treatement	Before treatement	After treatement
6	10^6	No growth	L3	L2	>30	<15
3	10^5	No growth	L2	0	15-30	<15
3	10^8	No growth	L4	L2	>50	15-30

Conclusion

Patients with genitourinary infection have increased sperm DNA fragmentation. The inflammatory mediators can be a direct cause of DNA fragmentation in ejaculated spermatozoa, which can ultimately lead to limited fertilizing abilities of the germ cells.This increase is proportionally greater than the influence on classical semen parameters and could result in a decreased fertility potential.Combination of antioxidant and antibiotic therapy appears to be important in providing a remedy for infection-induced high DNA fragmentation levels. In summary, there exists significant associations between sperm DNA fragmentation, oxidative stress and infection**.**

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