Original Article

Pyospermia; A Major Cause of Oxidative Stress in Sperms Having Repercussion in The Fertile Potential of Male by Reducing Its Motility and Count

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Abstract

Objective: To determine the frequency of Pathological pyospermia among male partners of infertile couples and to assess the association of Pyospermia with asthenospermia and azoospermia.

Methodology: This study was conducted in a private infertility center in Gujrat. Data collected was from January 2017 to Dec 2020. The semen parameters of 1393 men presenting to the infertility center were collected through interview and reviewing laboratory investigations after informed consent. The relationship of Pyospermia with Asthenospermia and Azoospermia was explored by applying Chi-square test. The P-value of less than 0.05 was considered statistically significant.

Results: The mean age of men were 33.62 ± 6.082 years. Pathological Pyospermia was detected in 78.2 % of men. 17% of men showed Asthenozoospermia with physiological pyospermia. 42.6 percent of men showed Asthenozoopspermia with pathological pyospermia. Regarding total sperm count 214 (15.4%) had oligospermia which is less than 15 million sperm per millileter of semen and 43 (3.1%) had azoospermia which is no sperm in ejaculate. The relationship of Pyospermia with decreased sperm motility and low sperm count was statistically significant.

Conclusion: The frequency of Pyospermia among male partners of 1393 infertile couples was found to be 78.2%. Pyospermia had statistically significant effect on reducing total sperm count and sperm motility causing Oligospermia, Azoospermia and Asthenospermia.

Key words: Pyospermia, Asthenospermia, Azoospermia. Oxidative stress. sperm motility.

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Introduction

A couple is said to be infertile if after living together of one year with unprotected intercourse they remain unable to conceive.[1] Infertility is a public health problem that affects approximately 48.5 million couples globally, with 50% of cases attributed to a male factor worldwide.[2]

"Human spermatozoa produce reactive oxygen species" this groundbreaking observation was by a Scottish scientist John Macleod in 1943. Reactive oxygen species are highly reactive derivatives of oxygen such as hydrogen peroxide (H_2O_2) and that these substances have harmful effects on various sperm functions.[3] Studies have shown that ROS not only have a profound effect on the functioning and vitality of sperm but also raised serious questions about health and wellbeing of the progeny, if the male germ cell is exposed to high concentration of oxidants.[4] The purpose of this study is to take a round trip by discussing the delicate morphology of sperm, the increased propensity to production of ROS in pyospermia, consequences of

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exposure of ROS produced by pyospermia to sperm, which render the male infertile by effecting total sperm motility.

As compared to somatic cells, there are certain characteristics that a sperm cell possesses that make it easily vulnerable to damage by ROS. In the process of spermatogenesis, the spermatid undergoes a certain morphological changes, which make it more prone to damage by ROS. Along with many other changes the spermatid cytoplasm is sequestered, the nucleus condenses and the flagellum extends rendering the sperm cell the smallest cell of the body. Along with loss of most of the cytoplasm there is also loss of intracellular scavengers of ROS. Scavengers like catalase, glutathione peroxidase or super oxide dismutase. Certain non-enzymatic molecules such as vitamin C or E, which counteract the effect of oxidants, are also lost.[5]

Sperm cells are the only cells in the world that carry the responsibility of working efficiently in the body of another individual. They carry the genome and travel at least 18 to 24 centimeters in the female genital tract efficiently facing many ordeals in the way. This magical and mysterious function of sperm is much related to its plasma membrane fluidity, so membrane integrity is directly related to the normal functioning of sperm.[6] It's the plasma membrane special lipid proportion having a high level of polyunsaturated lipid content that give its good membrane fluidity. Tevilani et al, have reported that the polyunsaturated fatty acids (PUFA) levels in poorly motile sperms as in the case of asthenozoospermic individuals and individuals with male genital tract infection were significantly lower.[7] A pathological sample show an altered amount of PUFA may it be decreased or increased as khosrowbeygi and zarghami reveal in their study that pathological semen samples with poor motility have high PUFA and DHA levels than normozoospermic samples. They concluded that due to higher content of PUFA in these pathological samples they are more prone to oxidative damage as compared to normozoospermic samples.[8] An excess amount of polyunsaturated fatty acids triggers mitochondrial ROS production and therefore create oxidative stress for these sperms[9], considering the high potential to generate high content of ROS in any pathological condition and decreased ability to clean out the mess due to decreased scavenging ability of sperms. The spermatozoa become extremely vulnerable to oxidative stress.

Leukocytosis or Pyospermia is a major cause of ROS. It is a natural defense mechanism of body to sequester leukocytes at an event of an attack by a pathogen. In leukocytes it is a physiological necessity to release large amount of super oxides into phagocytic vesicles to engulf and kill the pathogens. In the whole process of defense, 1000 times more ROS then spermatozoa are generated in the semen.[5] As a result of production of greater amount of leukocytes in an infected male which in return produce tremendous amount of oxidative radicals rendering the male infertile, by seriously affecting the sperm function. The semen parameters may fall to an extent that clinically it may appear as asthenospermia, oligospermia or even azoospermia. In this case ROS have shown to be associated with infertility by induction and stimulation of lipid membrane. Through this mechanism infection / inflammation do not only damage sperm DNA and reduce sperm count and seminal volume but also impair sperm functions like motility, acrosome reaction. In a study, strong positive correlation was observed between different indicators of ROS production and sperm motility and velocity alteration. The redox homeostasis of the cell thus leads to sperm senescence and finally death.[11]

Sperm DNA is highly compacted by protamine but is an important target by Reactive Oxygen Species. It leads to the formation of adducts between nitrogen basis, destabilizing the DNA molecule, resulting in DNA strand breakage and damage.[12] Monica Murtatori et al demonstrated in their study that all sperms that showed signs of DNA fragmentation / Damage expressed apoptotic enzymes,[13] actual caspases were also present in sperms with DNA fragmentation and such enzymes were present in very less amount without DNA damage. So it's the fate of a sperms, whose DNA is damaged to end up in apoptosis. Thus, Pyospermia can initiate a cascade of events which can eventually decrease the motility and number of sperms leading to decreased fertility potential of males partners in many of infertile couples.

Methodology

This is a descriptive cross sectional study of 1393 infertility cases presented to our gynecology/ infertility clinic located in a thickly populated city of central Punjab from January 2017 to December 2020. This study explores the data of all 1393 cases presenting to our clinic during study period of almost three years. After informed consent, a short interview of participants was conducted and Laboratory investigations were reviewed.

All the semen analysis was done using WHO standards and guidelines. The pathological Pyospermia was labelled in the presence of >1 million WBCs per millileter of semen. Oligospermia was taken as <15 million sperms per ml and Azoospermia was total absence of sperms in the ejaculate. Asthenospermia was labelled as <40% total sperm motility. The quantitative variables like age of male partners among infertile couples was presented as mean and standard deviation. Qualitative variables like Pyospermia, asthenospermia and azoospermia were presented as frequency and percentages. The relationship of Pyospermia with Asthenospermia and Azoospermia was explored by applying Chi-square test. The P-value of less than 0.05 was considered statistically significant.

Results

This survey was conducted on 1393 male partners of infertile couples presenting to a private infertility clinic in Central Punjab. Ages of the male patients ranged from 19 years to 64 years with the mean of 33.62 ± 6.082years. Among 1393 patients 1090 (78.2%) had pathological pyospermia. On Semen, analysis 519 (37.3%) male subjects had asthenospermia (por sperm motility, less than 40%). Regarding total sperm count 214 (15.4%) had oligospermia, which is less than 15 million sperm per millileter of semen and 43 (3.1%), had azoospermia, which is no sperm in ejaculate (Table I).

Table I: Distribution of different characteristics of study sample

Characteristics	Frequency	Percentage
Age of patients		
Mean ± SD	33.62 ± 6.082	
Pyospermia		
Physiologic Pyospermia	303	21.8
Pathologic Pyospermia	1090	78.2
Asthenospermia		
Asthenospermia	519	37.3
Normal Motility	874	62.7
Azoospermia		
Azoospermia	43	3.1
Oligospermia	214	15.4
Normospermia	1136	81.6
Total	**1393**	**100**

The effect of Pyospermia on sperm motility and total sperm count, the two most important determinants of fertility was evaluated by constructing two by two table and applying Chi square test. It was found that 465 subjects with pathological pyospermia showed Asthenospermia as compared to 54 asthenospermic with physiological pyospermia. The p-value was 0.000 making it highly statistically significant. Cross tabulation was done to determine the impact of Pyospermia on total sperm count. It was found that 181 subjects with Oligospermia and 37 with azoospermia had pathological Pyospermia. The p-value was 0.018 stating that the difference in the total sperm count of subjects with and without pathological pyospermia is statistically significant (Table II).

Table II: Association of Pyospermia with Sperm Motility and Total Sperm Count.

Variables	Pyospermia: Physiological Pyospermia	Pyospermia: Pathological Pyospermia	Total	P-value
Asthenozoospermia				
Asthenospermia	54	465	519	0.000
Normal Motility	249	625	874	
Total Sperm Count				
Azoospermia	6	37	43	0.018
Oligospermia	33	181	214	
Normospermia	264	872	1136	
Total	303	1090	1393	

Thus majority (78.2%) of male partners of infertile couples had Pathological Pyospermia which had statistically significant role in reducing sperm count and its motility leading to diminished fertile potential. (Figure 1)

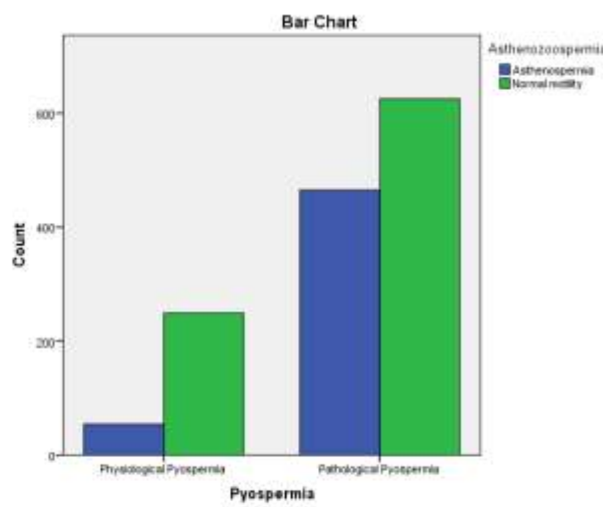

Figure 1. Association of Pyospermia with asthenospermia.

Discussion

Leukocytopspermia is a common abnormality that accounts for 30% of male factor infertility[14], making it one of the most common and most important causes of male infertility, but the distribution, origin and role of pus cells in semen still is controversial. The purpose of the present study was to establish the link and effect of pyospería on sperm motility and count. It is an established fact that leucocytes in semen is going to generate a cascade of reactive oxygen species (ROS) which will greatly break the function and integrity of sperm. Agarwal et al studied the relationship between ROS and semen parameter. Across time, semen samples were analyzed using basic semen parameters as per the 2010 World Health Organization guidelines and ROS measurement at 0 and 120 minutes after semen liquefaction. Those who exhibited poor motility and morphology, showed elevated ROS and vice versa. ROS was inversely related to semen parameters.[15] Similar relationships were established in our patients by measuring the effect of pyospermia. The patient found to have leucocytes in higher concentration showed reduced sperm count and reduced motility.

Pus cells showed an inverse relationship to sperm motility and count. Fewest pus cells were observed among groups where normal forms where significantly found. More pus cells were observed in cases where motility, and concentration or morphology was compromised. Similarly, low pus cell count were seen in cases where sperm had the fewest head and neck defects. In MS Khan Study in 2012, he found 36% of men having pyospermia, which has increased to 78% in our study. he showed that among the 36% infertile male population 10 percent have only isolated pyospermia while 26% were pyospermic in addition to having concentration and motility disturbances.[16]

It has been reported that a significant positive correlation exists between pyospermia and compromised sperm morphology, including motility defects, acrosome damage, and high sperm deformity index scores.[17] In another study the findings suggest that leukocytes had a positive association with normal morphology and progressive motility in semen samples at a concentration of 0-1x10 6/ml. These findings suggested that the association between leukocytes and semen quality might be concentration dependent. At higher concentration of Pus cells, semen motility grades tend to decrease and have greater sperm deformities at higher concentration of leukocytes. Our study showed a similar trend. At lower concentration of pus cells (Physiological Pyospermia) only 54 patients (17.8%) showed asthenozoospermia. At higher levels of pus cells (Pathological Pyospermia) the motility decreased significantly and 465 patients (42.6%) showed asthenozoospermia.

Leukocytospermia is so prevalent in infertile men; it can be questioned as to whether the presence of seminal leukocytes correlated with semen quality. Early studies found leukocytes to have a positive effect on semen quanlity.[18,19] Kiessling et al found an improvement in sperm motility and semen samples with a leukocyte concentration of 2x10 6/ml had better parameters. Tomlinson et al reported that leukocytes phagocytose abnormal spermatozoa. However, with the Advancement of time the newer studies suggest that leukocyte negatively affects semen quality because of the presence of reactive oxygen species (ROS), which are primary products of leukocytes. Like Aziz et al Ziyyat et al reported an increase in sperm motility in semen samples with moderate leukocytes <1x10 6/ml (physiological pyospermia). However, observed a paradoxical decrease in sperm motility with increasing amount of leukocytes in the semen (pathological pyospermia).[20] Another study showed that pyospermia affects the quality of sperm, and is one of the characteristic source of male infertility. The studies also showed similar results like ours and confirmed that infection in the semen is most likely associated with low and abnormal sperm motility.[21] Data of various studies also showed that leukocytospermia had a negative effect on the quality of the semen samples. The sperm concentration and forward progressive motility rates were lower in the leukocytospermic group than the non-leukocytospermic group before and after semen preparation.[22]

Researchers have reported pyospermia ‘s negative effects on semen parameters and even in in vitro fertilization (IVF). The modern reproduction techniques employed in assisted reproduction units is an iatrogenic source of ROS. During sperm preparation and manipulation, excessive production of ROS w damages the sperm. Cryopreservation is an extensively used procedure in an IVF lab; it is essentially an integral part of future of IVF laboratory. However, cryopreservation intensifies oxidative damage and influences IVF results. The process of cryopreservation has been related to diluting and removing seminal plasma, which is an important reservoir of antioxidants. As previously discussed Electron transport chain or NADPH, oxidase activity is the main source of production of oxidative radicals. Sperm energy demand is tremendously high leading to compensatory elevated mitochondrial activity.

Most probably in the environment created during cryopreservation, excessive mitochondrial ROS production overcomes limited antioxidant machinery almost instantly. Successive washes and sperm cryoprotectants anticipate the capacitation process by removing capacitation inhibition factors present in the seminal fluid. Thus, cryopreserved spermatozoa suffer injuries that will impair motility, mitochondrial and DNA integrity, which impairs fertilizing ability and embryo development. Already compromised sperm with pyospermia withstands the procedures of IVF with difficulty.

Pyospermic, Sperm oxidative stress is even detrimental to embryo development. As discussed earlier that DNA integrity is damaged by oxidative challenge. Nevertheless, even after DNA damage the sperm is able to fertilize the oocyte and carry on the effect of oxidative stress to the progeny. Leticia et al studied the effect of oxidative damaged bovine sperm on the future embryo. Their results verified that oxidative stress suffered by sperm prior to IVF has a dose dependent effect on both early (cleavage rate) and late (developmental rate and blastocyst) embryo development. They also observed that exposure to H_2O_2, lead to increased rate of capacitation of sperm. But this increased rate of capacitation of sperm had no effect on the fertilizing potential of sperm. Under the influence of oxidative challenge, the sperms would capacitate prematurely indicating that these sperms are dying as shown by decreased sperm motility.[23]

Conclusion

The rate of Pyospermia was found to be 78.2%. Pyospermia had statistically significant effect on reducing total sperm count and sperm motility causing Oligospermia, Azoospermia and Asthenospermia. Many tests like blood redox status monitoring, leukocyte ROS levels, and seminal fluid oxidative stress assessment, may provide clinicians a novel, less invasive way to examine the integrity of sperm cells and their capacity for fertilization. Novel therapeutic options based on antioxidant supplements may be helpful to lower systemic oxidative stress in infertile males which would improve the diagnosis and management of male infertility.

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