Correlation Of Bacterospermia With Primary Infertility In Males

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Introduction –

Male Urogenital Tract Infections is one of the most important causes of male infertility, worldwide. Genital tract infection and inflammation have been associated to 8-35% of male infertility cases. Asymptomatic bacteriospermia may play a major role. Male accessory sex glands infection is a major risk factor in infertility [(1).] The significant of Pathophysiology of bacteriospermia has been discussed in recent years. Some possible patho-mechanisms of the development of infertility linked with infection are considered: direct effect on sperm function (motility, morphology, etc), deterioration of spermatogenesis, autoimmune processes induced by inflammation and dysfunction of accessory sex glands [(2)].

Measurement of leukocytes in semen has been a standard component of the semen analysis, but its true significance is still unknown. The issue of whether or not leukocytospermia is detrimental to fertility is unsettled. Some studies found no detrimental effects of leukocytospermia but several others correlated seminal leukocytes with impaired semen parameters, especially sperm morphology and motility. Adding to the confusion is an older study that suggested that seminal leukocytes at concentrations between 1 and 3 million/mL (M/ml) are beneficial for sperm function, arguably due to effects of cytokines or scavenging of abnormal sperm. Leukocytospermia is thought to have multifactorial origin. In addition to genital tract infections, other aetiologies such as smoking, alcohol consumption, and marijuana use increase WBC in semen [(3)]

The normal sperm count is 20×10^6 spermatozoa/ml or more. The counts less than 20×10^6 cells/ml is known to be associated with male sterility.Normal spermatozoa measure 50 to 70 µm in length. Each consists of an oval-shaped head (with acrosomal cap), which measures 3-5 × 2-3 µm, a short middle piece, and a long thin tail (at least 45 µm in length). At least 50% of spermatozoa should show normal morphology in normal semen. Infertility constitutes a grave emotional and social problem in societies where great importance is attached to having children [(4)].

Hence, microbiology investigation of male partners in infertile couple can be useful to detect the male urogenital tract infection, especially asmptomatic infections**.**

Aims & Objectives- To study seminal parameter and bacteriological isolates in primary infertile patients and their clinical relevance to infertility

Material & Method - The seminal fluid was collected from males with primary infertility in the age group of 25-45 years age attending the fertility clinic, Department of Physiology, Government Medical College and Aurangabad. Fifty samples were collected by masturbation in a sterile wide mouth container, under aseptic precautions, after 5 days abstinence. Mid-stream urine was collected for culture two days after the semen collection and in cases with and without urinary complains**.** The patients without urinary complains were taken as control. The semen was allowed to liquefy completely and then inoculations were done with undiluted, 1:10 diluted, and 1:100 diluted samples using standard loop method. The diluent used was sterile distilled water and the bacterial count was done by the method described by Hoeprich4 Undiluted samples were inoculated on nutrient agar, blood agar, McConkey's agar, and chocolate agar and glucose and thioglycollate broths. Diluted samples were inoculated separately on nutrient agar and blood agar media. All the media were incubated at 37°C for 18 to 24 hours and then examined for any evidence of growth. Subcultures on solid media and biochemical tests were employed for the proper identification and confirmation of the organisms isolated 2 Urine samples were cultured in a similar manner on nutrient agar, McConkey's agar and glucose broth [(5).] The presence of growth of single type with a colony count of 10^5CFU/ml was considered significant. Mixed growths were not processed further considering it as urethral contamination. The urine sample was inoculated on CLED medium and presence of pus cells in the microscopy with colony count of 10^5 CFU/ml was considered significant Routine semen examination was done for sperm count, motility, morphology and presence of pus cells and R.B.C.

Antibiotic susceptibility test of the isolates was tested by Kirby bauer disc diffusion method. The patient with urinary complains and significant colony count were treated according to the

AST pattern of the isolate. The sperm count before and after antibiotic therapy was compared.

Results-

In all the 50 samples processed~~, significant colony count was found in 19 cases~~. The patients were grouped in three categories- azoospermia, oligospermia and normozoospermia based on their sperm count as per WHO manual [6].

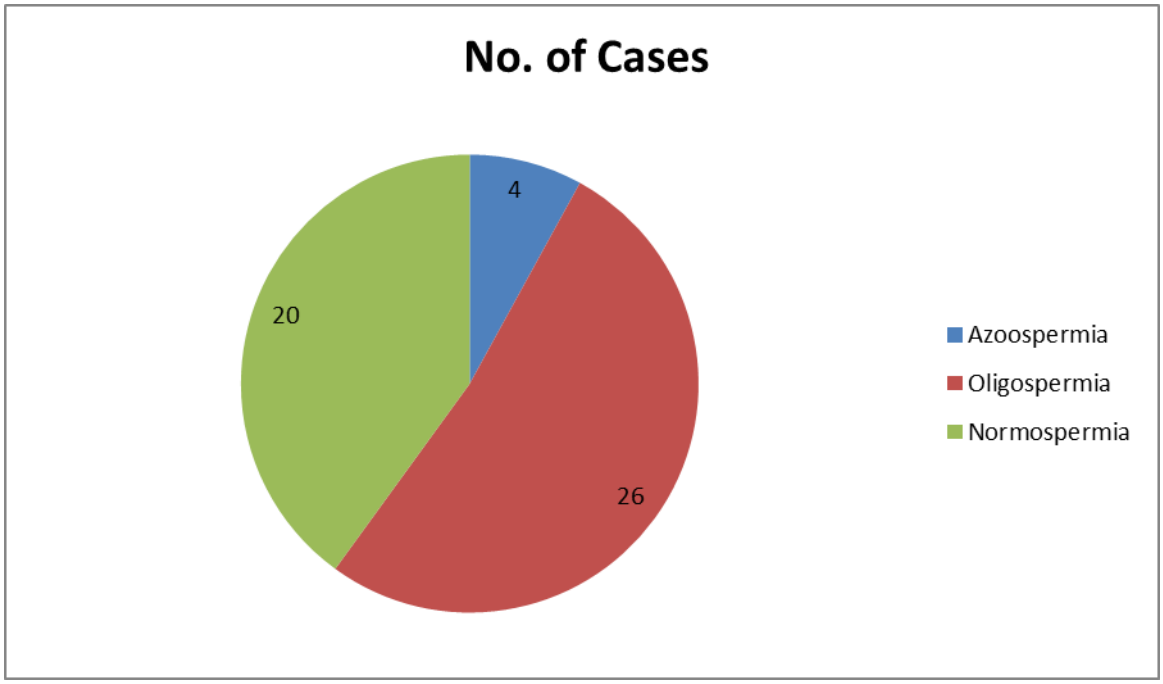

Chart- 1: Patients with their sperm count

We observed that, 26 patients were oligozoospermic, 20 Normozoospermic and four patients had azoospermia. The main bulk of infertile patient was due to decreased sperm count (52%). The Normozoospermic patient constituted 40% 0f the cases.

Table -1. Age distribution and history of addiction-

Age group (years)	25-30	30-35	35-40	40-45
No. of patients	20	18	8	4
Smokers/Nicotine Chewing	15	11	3	1
Alcoholics	5	3	1	1

The history of addiction was also taken in to account. In the age group of 25-30 years the history of smoking or addiction to nicotine addiction was present in 75% of the cases while 20% of the patients were addicted smoking and were alcoholic.

In the age group of 30-35 years the habit of smoking was seen in 61% patients and 16% were alcoholic as well as smokers.

In age group of 35-45 years 37.5% were smokers and in one case we observed addiction to both alcohol and tobacco.

In 40-45 years age group there were four patients one patient was addicted to both alcohol and tobacco.

Following culture, in 19 cases we found a pure colony count of 10^5 CFU/ml (Colony Forming Units). The distribution of the isolates is as shown in the pie chart.

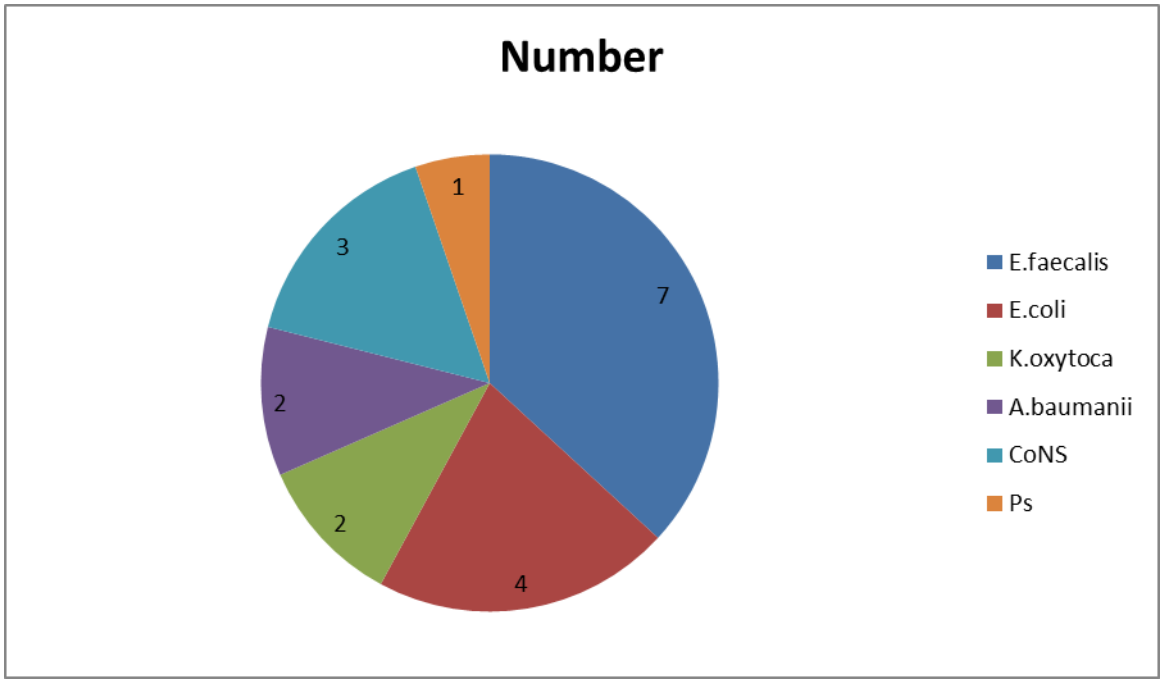

Chart- 2: Distribution of isolates.

The most common isolate was *Enterococcus faecalis* (7) followed by *E.coli* (4), *Staphylococcus saprophyticus* (3), *Klebsiella oxytoca* (2), *Acinetobacter baumanii* (2) and *Pseudomonas aeruginosa* (1).

Table- 2: showing the distribution of the isolates according to the sperm count

Isolates	Azoospermia	Oligospermia	Normozoospermia
Enterococcus faecalis	1	4	2`
Staphylococcus saprophytcus	0	1	2
E.coli	0	4	0

Klebsiella oxytoca	1	0	1
Acinetobacter baumanii	0	1	1
Pseudomonas aeruginosa	0	1	0

Of the 19 isolates totally recovered 11 were recovered from the oligozoospermic patients six from the normozoospermic and two from azoospermic cases.

Table 3 Antibiogram of the isolates (% sensitivity)

Antibiotics	Ampicillin	Vancomycin	Gentamycin	Cefotaxime	Meropenem	Ceftazime-clavalunic acid
Enterococcus faecalis	57 %	100%	-	-	-	-
Staphylococcus saprophyticus	100%	100%	-	-	-	-
E.coli	50%	-	50%	50%	100%	100%
Klebsiella oxytoca	100%	-	100%	100%	100%	100%
Acinetobacter baumanii	-	-	0%	100%	100%	100%
Pseudomonas aeruginosa	-	-	100%	100%	100%	100%

There was marked resistance seen in the *E.coli* isolates with two strains being resistant to third generation cephalosporin. These strains were tested for ESBL (Extended spectrum Metallo b- lactamase) production and were found positive by double disc synergy test (DDST) with a zone enhancement of more than 5mm for ceftazidime -clavulanic acid combination as per CLSI 2013 Guidelines [(8).]

Urinary isolates –

In all the cases microscopic examination of urine was done and the samples showing pyuria along with the clinical manifestation of urinary tract infection were cultured. In all four patients met the above criteria's along with it four age matched patients were randomly selected with no pyuria and no complains of urinary tract infection were taken as control.

Urine culture was positive in four cases with pyuria. *E.coli* was isolated in all these cases. The cases in which urinary complaints were present were associated with oligozosprmia, the

findings of semen culture and urine culture were identical in their cases. The Antibiogram of the *E.coli* isolates recovered in semen and urine was identical. In control group urine samples were sterile

In addition to above drugs for urinary isolates of *E.coli* AST was done for Nitrofurantoin, Nalidixic acid, Cotrimoxazole and Norfloxacin. All of the isolates were sensitive to Norfloxacin.

Following the AST report all the four patients were prescribed Norfloxacin 400 mg BD X 5 days. After the symptoms were relieved. A repeat urine culture was done after the culture was sterile patient was asked to give a repeat semen sample. Findings are as follows –

Table -4 Comparison of sperm motility before and after treating

Patients	Motility (before T/t)	Sperm count (before T/t)	Motility After (T/t)	Sperm count After (T/t)
Oligozoospermic -1	10%	10 millions	20%	10 million
Oligozoospermic-2	20%	10 million	35%	10 million
Oligozoospermic-3	10%	10 million	30%	10 million
Oligozoospermic-4	10%	10 million	20 %	10 million

P = <0.05 by paired t test. The above table shows comparison of sperm count and motility before and after the treatment. This shows that presence of *E.coli* in semen has profound effect on sperm motility.

Table – 5 Comparison of E.coli isolates in semen and urine culture.

	Semen culture positive	Urine culture positive
E.coli present	4	4
E.coli absent	15	0

P = <0.0079 the value is highly significant by Fischer t test. The table shows presence of *E.coli* was statistically significant both in urine and semen.

Discussion-

In this study 19 (38%) out of a total number of 50 semen samples from infertile males collected yielded bacterial growth. *E. faecalis* (37%) and Escherichia *coli* (21%) were the main organisms. *E.coli* was found to have the most negative influence on sperm motility and morphology. The immobilization effect of *Escherichia coli* had been reported Ibadan et al (2008) and Teague *et al* (1991). The bacterial infection may be partly responsible for male infertility arises from the clinical clinical observation of the patient male reproductive system. The mechanism that results in infertility through infection is not clear. It is assumed that bacterial infections may stimulate the immune system and attack on sperm cell membrane with exposure of the spermatozoa to immunologically competent cells in inflammatory conditions. This infection may cause occlusion in the canalicular system of male genital tract, may damage the epithelial cells involved in the spermatogenesis. This might be the cause in low sperm count in these cases [(9).]

Male Urogenital Tract Infection is one of the most important causes of male infertility worldwide. Infection processes may lead to deterioration of spermatogenesis, impairment of sperm functions, and obstruction of the seminal tract. Consequently in presence or absence of the Leukocytospermia, microbiological investigation should be performed on all semen samples, as a routine test, from infertile male attending infertility clinics.

It should be noted that presence of Urogenital Tract Infection and inflammation posed a danger to the fertility profile of male patient and should be eradicated by antibiotics and anti-inflammatory treatment, especially during Assisted Reproductive Technique (ART). This is because genital bacteria can attach to sperms and some of them cannot be removed even during the process of sperm washing in the *In vitro* Fertilisation Lab (IVF). The most common organisms isolated form IVF culture system is *Escherichia coli*. Microbial contamination of the IVF culture media may lead to fertilization failure [(2,7)]

The age group most commonly affected was found to be in and around 30s. Smoking or tobacco chewing was found to the most prevalent habit in the patients in the age group of 25-35 years. Out of the 50 infertile patients history of either tobacco chewing or smoking was present in 30 individuals. The co -addiction of alcohol and smoking had a more profound effect on sperm count.

Conclusion – in our study we compared the outcome of treating UTI and effect of presence of bacteria in semen. The presence of *E.coli* in semen and urine was strongly associated with oligozoospermia. *E.coli* was found to have profound effect on motility of sperm. Regarding the other isolated in semen though not isolated in urine and were not accompanied with any clinical symptoms their role in pathogenesis of infertility cannot be ruled out due to the above mentioned mechanisms. Smoking and nicotine addiction was also associated with infertility in majority of the cases. Leukocytospermia along with pyuria or UTI is a significant cause of male infertility

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