Bacteriological Profile and Seminal Fluid Analysis of Men Attending Infertility Clinic of A Teaching Hospital: A Study From Eastern India

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ABSTRACT

Infertility is a matter of concern in India because of its medical and social problems. Semen analysis is usually considered as the surrogate marker of male infertility because there are several factors that affect semen quality and bacteriospermia. During the study, 226 semen samples were processed and analyzed as per WHO guidelines. Samples were cultured and isolates were identified using standard biochemical tests. Out of 226 cultured samples, 110 bacteria were isolated. *Staphylococcus aureus* was the most common isolate followed by *Escherichia coli* and *Enterococcus spp*. 59.7% (135/226) samples were normospermic, 32.7% (74/226) oligospermic and 7.5% (17/226) azoospermic. 48.6% semen samples were infected and these significantly affected the semen parameters. Infertile male couples should be investigated for infections and appropriate treatment should be given based on antibiotic sensitivity report.

Key words: Bacteriospermia, Male infertility, Motility, Semen analysis,

INTRODUCTION

Infertility can be defined as the biological inability of a man or woman to contribute to conception. In other words, infertility is the state of a woman who is unable to carry a pregnancy to full term.[1] Normally a woman is fertile around their ovulation period (48 hours before to 48 hours after the ovulation) before returning to a normal state of infertility for the rest of their menstrual cycle.[1] According to the study done by American National Institute of Health on infertility, male factors are responsible in 1/3rd of the cases, female factor in another 1/3rd of the cases, and in rest 1/3rd of the cases both male and female factors are responsible or no apparent cause is detected. [2] In other words we can say in around 40% of infertile couples, male is either sole cause or a contributing cause of infertility. In males, sperm deficit is the main factor responsible for infertility whereas in female it is more complex. Some evidence revealed that untreated urogenital infections in male and female can lead to infertility. Perhaps semen analysis is the most important laboratory investigation of male partner of an infertile couple.[3] Several studies have found that sperm parameters such as low concentration, sluggish motility and morphological abnormalities of sperm contribute in male infertility.[4] These factors sometimes associated with presence of nonspecific seminal tract infections.[5] On the basis of clinical and experimental research it is found that there is association between isolation of bacteria from semen and deterioration of spermatogenesis and spermatozoal function which can ultimately lead to infertility.[6] In our society for every case of infertile marriage, female partners are usually blamed because of lots of misconceptions about what a fertile man is. People think that once a man is able to have intercourse, ejaculate semen, then problem must lie in the wife and not in the male partner. However, with improvement in the level of education and awareness these days, trends are gradually changing. Many male partners are now visiting infertility clinics to verify their reproductive status if they are in doubt. So keeping in mind the entire present scenario, present study aims at isolating aerobic bacterial pathogens from semen and assesses its correlation with semen parameters in infertile male partners.

MATERIALS AND METHODS

The present study was done in a teaching hospital in the Eastern part of India from July 2013 to July 2015, for a period of 2 years. The study includes all males who attended the infertility clinic of the hospital with their mates. Before collection of semen sample, counseling was done. The counseling included abstinence from sexual intercourse or masturbation for a period 3-5 days and also avoid use of antibiotics before collection of samples, especially in those cases where culture was required. All samples were collected through masturbation aseptically in a sterile container and then sent to the laboratory within an hour for physical and microscopic examination. Samples were allowed to liquefy at 37^0c for 30 minutes before examination. Physical parameter included -

Appearance: Normal semen sample appears homogenous gray opalescent. It may be less opaque if the sperm concentration is very low. Volume was measured into a graduated centrifuge tube and the level was recorded in ml. It was measured using Pasteur pipette. Normal semen sample leaves the pipette as small discrete drops whereas in abnormal cases the semen drop forms a thread of >2 cm length.

Motility: It was done by applying a drop of semen sample onto a slide covered with cover slip and then examined under high power (×40) objective lens. Motility was graded active motile, sluggish motile and non motile as per WHO criteria [7]. Samples were stained for morphological assessment using Giemsa stain. Bacteriological examinations of the samples were done using blood agar and Mac-conkey agar. After inoculation, plates were incubated at 37^0c for 24 -48 hours. Identifi cation of bacteriological isolates was done using standard biochemical tests.[8] Antibiotic sensitivity was done using Kirby-Bauer disc diffusion method.

RESULTS

Out of 226 semen samples analyzed, 17(7.5%) were azoospermic (no spermatozoa in semen), 74 (32.7%) were oligospermic (sperm concentration ≤ 20 million/ml) whereas in 135(59.7%) samples there was normal sperm concentration (≥ 20 million/ml). **(Table-1)**

Table-1: Age variation with sperm concentration (million/ml)

Age in years	Nil (0/ml)	< 20million/ml	>20million/ml	Total
< 30	4	21	26	51 (22.5%)
31 – 40	7	33	78	118 (55.2%)
41 – 50	5	16	23	44 (19.4%)
51 – 60	`1	3	6	10 (4.42%)
61 – 70	0	1	2	3 (1.32%)
Total	17 (7.5%)	74 (32.7%)	135 (59.7%)	226 (100%)

Sperm motility is an important factor while assessing quality of seminal fluid. In our study, sperm motility was 50% or above in 106(46.9%) samples. In 49(21.6%) sperm motility was between 20-49%, in 66(29.2%) samples, motility was 1-19%. In 5(2.2%) samples, spermatozoa were totally non-motile. **(Table-2)**

Table-2: Sperm motility assessment of semen

% of motility	>50%	20-40%	1-19%	0%	Total
No of men	106	49	66	5	226
% age	46.9%	21.6	29.2	2.2	100%

In our study, 110 bacteria were isolated from 226 processed semen samples. *Staphylococcus aureus (S. aureus)* was the most common isolate (28.1%) followed by *Escherichia coli (E.coli)* (21.8%), *Enterococcus spp*. (14.5%) and *Coagulase negative Staphylococci (CONS)* (10.9%).**(Table-3).**

Table-3: Bacterial isolates from cultured semen samples (n=110)

Microorganism	Frequency of isolate	Percentage
S.aureus	31	28.1%
E.coli	24	21.8%
Klebsiellapneumoniae	10	9%
Enterococcus spp.	16	14.5%
Coagulase Negative Staphylococci (CONS)	12	10.9%
Pseudomonas aeruginosa	8	7.27%
Acinetobacter spp.	6	5.45%
Proteus vulgaris	3	2.72%
Total	110	100%

Out of 17 Azoospermia semen samples growth was seen in 13(76.5%) cases. Similarly, out of 74 oligospermic samples, growth was seen in 73% (54/74). Normospermic patients showing lowest growth rate (43/135). **(Table-4)**

Table-4:- Frequency of isolation of bacteria in relation withSperm concentration

Bacterial isolates	Sperm concentration		
	Normal sperm concentration (≥20 million/ml) n=135	Oligospermia (<20 million/ml) n=74	Azoospermia (0/ml) n=17
Staphylococcus aureus (n=31)	13	11	07
Escherichia coli (n=24)	09	12	03
Enterococcus spp. (n=16)	07	08	01
Cogulase Negative Staphylococci (n=12)	04	08	0
Klebsiellapneumoniae (n=10)	03	05	02
Pseudomonas aeruginosa (n=8)	03	05	--
Acinetobacter spp. (n=6)	03	03	--
Proteus vulgaris (n=3)	01	02	--
Total growth	43 (32%)	54 (73%)	13 (76.5%)
No growth	n=92(68%)	n=20(27%)	n=4(23.5%)

Table 5 : ntibiotic Susceptibility Pattern of Bacterial Isolates

Bacterial isolates	Antibiotic Susceptibility Pattern Of Bacterial Isolates									
	Va	**Lz**	**Le**	**Gen**	**Ak**	**Amp**	**I**	**Caz**	**Cot**	**Cip**
Staphylococcus aureus (n=31)	30 (96.7%)	31 (100%)	19 (61.3%)	21 (67.7%)	--	--	--	--	--	18 (58%)
CogulaseNegative Staphylococci (CONS) (n=12)	10 (83.3%)	12 (100%)	7 (58.3%)	8 (66.6%)	--	--	--	--	--	8 (66.6%)
Enterococcus spp. (n=16)	14 (87.5%)	16 (100%)	10 (62.5%)	--	--	8 (50%)	--	--	--	9 (75%)
Escherichia coli (n=24)	--	--	13 (54.3%)	15 (62.5%)	18 (75%)	11 (45.8%)	22 (91.6%)	16 (66.6%)	20 (83.3%)	12 (50%)
Klebsiellapneumoniae(n=10)	--	--	4 (40%)	5 (50%)	7 (70%)	4 (40%)	9 (70%)	6 (60 %)	7 (70%)	6 (60%)
Pseudomonas aeruginosa (n=8)	--	--	--	4 (50%)	5 (62.5%)	3 (37.5%)	7 (87.5%)	6 (75%)	--	3 (37.5%)
Acinetobacter spp. (n=6)	--	--	4 (66.6%)	3 (50%)	4 (66.6%)	2 (33.3%)	5 (83.3%)	3 (50%)	2 (33.3%)	1 (16.6%)
Proteus vulgaris (n=3)	--	--	1 (33.3%)	2 (66.6%)	2 (66.6%)	1 (33.3%)	3 (100%)	1 (33.3%)	1 (33.3%)	1 (33.3%)

Linezolid was 100% sensitive antibiotic against gram positive isolates, whereas Vancomycin was 96.7% sensitive against *S.aureus*, 87.5% against *Enterococcus spp*. and 83.3% against *Coagulase negative Staphylococci (CONS)*. For Gram negative isolates, Imipenem was the most sensitive antibiotic (90%) followed by Amikacin (70.5%) and Ceftazidime (62.7%). **[Table-5] Abbreviation:**Va (Vancomycin), Lz (Linezolid), Le (Levofloxacin), Gen (Gentamicin), Ak (Amikacin), Amp (Ampicillin), I (Imipenem), Caz (Ceftazidime), Cot (Cotrimoxazole), Cip (Ciprofloxacin)

DISCUSSION

The incidence of infertility increases as age of both the partners increases. Due to various reasons marriages and subsequently the first child birth are going delayed, there by subsequently increasing the infertility rates. On the basis of sperm count, it was found that >40% study population was either oligospermic or azoospermic, as normal sperm count (>20 million/ml) was seen only in 59.7 % of male partner. Men either alone or along with their female partners contribute to 40-50 % cases of infertility.[9] Infection etiologies involving bacteria, virus, fungi and protozoa also contribute to around 15 % of male factor infertility.[10-12] Nonspecific seminal tract infection affects the normal fertility process by following mechanisms – deterioration of spermatogenesis, decreased sperm motility, altered chemical composition of seminal fluid, altered morphology, formation of anti sperm antibodies due to breach in the blood- testis barrier and genital tract obstruction due to inflammation and fibrosis. [13]

In the present study, 226 semen samples were processed, out of which 110 (48.6 %) samples were found to be infected which was more or less similar to study done by Mogra *et al,*[14] where infection rate was 42.9 % .110 bacterial growth were obtained from the infected samples, and *Staphylococcus aureus (S. aureus)* was the most common isolate followed by *Escherichia coli (E. coli)*, *Enterococcus spp. and Coagulase negative staphylococci (CONS*), which was similar to study done by Olajubu F.A *et al,*[15] Oiyeyipo *et al*,[16] found that *S. aureus*, *E. coli, Enterococcus spp*. and *CONS* etc. have most negative effect on sperm morphology and motility. Of these 110 bacterial isolates 66 (60 %) were from oligospermic, 3 (2.73 %) from azoospermic and 41 (37.27 %) were from normospermic male partners. Isolation of S. *aureus* and *Coagulase negative staphylococci (CONS)* in this study might be associated with body hygiene of the couple involved. Isolates from semen samples have any clinical significance or not needs careful evaluation and because there is possibility of contamination during sample collection. Distribution of bacterial isolates according to sperm cell concentration revealed that azoospermic patients have highest isolation rate 13/17 (76.5 %) followed by oligospermic patients 54/74 (73%). Normospermic patients have lowest isolation rate (32%). This study was in agreement with Onemu S.O *et al.*[17] Gomez *et al* reported that, microbial infections of the semen are major causes of male infertility.[18]It has been observed that presence of pathogenic organism may interfere with treatment of infertility involving the application of in-vitro fertilization (IVF) and intrauterine insemination.[19] Occupational exposures

to heavy metals like lead, zinc and arsenic have been reported to impair spermatogenesis. Certain herbicides and pesticides have also found to be toxic to spermatogenesis. Smoking has also been associated with lower sperm concentration.[20]

There are various factors associated with infertility

in man. These includes; presence of varicocele, sexual dysfunction, genitourinary infections, urospermia, age and nutrition. Apart from this other factors are stress, endocrine and chromosomal abnormalities, excessive alcohol intake etc. In the present study 48.6% semen samples show bacterial growth. Certain other pathogens like *anaerobes*, *Chlamydia, Ureaplasma, Mycoplasma* etc. were not included in the study due to lack of facilities. Therefore, it is necessary to investigate all male partners of infertile couple for the presence of bacteria having proven pathogenic potentials. Prompt antibiotic therapy should be started based on antibiotic susceptibility report. We recommend that further study should be done to establish correlation between bacterial pathogens from azoospermic and oligospermic patients and its role in infertile couples.

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