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An Influential Relationship of Seminal Fluid Microbial Infections and Infertility, Taif Region, KSA

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Abstract: Search was carried up on male patients who attended to Male Fertility Clinic (MFC), from private polyclinics at Taif Region, KSA during 2013. Male patients under study suffered from infertility problems after married. The search was carried on seminal fluid specimens as stress point for analysis.Total seminal fluid specimens examined were 856, pyospermia were reported in 17.3%. Total microbial isolates were in 87.2% out of the pyospermia specimens that was differentiated into Gram positive which included *S. aureus, S. faecalis and S. pyogenes* (14.2, 12.8 and 8.8% respectively), Gram negative included *E. coli, Gardnerella vaginalis, Chlamydia spp., Pseudomonas spp., Proteus spp. and Mycoplasma spp.* as (14.9, 9.5, 8.1, 5.4, 4.7 and 1.4% respectively). As well as fungi were isolated also as *Candida spp.*in 7.4% of the examined specimens. Spermatozoa count from pyospermia seminal fluid specimens were either oligospermia in 61.5% as and azoospermia in 38.5% of the examined specimens.

Key word: Infertility · Seminal Fluid · Pyospermia · Infection · Oligospermia, Azoospermia

INTRODUCTION

Microbial infections may affect fertility in several ways: by damaging sperm, hampering their motility, altering the chemical composition of the seminal fluid, or by producing an inflammatory structure in the tract. Seminal fluid infection could also be the cause of the chronicity of urinary tract infection by acting as the reservoir of microbial infection [1]. Infections may affect fertility in different ways, possible consequences are impairment of the spermatogenesis, the induction of an autoimmune mechanism. Reduced motility of spermatozoa has been found in seminal fluid samples which contain high concentrations of bacteria [2]. High WBCs concentrations within seminal fluid are an indicator of infection; this condition, marked by pus cells in the seminal fluid, is termed pyospermia [3]. Infertility dangers about 10-15% of couples all over the world and is always considered a female issue. Husbands are not willing to go for making many tests nevertheless, male factor responsible for 45% out of the cases [4]. Pyospermia (WBCs over 5/HPF) leading the other causes of male infertility that indicates infection and then manifested by either oligospermia or azoospermia [5]. There is difference as to the influence of certain microbial infection on male infertility [6]. Urinary tract infections are common in men and clinicians working with infertility frequently encounter patients with these diseases [7]. Male infertility is a multifactorial problem. More than 90% of male infertility cases is due to either low sperm count (oligospermia); no sperm at all (azoospermia) or poor seminal fluid quality or combination of the two and this claimed to the increase prevalence of sexually transmitted diseases (STDs) and urogenital infections alarmed since 1992 [8]. Infections may affect fertility in several ways: damage sperm, hamper sperm motility, alter the seminal fluid chemical composition, or produce an inflammatory structure in the tract. Moreover, infections may impair the spermatogenesis; induce an autoimmune illness; result in spermatozoa dysfunction and cause inflammatory occlusion of the ejaculatory duct [2-3]. WHO defined the seminal fluid infection as the presence of significant bacteriospermia (=10^3 bacteria/ml semen). Bacteriological investigation of pyospermia sperm samples revealed significant growth of *Streptococcus faecalis*, *E coli*, Coagulase positive *Staphylococcus*, *Proteus valgaris*,

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Pseudomonas. *Pyocyanea* and beta hemolytic *Streptococci* in 42.9% out of the examined cases. Other bacterial species isolated from patient with pyospermia; *Enterobacteriaceae*, *Gardnerella vaginalis*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Neisseria gonorrhoeae*, *C. trachomatis*, *Ureaplasma parvum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *S. pyogenes*, *S. aureus*, *S. epidermidis*, *S saprophyticus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Ps. aeruginosa*, Toxoplasma gondii and *Candida*; with considerable difference to influence male infertility [6]. WBCs may have a valuable effect on sperms function when leukocyte levels range from 1-3x10^6/ml [9]. According to WHO, seminal fluid infection was defined as the presence of significant bacteriospermia (=10^3 bacteria/ml semen), detection of *Neisseria gonorrhoeae, C. trachomatis, U. urealyticum*; significant leukocytospermia (10^6 peroxidase positive leukocyte/ml semen). It therefore follows that if some or all the conditions above are not met, the isolation of bacteria in seminal fluids are often regarded as contaminants by most practitioners [10]. Oligospermia and azoospermia are most common causes of male infertility which has been reported due to bacterial infections [11]. The mean of pus cells was 3.25 ± 0.26 and 3.10 ± 0.19/HPF in azoospermia and oligozoospermia respectively. Pus cells showed an inverse relationship to sperms motility and count, except in azoospermia cases. Similarly, the fewest pus cells were observed among groups where normal forms where significantly more frequent. More pus cells were observed in cases where motility and concentration or morphology was compromised. Similarly, low pus cell counts were seen in cases where sperm had the fewest head and neck defects. All kinds of sperm defects varied no significantly between proven fathers and normal concentration cases [12]. The results had shown that from 17 microbial species there are, *Ureaplasma urealyticum* 4.9%, *Ureaplasma parvum* 2.2 %, *Mycoplasma hominis* 2.5 %, *Mycoplasma genitalium* 5.9%, *Chlamydia trachomatis* 9.9 %, *Strept. pyogenes* 8.6%, *Staph. aureus* 11.1 %, *Staph. epidermidis* 12%, *Staph. saprophyticus* 0.9%, *E. coli* 20%, *Proteus mirabilis* 1.2%, *Proteus vulgaris* 2.5%, *Klebsiella pneumoniae* 0.9%, *Pseudomonas aeruginosa* 1.5%, *Neisseria gonorrhoeae* 2.8%, *Toxoplasma gondii* 6.2% and *Candida* 6.8%. Also the infection with micro-organisms revealed that it is higher in azoospermia patients [13]. Abnormal seminal fluid quality is a major factor in our rural setup with 52% of male partners of infertile couples having abnormal semen parameters [14]. Azoospermia showed in 14.89% and oligospermia in 11.11% [15].

The Aim: This cross-sectional study aimed to investigate the prevalence of microbial infection in patients with oligospermia or azoospermia.

MATERIALS AND METHODS

Study Region: Search was carried up on target male patients who attended to Male Fertility Clinic (MFC), from private polyclinics at Taif region, they suffered from infertility problems after married. Getting an informed consent from understudy male patients, also the search aim was explained to them, as well as the medical data will be participated in search without recording any personal information. The search was carried on seminal fluid specimens' analysis which collected from selected target male patients during 2013.

Understudy Male Patients: Seminal fluid specimens were collected (856). All specimens were examined for pyospermia, microbial infections and seminal fluid analysis. Group preparation was carried on understudy male patients for collection of data and dates for laboratory analysis.

Specimen Collection: Each seminal fluid specimen was collected by patient himself into sterile container. The subjects were instructed on how to collect the specimens and submit to the laboratory within 1h of collection.

Microbial Analysis: The seminal fluid specimens were cultured after liquefaction on bacterial and fungal media and then incubated for 24-48 h at 37°C, to confirm the culture results Microbial Identification (Micro-Scan System) was used. Others which are not cultivable micro-organisms were detected by ELISA technique such as Chlamydia, Ureaplasma and Mycoplasma [16].

Seminal Analysis: Parameters of semen outlined general included: Appearance, grey/opalescent; Volume, 2.0ml or more; pH, 7.2-7.8; Sperm concentration, >15x10^6 sperm/ml; total sperm count, 39x10^6/ejaculate or more; Motility, 50% or more with forward progression; morphology, 4% or more with normal form; and white cell count, <1x10^6/ml.

Semen analysis was performed within 60 minutes of their collection. Specimen considered pyospermia as long as the WBCs concentration remains higher than 5/HPF [3]. After liquefaction, the semen specimen was thoroughly mixed with the help of a glass road and a thin drop was spread on a glass slide by placing a cover slip

on it. Counting of sperm was done using Meckler's counting chamber. Semen specimens well mixed and thoroughly homogenized then checked for the pus cells/HPF and sperms count/ml. Pus cells were confirmed by the Giemsa staining technique. Specimen having no sperm is (Azoospermia) and having less than 15million/ml (Oligozoospermia) [16].

Data: The results obtained were subjected to statistical analysis by using Statistical Package for the Social Sciences [17].

RESULTS

Table 1 and figure 1 show prevalence of pyospermia in the examined seminal fluid specimens. The overall prevalence of pyospermia was 17.3%. The higher prevalence pyospermia were appeared at Jan., Feb. Sep. and Ma. months.

Table 2 and figure 2 show the prevalence of main microbial species were isolated from pyospermia specimens, the total isolates were 87.2%, differentiated into Gram positive, Gram negative and fungi. Gram positive were isolated as *Staph. aureus, Strept. faecalis and Strept. pyogenes* as (14.2, 12.8 and 8.8%) respectively. Gram negative were isolated as *E. coli, Gardnerella vaginalis, Chlamydia spp., Pseudomonas spp., Proteus spp.* and *Mycoplasma spp.* as (14.9, 9.5, 8.1, 5.4, 4.7 and 1.4%) respectively. As well as fungi were represented by *Candida spp*. as 7.4%.

Table 3 and figure 3 show sperms count from pyospermia specimens. Oligospermia was 61.5% while azoospermia was 38.5% out of the examined 148 pyospermia specimens.

DISCUSSION

Table 1 and figure 1 show prevalence of pyospermia in the examined seminal fluid specimens, total were recorded in 148 (17.3%) out of 856 seminal fluid examined specimens. The higher prevalence of pyospermia was appeared at Jan., Feb., Sep. and Ma. months. High WBCs concentrations within semen fluid are an indicator of infection, marked by pus in the semen, is termed pyospermia. Although small numbers of WBCs are a normal constituent of the semen, patients are only considered non-pyospermia as long as the WBC concentration remains below 5/HPF [3]. The relationship between seminal fluid quality, pyospermia and microbiology were studied in semen isolates, which showed reduced fertility in 43% of patients who were pyospermia [5]. WBCs may have a valuable effect on sperms function when leukocyte levels range from 1-3x10^6/ml [9]. According to WHO, seminal fluid infection

Table 1: Prevalence of pyospermia in the examined seminal fluid specimens

Month	Jan.	Feb.	Mar.	Ap.	Ma.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Total *No. examined	72	69	69	68	73	69	74	75	77	68	72	70	856
Positive No.	14	13	12	11	13	11	12	13	14	11	12	12	148
Percentage%	19.4	18.5	17.4	16.2	17.8	15.9	16.2	17.3	18.2	16.2	16.7	17.1	17.3

**No.: Number*

Table 2: Prevalence of main microbial species were isolated from pyospermia specimens

Pyospermia Positive (*No.=148)	Positive *No. for microbial isolation	Total*No.	Percentage%
Microbial species:			
-Gram positive:			
*Staph. aureus	21	148	14.2
*Strept. pyogenes	13	148	8.8
*Strept faecalis	19	148	12.8
-Gram negative:			
*E. coli	22	148	14.9
Proteus *spp.	7	48	4.7
Pseudomonas* spp.	8	148	5.4
Gardnerella vaginalis	14	148	9.5
Chlamydia *spp.	12	148	8.15
Mycoplasma *spp.	2	148	1.4
-Fungi:			
Candida* spp.	11	148	7.4
Total	129	148	87.2

*No.: Number

Table 3: Sperms count from pyospermia specimens

Pyospermia Positive (*No.=148)	Positive*No.	Total*No.	Percentage%
Oligospermia	91	148	61.5
Azoospermia	57	148	38.5
Total	148	148	100

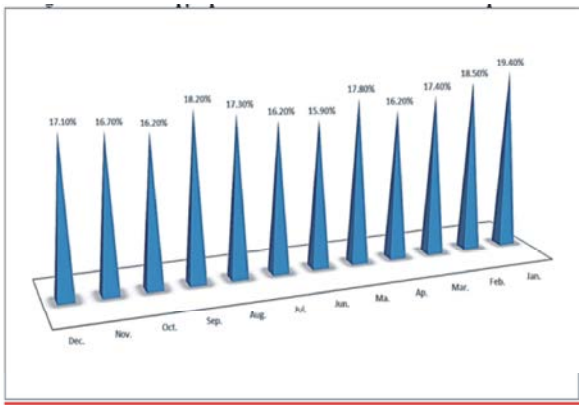

Fig 1: Prevalence of pyospermia in the examined seminal fluid specimens

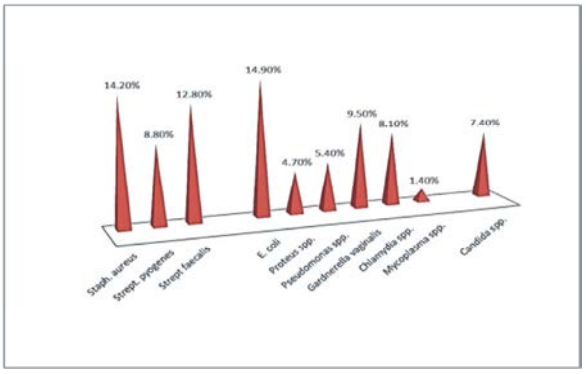

Fig 2: Prevalence of main microbial species were isolated from pyospermia specimens

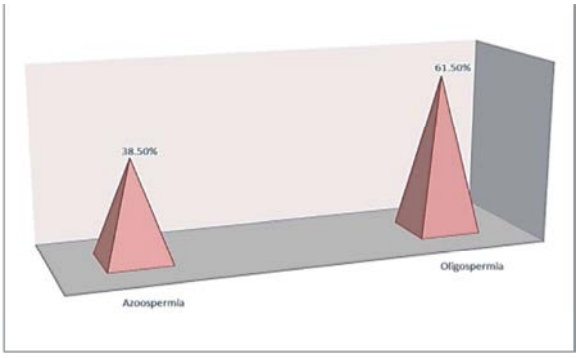

Fig 3: Sperms count from pyospermia specimens

was defined as the presence of significant bacteriospermia (=10^3 bacteria/ml semen) [10]. Pyospermia where in azoospermia and oligozoospermia specimens. More pus cells were observed in cases where motility and concentration or morphology was compromised [12].

Table 2 and figure 2 show the prevalence of main microbial species were isolated from pyospermia specimens, the total isolates were 87.2%, differentiated into Gram positive, Gram negative and fungi. Gram positive were isolated as *S. aureus, S. faecalis and S. pyogenes* as (14.2, 12.8 and 8.8% respectively). Gram negative were isolated as *E. coli, Gardnerella vaginalis, Chlamydia spp., Pseudomonas spp., Proteus spp.* and *Mycoplasma spp.* as (14.9, 9.5, 8.1, 5.4, 4.7 and 1.4% respectively). As well as fungi isolated were *Candida spp*. as 7.4%. Our results was in concordance with many authors investigated such problem. *S. faecalis, E. coli*, Coagulase positive *Staphylococcus*, *Proteus valgaris*, *Pseudomonas pyocyanea* and beta hemolytic *Streptococcus* were found in 42.9% of cases with pyospermia [1]. In addition, another study isolated *Enterobacteriaceae* 2.8%, *Gardnerella vaginalis* 9.6%, *Chlamydia trachomatis* 1.6%, *Mycoplasma genitalium* 0.9% and *Ureaplasma urealyticum* 11.9% [5]. The microbial species were *Mycoplasma hominis* 2.5 %, *Mycoplasma genitalium* 5.9%, *Chlamydia trachomatis* 9.9 %, *Strept. pyogenes* 8.6%, *Staph. aureus* 11.1 %, *E. coli* 20%, *Proteus mirabilis* 1.2%, *Proteus vulgaris* 2.5%, *Pseudomonas aeruginosa* 1.5% and *Candida* 6.8%. The infection with micro-organisms revealed that it is higher in azoospermia patients [13].

Table 3 and figure 3 show pyospermia specimens sperm count and judged as sample has less than 20 million sperm/ml, considered to be a low sperm count and Less than 10 million is very low. The technical term for this is oligospermia (Oligo means few), if no sperm at all said to be azoospermia. Out of the examined 148 specimens, 61.5% suffered from oligospermia while the other 38.5% suffered from azoospermia and these results explained as there is an inverse relationship between pus cell and sperm/ml count. Microbial infections may affect fertility in different ways, possible consequences are impairment of the spermatogenesis, the induction of an autoimmune mechanism. Reduced motility of spermatozoa has been found in seminal fluid samples which contain high concentrations of bacteria [2]. While male infertility is a serious global problem., although the problem is mainly due to sperm defects, other factors are also responsible for male infertility, of which only one is pyospermia [5]. Also, there is difference as to the influence of certain

microbial infection on male infertility [6]. Urinary tract infections are common in men and clinicians working with infertility frequently encounter patients with these diseases. The possible relationship between infection and infertility has been the subject of controversy since the second half of the 1970s and several therapeutic trials have been initiated since then [7]. While recent data confirm the decline in seminal fluid quality and quantity all over the world probably due to increased prevalence of sexually transmitted diseases (STDs) and urogenital infections. A report in 1992 alarmed the world about the problem and led others to investigate the phenomenon [8]. WBCs may have a valuable effect on sperms function when leukocyte levels range from 1-3x10^6/ml [9]. In some parts of the world, oligospermia and azoospermia are most common causes of male infertility which has been reported due to bacterial infections [11]. Also the infection with micro-organisms revealed that it is higher in azoospermia patients [13]. There was 52% of male partners of infertile couples having abnormal semen parameters [14]. Azoospermia were in 14.89% and oligospermia in 11.11%. Oligospermia [15].

CONCLUSIONS

Pyospermia is a leading cause for male infertility. Therefore, it is suggested that the presence of pus cells in the seminal fluid should not be ignored by the treating physician and must be considered as a factor limiting male fertility. Positive seminal fluid cultures should be interpreted with caution, taking into account both raised colony counts of single isolates in the semen.

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REFERENCES

1. Mogra, N., A. Dhruva and L.K. Kothari, 1981. Non-specific seminal tract infection and male infertility: a bacteriological study. J. Postgrad. Med., 27: 99-104.
2. Krause, W. and W. Weidner, 1989. Sexually transmitted diseases as causes of disorders of male fertility. Z. Hautkr., 64: 596-601.
3. Satoh, S., K. Satoh, S. Orikasa, I. Maehara, M. Takahashi and M. Hiramatsu, 1990. Studies on pyospermia in male infertility. Nihon Hinyokika Gakkai Zasshi, 81: 170-177.
4. Carlsen, E., A. Giwercman, N. Keiding and N.E. Skakkebaek, 1992. Evidence for decreasing quality of semen during past 50 years. BMJ., 305: 609-613.
5. Kjaergaard, N., B. Kristensen, E.S. Hansen, S. Farholt, H.C. Schonheyder, N. Uldbjerg and H. Madsen, 1997. Microbiology of semen specimens from males attending a fertility clinic. APMIS., 105: 566-570.
6. Ajabor, L.M., M. Ezimokhai and A. Kadiri, 1981. Male contribution to subfertility in Benin City, Nigeria. Trop J. Obst. Gynaecol., 2: 53-56.
7. Rowe, P., 2000. WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male, 1st ed. Cambridge: Cambridge University Press.
8. Wong, W.Y., C.M. Thomas, J.M. Merkus, G.A. Zielhius and R.P. Steegers-Theunissen, 2000. Male factor subfertility: possible causes and impact of nutritional factors. Fertile. Sterile., 73: 435-442.
9. Kaleli, S., F. Oçer, T. Irez, E. Budak and M.F. Aksu, 2000. Does leukocytospermia associate with poor semen parameters and sperm functions in male infertility? The role of different seminal leukocyte concentrations. Eur. J. Obstet. Gynecol. Reprod. Biol., 89: 185-191.
10. Okon, K., M. Nwaogwu, S. Zailani and C. Chama, 2005. Pattern of Seminal fluid indices among infertile male partners attending the infertility clinic of University of Maiduguri Teaching Hospital, Maiduguri, Nigeria. Highland Medical Research Journal, 3: 18-23.
11. Megafu, U., 2007. Seminal fluid infection and oligospermia. Trop. J. Obst. Gyne., 9: 10-12.
12. Khan, M.S., S.H. Mohammad, F. Deepa and F. Tahir, 2012. Association between Pus Cells and Semen Parameters in Infertile Pakistani Males. Sultan Qaboos University Med. J., 12: 479-484.
13. Al-Marzoqi, A.H., M.M. Aboud and M.A. Sabri, 2012. Study of Bacterial infection associated with male infertility in Hillah city-Iraq. Journal of Biology, Agriculture and Healthcare, 2: 10-17.
14. Jajoo, S. and K.R. Kalyani, 2013. Prevalence of abnormal semen analysis in patients of infertility at a rural setup in Central India. Int. J. Reprod. Contracept. Obstet. Gynecol., 2: 161-164.

15. Butt, F. and N. Akram, 2013. Semen analysis parameters: experiences and insight into male infertility at a tertiary care hospital in Punjab. J. Pak. Med. Assoc., 63: 558-562.
16. World Health Organization, 2004. WHO Laboratory Biosafety Manual. 3rd ed. Geneva: World Health Organization.
17. Coulombier, D., R. Fagan, L. Hathcock and C. Smith, 2001. Epi Info 6 Version 6.04. A Word Processing, Database and Statistical Program for Public Health. Centers for Disease Control and Prevention, Atlanta, Delaware, USA.