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RESEARCH ARTICLE

The Effect of Pyospermia on Male Infertility

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Abstract: A cross sectional study was carried out on written consent in a fertility clinic of Sri Lanka during the period of August 2014 to August 2017 to find out the relationship of pyospermia with seminal parameters such as seminal volume, count, mortality and morphology. The amount of 105 subjects who had fertility issues were screened for seminal analysis (pus cells were counted separately with Giemsa staining technique) and found to have 40 of them were pyospermic (over 5 seminal pus cells per high power field/HPF). Out of them 15 were having over 10 seminal pus cells per HPF. The seminal parameters of Indnivuals with pus cells (5-10 /HPF) were compared with that of control group and found to have no statistical significance between the two groups. Anyway a dramatically negative relationship was found between pyospermia (over 10 pus cells / HPF) and seminal parameters such as motility and morphology. This could be due to the secretary materials of pus cells (harmful to sperms) such as collagenase, reactive oxygen species which are proportionate to the number of pus cells found in the semen. Thus, it is important to suppress the pyospermia condition especially with over 10 pus cells/HPF in the management of male infertility.

Keywords: *Male infertility; Motility; Morphology; Pyospermia; Semen parameter.*

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Introduction

Male factor infertility, which accounts 30-40% of the infertility [1] has become a psychosocial issue in the modern society. The men's fertility basically depends on the quality of seminal parameters such as seminal volume, count, motility and morphology. Thus the deviation of these parameters from their normal reference range could lead to poor quality semen, hence the male infertility. Various conditions can cause poor quality semen. Some are yet to be detected [2-3].

Sometime it could be due to biological reason such as pyospermia, which is defined as the presence of more than 1 million leukocytes in 1 ml semen or more than 5 pus cell/HPF of microscope [4-5]. Anyway, the finding of few leucocytes in semen (below the pyospermia level) is considered as normal and it can contribute immunity to the semen.

The pyospermia condition could compromise male fertility, as it increases the level of oxidative stress which badly affect on the sperm quality [4]. Infections or inflammation of the accessory sex glands have been found as the prominent causes of contribution of pus cells to the semen [4].

Thus, it is very much important to investigate the effect of pyospermia on seminal parameters as it helpful to prevent or cure the condition from male partners. In the present study the effect of pyospermia as well as the severity of pyospermia on seminal parameters is investigated in a selected population of Sri Lanka. The study was carried out from my personal fund.

Research Problem

Is pyospermia negatively affected on seminal parameters?

Hypothesis

The pyospermia has a significant negative effect on seminal volume, sperm count, sperm motility and sperm morphology.

Methods and Methodology

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The ethical approval was obtained from the ethic review committee of the institute where the study was carried out.

Design [6]

Cross Sectional Study

Venue and Time Period

The male partner of infertile couples who visited the fertility clinic of an institute of Sri Lanka during the period of August 2014 – August 2017 was involved in the study. The individuals who wished to take part in the study were evaluated on exclusive and inclusive criteria on the consent.

The Sample size [7]

$$\text{Sample size} = \frac{4 Z_{\alpha}^2 P (1-P)}{D^2}$$

Z_{α}= standard normal deviate (at 95% confidence interval = 1.96)

P = prevalence of male infertility (8%) [8]

D = Total width of confidence (0.125)

Thus, the sample size at 95% of confidence interval = $\frac{4 (1.96)^2 \text{ X } 0.08 \text{ X } 0.92}{0.015625} = 72$

N = 72

The sample size of pyospermic individuals with fertility issues was found as follows

$$\text{Sample size} = \frac{4 Z_{\alpha}^2 P (1-P)}{D^2}$$

Z_{α}= standard normal deviate (at 95% confidence interval = 1.96)

P = prevalence of pyopsermia among infertile male (5%) [9]

D = Total width of confidence (0.125)

Thus, the sample size at 95% of confidence interval = $\frac{4 (1.96)^2 \text{ X } 0.05 \text{ X } 0.95}{0.015625} = 46$

N = 46. Anyway, the values of calculated sample sizes were exceeded during the period of study.

The Control Group

The age matching, number equal (as much as possible) non-pyospermic healthy men who visited the clinic were selected for the control group [10]. These indnivuals had the normal semen profiles and the infertility issues were with their wives. The control was considered from the same population to maintain the location equality [10].

Inclusive Criteria

- All the male came to fertility clinic and who were over 18 years old. 4.6 Exclusive criteria [11-12-13].
- Individuals with diseases (diabetes, hypertension, cancer, arthritis, varicocele) or ones on drug for those diseases during the period.
- Individuals, take marijuana, abin and ganja (these can cause negative effect on the sperms synthesis).
- Individuals, who had been on anti-gastric drugs such as cimetidine or any steroidal drugs (increase the hormone, prolactin which negatively affect on sperm production).
- Individuals, who were dislike to participate or unable to communicate.

The selected subjects were interviewed orally to gather the demographic data such as age, residence, contact numbers and were asked to provide a semen sample (3 days abstinent from ejaculation).

The Semen Analysis [14]

Liquefied semen was analyzed on the room temperature under WHO method as the method was concordant with the strict criteria [15].

- Measuring of semen volume (normal reference volume is over 2 ml) the volume of liquefied semen was measured with 10 ml of measuring cylinder.
- Detection of sperm motility

Seminal volume (10.00 µl) was placed on a clean slide and observed several field under the high power field (x 40) of the microscope to count total and motile sperms. During the observation, rapidly and slowly movable sperms were counted separately. Following situations were considered as normal.

(1) Availability of 25% or above of rapidly motile sperms in the semen drop.

(11) Finding of 50% or above of rapidly and slowly movable sperms (as a collection).

- Measuring of sperm count **(**25 million sperms /ml of semen was the normal reference value)

The semen was diluted 1:20 with semen diluting fluid (5% sodium bicarbonate and phenol) and counted the sperm cells with Improved Neubauer counting chamber following the way of counting of White blood cells.

$$\text{Number of sperm/ml} = \frac{n \times 10 \times 20 \times 1000}{4}$$

n = number of sperm counted in all four corner squares

- Finding of sperm morphology (over 30% of normal sperms was considered as a normal situation)

Semen (10.00 µl) was placed on a clean slide and made a thin smear and the smear was air dried. The dried smear was stained with Leishman stain and observed for sperm morphology under the high power field of the microscope. Sperms with characteristic head and tail (tad pole shape) were considered as normal sperms. The value of 30% or more of normal sperm cells was considered the normal value of seminal morphology.

- The counting of pus cells in the semen with Giemsa staining

The number of pus cells in the semen sample was also counted roughly in the occasion of sperm morphology testing and as a confirmatory step the smears of semen from each individual were separately stained with Giemsa staining as well [15]. Thus, the double checking improved the sensitivity as well as accuracy of the outcome.

Table 1: Statistical comparison of the seminal parameters of the test group 1 (pyospermia with 5-10/HPF of pus cells) and control

Semen parameter	Test group 1 (the pyospermic group, n = 25)	Control group (the non pyospermic group, n = 40)	P value (Wilcoxon signed rank)
Volume (ml)	2.30 ± 00.40	2.04 ± 00.43	P > 0.05
Count (10^6cells/ml)	65.33 ± 11.03	69.58 ± 13.10	P > 0.05
Morphology (normal form %)	35.00 ± 8.60	44.33 ± 8.80	P > 0.05
Motility (%)	51.20 ± 12.18	61.66 ± 12.74	P > 0.05

Table 2: Linear regression analysis of the number of pus cells against each seminal parameter for the test group 1

Variables	R value	R square	P value
Number of pus cells, Semen volume	0.020	0.001	P > 0.05
Number of pus cells, Semen count	0.092	0.009	P > 0.05
Number of pus cells, Sperm motility	0.216	0.047	P > 0.05
Number of pus cells, Sperm morphology	0.320	0.146	P > 0.05

Table 3: The statistical comparison of the seminal parameters of test group 2 and control

Semen parameter	Test group 2 (pyospermia with pus cells over 10/HPF, n = 15)	Control group (the non pyospermic group, n = 40)	P value (Wilcoxon signed rank)
Volume (ml)	2.29 ± 00.51	2.04 ± 00.43	P > 0.05
Count (10^6cells/ml)	34.33 ± 9.13	69.58 ± 13.10	P < 0.05, (P = 0.006)
Morphology (Normal form %)	29.00 ± 6.60	44.33 ± 8.80	P < 0.05, (P = 0.001)
Motility (%)	40.20 ± 10.38	61.66 ± 12.74	P < 0.05, (P = 0.004)

Table 4: Linear regression analysis of the number of pus cells against each seminal parameters for the group 2

Variables	R value	R square	P value
Number of pus cells, Semen volume	0.132	0.017	P > 0.05
Number of pus cells, Semen count	0.633	0.400	P < 0.05 (P = 0.002)
Number of pus cells, Sperm motility	0.845	0.714	P < 0.05 (P = 0.001)
Number of pus cells, Sperm morphology	0.900	0.896	P < 0.05 (P = 0.001)

Result and Discussion

The average seminal parameters such as semen volume, count, motility and morphology of the pyospermic group (pus cells 5-10/HPF-test group 1) was compared with that of the control group under Wilcoxon signed rank test. In addition, the average seminal parameters of the individuals with pus cells over 10/HPF (test group 2) were also compared with the control group. This was carried out to detect the effect of severity of pyospermia on seminal parameters. Upon the result (Table 1), it was obvious that the seminal volume was independent of pyospermia with pus cells 5-10/HPF.

The average seminal volume of the test group 1 (2.30 ± 00.40 ml) was higher than that of the control group (2.04 ± 00.43 ml). The outcome implies that the pyospermia with pus cells 5-10 HPF has no significant effect on the seminal volume. Further, the values of both groups were within the normal reference range of above 2 ml as well. The result of the linear regression analysis (Table 2) had also proved that there was no relationship between the two variables (R = 0.003).

The same result was seen in the test group 2 (pyospermia with over 10 cells/HPF) where the semen volume was independent of severity of pyospermia. No statistically significant difference was found between the average seminal count of test group 1 and control group (Table 1). Anyway, though there was a statically significant difference was found between the average count of test group 2 and control group (Table 3), the difference cannot be considered as a true different as the both values were within the normal reference range of sperm count. Thus, it can be mentioned that the pyospermia had no effect on seminal count according to the test. The decision was proved even with the linear regression analysis (Table 2 and 4). The outcome was completely different regarding the seminal motility and morphology.

In the comparison of average motility and morphology of group 1 with that of the control group (Table 1) it was obvious that there was no statistically significant difference. However, in the statistical comparison of average motility and morphology levels of group 2 with that of the control group, it was found to have a statistically significant reduction of them in the test group (group 2). Thus, it can be implied that the seminal motility and morphology is dependent (negatively) on pyospermia with pus cells over 10/HPF.

The relationship was proved even with the linear regression analysis (Table 2 and 4). In the study of Sudan [16], which was carried to find the relationship of bacteriospermia and pyospermia, the investigators had considered pyospermia as the occurrence of pus cells more than 10/HPF of semen instead of more than 5/HPF. This could be due to the prior acknowledgement of the investigators that the pus cells has an effect only when they are found in high numbers in semen. Anyway the pre acknowledgment of them had been true,

according to the current study. In the Pakistan study of Sultan et al [17] to find the number of average pus cells in each group with teratozoospermia, oligozoospermia, asthenozoospermia and normospermia had found that the pus cells in the teratozoospermic group (7.43 ± 0.43) were higher than that of the other groups (around 4 cells in each). Thus, according to the outcome of the study, the higher number of pus cells could cause the morphological change of the spermatozoa (teratozoospermia) in the group.

This had been proved even in the present study also, where the high pus cell loads had showed a significant negative effect on the seminal morphology. Keith Jarvi in Mount Sinai Hospital, Toronto had expressed that the presence of significant numbers of white blood cells in the semen was correlated with poorer sperm parameters and diminished fertility. Further, he mentioned that it did not prove yet that the changes in seminal parameters were exactly due to the pyopsermia or the underlining etiology which gave rise to pyospermia.

However, the concept is collapsed with the outcome of the current study, where the etiology of most cases of pyospermia was unknown. Only a few cases of pyospermia had bacterial infections. In the study of Hinyokika *et. al.* [18], pyospermia had been categorized as the occurrence of pus cells greater than or equal to 10/HPF semen [(18)]. Thus, they found 72 pyospermic individuals from 670 infertile males and detected that the pyospemia significantly affect on the motility of spermatozoa.

It was detected that the granulocyte elastase of pyospermic group was comparatively higher in the pyopsermic group. Further, pyospermia can be caused by inflammation of the genitourinary tract [19]. Finally, among the causes of negative effect of high number of pus cells on seminal parameters following facts can be mentioned.

Secretion of high concentration of collagenase, elastase that digests the protein of sperm can alter the morphology/shape of sperms. Further the secretion of high level of free radicles from the pus cells can react with macromolecules of sperms such as lipid, protein and nucleic acid.

Free radicle attacks on mitochondria of spermatozoa can reduce the function of them (ATP synthesis) which ultimately causes low motile sperms. Free radicle attack on sperm membrane components such as lipid, protein, carbohydrate can also cause alteration of morphology [20] and destruction of the cells. Thus, high pus cell can negatively affect on semen morphology and motility.

Conclusion

According to the outcome of the scientific study, it is clear that pyospermia due to over 10 pus cells per HPF can have a negative effect on seminal motility a morphology. Anyway the effect is null with the pyospermia below 10 pus cells /HPF. The higher secretion of sperm unfavorable materials such as collagenase, reactive oxygen species from the higher number of pus cells could be the reason for this fact. The study was carried out from my personnel fund.

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