Scientific Research Publishing

Advances in Reproductive Sciences, 2019, 7, 106-112
https://www.scirp.org/journal/arsci
ISSN Online: 2330-0752
ISSN Print: 2330-0744

Does Leukocytospermia Considered as an Indicator of Infection?

Hashim Howaida

IVF/Reproductive Endocrinology Unit, Obstetrics and Gynaecology Department, Dr Erfan & Bagedo General Hospital, Jeddah, Saudi Arabia

Email: hhashimh@aol.com

How to cite this paper: Howaida, H. (2019) Does Leukocytospermia Considered as an Indicator of Infection? *Advances in Reproductive Sciences*, **7**, 106-112.
https://doi.org/10.4236/arsci.2019.74012

Received: May 16, 2019
Accepted: November 22, 2019
Published: November 25, 2019

Abstract

Background: The acceptable count of Leukocytospermia in semen sample has been defined by the World Health Organization (WHO) as $\leq 1 \times 10^6$ WBC/ml. The presence of more than one million peroxidase positive white blood cells (WBC) per ml is considered abnormal and is labeled as "leukocytospermia" [1]. But, is there any deterioration in the semen parameters in cases where the number of leukocytes is higher than "normal", or is it an indication for infection? **Objective:** To test the significance of the presence of leukocytes in the semen sample. **Design:** A retrospective randomised study. **Materials and Methods:** 6481 semen samples were divided into two groups according to the availability of the leukocytes. Group I includes samples with $\leq 1 \times 10^6$ leukocyte/ml (n = 3948) and group II includes samples with $>1 \times 10^6$ leukocyte/ml (n = 2533). Semen samples were from partners of couples undergoing infertility evaluation. Specimens were collected by masturbation, and subjected to routine semen analysis including count, motility, sperm morphology (assessments were performed according to the Tygerberg strict criteria), number of leukocytes present (identification of Leukocytes was done using peroxidase staining) and Mixed Antiglobin Reaction (MAR) test was done for detection of sperm surface antibodies. All semen samples from group II were sent for culture. **Results:** A comparison in the mean ± standard deviation (mean ± SD) between the two groups showed statistical significant differences in the mean ± SD motility (55.9 ± 27.8 vs 53.4 ± 27.3, $P < 0.001$), mean ± SD progressive motility (19.5 ± 14.7 vs 18.3 ± 14.2, $P < 0.001$) and mean ± SD rapid sperm movement (35.6 ± 54.9 vs 31.4 ± 19.9, $P < 0.001$), which could be mediated by the induction of lipid peroxidation by the Radical Oxygen Species (ROS) where the effect could be directed to nuclear DNA of sperm. All samples sent for culture were microbiologically negative. **Conclusion:** From the results obtained, we support the studies showing that the presence of leukocytes may not be linked to infection. Future studies are

needed to verify the effect of lipoperoxidation process as a result of leukocytes' presence and to determine the cut off number of leukocytes in semen to be considered of importance since the semen parameters were affected significantly.

Keywords

Leukocytospermia, Semen Analysis, Leukocytes

1. Introduction

When a typical semen analysis is performed, it is very difficult to differentiate white blood cells from other types of cells in the semen sample (such as spermatogenic originator cells). A relatively rapid and inexpensive method of differentiating peroxidase positive white blood cells from other round cells in a semen sample makes use of the intrinsic peroxidase activity of these cells. Leuco Screen peroxidase stain (Leuco Screen test, FertiPro, Belgium) is based on this technique and is used to stain white blood cells in semen samples [1].

The acceptable count of leukocytospermia in semen sample has been defined by the World Health Organization (WHO) as $\leq 1 \times 10^6$ WBC/ml. The presence of more than one million peroxidase positive white blood cells (WBC) per ml is considered abnormal and is labeled as "leukocytospermia" [1]. Elevated count of leukocyte is found in the seminal fluid in 20% - 30% of men with infertility, even in the lack of infection in semen [2]. Several studies showed that leukocytospermia could significantly be a cause of male infertility by decreasing sperm motility and forward progression or by increasing the sperm DNA deficiency and hence affecting the fertilizing ability of sperm and possibly the embryo quality [3] [4].

It is well known that semen analysis could be done either using manual scoring of parameters [3] [4] [5] or by using computer-aided semen analysis (CASA) [6] which has been applied widely in andrology laboratories [7]. Although CASA helps in providing accurate information about sperm motility and dynamics [8] [9] [10], but extra care should be taken in samples with low count (<1 million sperm/ml) because computer may count other round cells as sperm especially if they (the cells) have diameters close to the sperm head size, which will affect the total count of sperm and hence the motility and give false results. Because of that, it is always recommended to make a manual reading and scoring of samples with severe oligozoospermia [11] [12] [13].

It was noticed the elevated number of semen samples with high number of WBC's in our patients who were going through infertility evaluation in the National Guard Health Affairs, King Fahad Hospital, Riyadh, Saudi Arabia, where this retrospective study took place in the period from June 2010 to December 2011.

The current study aims to test the significance of the presence of leukocytospermia on the sperm parameters, and detect if it has any relationship with infection.

2. Material and Methods

Six thousand, four hundred and eighty one (6481) semen sample were analyzed in this retrospective study and divided into two groups according to the number of leukocytes available. Group I, samples with ≤1 million/ml leukocytospermia (n = 3948) and Group II, samples with >1 million/ml leukocytospermia (n = 2533). Semen sample were from partners of couples undergoing infertility evaluation. All results were collected from the files in the database system after the approval from the ethical committee. Semen collection was done by masturbation and after an abstinence period of 2 - 5 days, Semen analysis was done according to the WHO 2010 guidelines [14]. Sperm morphology was done according to Kruger Tygerberg strict criteria. Identification of leukocytes was carried out using peroxidase stain (Leuco Screen test, FertiPro, Belgium) [14] and Mixed Antiglobin Reaction (MAR) (FertiPro N.V. 8730 Beernem, Belgium) test was done for detection of any sperm surface antibody. Patients with leukocyte concentration in semen more than one million/ml were sent for culture [15].

3. Statistical Analysis

The data were analyzed and expressed as mean values ± standard deviations using SPSS version 17 program (SPSS, Inc., IBM, Armonk, NY). Unpaired t-test was used in comparisons of numerical parametric data between different groups. The Mann-Whitney test was used in comparison of numerical non-parametric data between groups. Fisher's exact test was used to compare percentages. Spearman correlation test was applied to analyze correlations between different quantitative variables. Values were considered significant when P-values were equal to or less than 0.05.

Semen Analysis

Was done following the WHO 2010 guidelines. After leaving the sample to liquefy (liquefaction time was noted), Sperm concentration was done using the Spermtrack counting chamber (Projects i Services R + D S.L. Cat. Agustin Escardino 9, Edificiio3-Planta 1, 46,980 Paterna, Espana/Spain), sperm morphology was done using the strict Tygerburg criteria after staining using the Sperm MAC staining and counting at least 200 sperm. For the detection of the leukocytes, each sample was stained using the peroxidase staining [16] [17]. Peroxidase positive: were the round cells stained brown/brown-reddish, these are polymorph nuclear white blood cells, where the peroxidase negative: are the round cells stained pink, these are other round cells (e.g. spermatides).

4. Results

A comparison was done between the two groups using the mean ± SD and percentages. P-value of <0.05 was considered significant.

There was a significant difference noticed between group I and group II in the motility 55.9 ± 27.8 vs 53.4 ± 27.3 ($P < 0.001$), normal morphology 15.00 ±

6.99 vs 13.44 ± 9.19 (P < 0.001) and in the progressive motility 39.1 ± 14.7 vs 22.04 ± 8.67 (P < 0.001) respectively. There was also a significant difference in the rapid motility movement between the two groups 35.6 ± 54.9 vs 31.4 ± 19.9 (P < 0.001) which could be explained by the induction of lipid peroxidation by the Radical Oxygen Species (ROS) where the effect could be directed to nuclear DNA of sperm. All samples that were sent for culture were microbiologically negative. Comparison of physical and microscopic semen variables in group I (non-leukocytospermia) and group II (leukocytospermia) group is explained in Table 1. There was no significant difference noticed in the anti-sperm antibody between the two groups 4.54 ± 15.99 vs 4.53 ± 15.24 for the Sperm Mar (IgG) test and 19.96 ± 26.35 vs 17.6 ± 24.37 for the Sperm Mar (IgA) test (Table 2).

5. Discussion

The presence of leukocytes became a regular finding in semen samples of husbands suffering from infertility. This could be an indication of an accessory gland or genitourinary infection [1]-[7], it may also be connected with other factors for example; autoimmune disorders, lifestyle habits such as smoking, obesity or exposure to environmental pollution and defective spermatogenesis [18] [19].

In contrast, the variation in semen parameters in leukocytospermic semen samples compared with the non-leukocytospermic samples may be an expression of oxidative stress due to excessive creation of Reactive Oxygen Species (ROS)

Table 1. Comparison of physical and microscopic semen variables.

Semen variable	Non leukocytospermia group (n = 3948)	Leukocytospermia group (n = 2533)	P-value
Semen volume (ml) Mean ± SD	1 - 8 2.56 ± 1.67	1 - 6.5 2.61 ± 1.40	NS*
Liquefaction time (min) Mean ± SD	20 - 45 28.60 ± 8.10	20 - 60 39.00 ± 12.75	<0.01
Percentages of highly viscous samples	28 (7/25)	60 (15/25)	<0.05
Sperm count (million/ml) Mean ± SD	72.63 ± 85.00	81.90 ± 87.26	NS
Normal sperm morphology* Mean ± SD	15.00 ± 6.99	13.44 ± 9.19	NS
Progressive sperm movement Mean ± SD	39.1 ± 14.7	22.04 ± 8.67	<0.001
Rapid sperm movement Mean ± SD	35.6 ± 54.9	31.4 ± 19.9	<0.001
Total sperm motility Mean ± SD	55.9 ± 27.8	53.4 ± 27.3	<0.001

*NS: Not significant; *Sperm morphology was scored using the strict criteria.

Table 2. Comparison in the anti-sperm antibody testing.

	Non-leukocytospermic (n = 3948)	Leukocytospermic (n = 2533)	P-value
Sperm Mar (IgG) %	4.54 ± 15.99	4.53 ± 15.24	NS
Sperm Mar (IgA) %	19.96 ± 26.35	17.6 ± 24.37	NS

by the leukocytes or possibly due to dysfunction of the genital ducts and glands by an infectious process [20].

In the current study, there was a statistically significant increase in semen liquefaction time in the leukocytospermia group when compared with the non leukocytospermia group. This result supports previous studies finding reporting seminal hyper-viscosity in semen with >1 million/ml leukocytes. Semen hyper-viscosity is mostly explained by gland infection that commonly associates semen samples with high inflammation, and the dysfunction of the sex glands or possibly the male immune system [16] [17] [18] [19] [20].

Possibly, there is a limitation in our study as we wanted to include the IVF/ICSI trials finding for patients of our groups that had IVF/ICSI cycles and to find out the impact of the "high" number of leukocytes on the fertilization, blastocyst formation, pregnancy and abortion. It was not possible because at the time of writing the abstract there were not enough data available for their IVF/ICSI cycles. An opportunity for future study could include further data.

6. Conclusion

In summary, and from the results obtained, we support the studies showing that the presence of leukocytes may not be linked to infection and future studies are needed to verify the effect of lipoperoxidation process as a result of leukocytes presence and to determine the cut off number of leukocytes in semen to be considered of significance. Also, further studies are required to obtain more information about the association of the presence of leukocytes in semen samples with the IVF/ICSI pregnancy and abortion.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] World Health Organization (2010) WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th Edition, Cambridge University Press, Cambridge.

[2] Gambera, L., Serafini, F., Morgante, G., Focarelli, R., De Leo, V. and Piomboni, P. (2007) Sperm Quality and Pregnancy Rate after COX-2 Inhibitor Therapy of Infertile Males with Abacterial Leukocytospermia. *Human Reproduction*, **22**, 1047-1051. https://doi.org/10.1093/humrep/del490

[3] Arata de Bellabarba, G., Tortolero, I., Villarroel, V., Molina, C.Z., Bellabarba, C. and Velazquez, E. (2000) Non Sperm Cells in Human Semen and Their Relationship with Semen Parameters. *Archives of Andrology*, **45**, 131-136. https://doi.org/10.1080/01485010050193896

[4] Fariello, R.M., Del Giudice, P.T., Spaine, D.M., Fraietta, R., Bertolla, R.P. and Cedenho, A.P. (2009) Effect of Leukocytospermia and Processing by Discontinuous Density Gradient on Sperm Nuclear DNA Fragmentation and Mitochondrial Activity. *Journal of Assisted Reproduction and Genetics*, **26**, 151-157.

https://doi.org/10.1007/s10815-008-9288-0

[5] Domes, T., Lo, K.C., Grober, E.D., Mullen, J.B., Mazzulli, T. and Jarvi, K. (2012) The Incidence and Effect of Bacteriospermia and Elevated Seminal Leukocytes on Semen Parameters. *Fertility and Sterility*, **97**, 1050-1055. https://doi.org/10.1016/j.fertnstert.2012.01.124

[6] Depuydt, C.E., Bosmans, E., Zalata, A., Schoonjans, F. and Comhaire, F.H. (1996) The Relation between Reactive Oxygen Species and Cytokines in Andrological Patients with or without Male Accessory Gland Infection. *Journal of Andrology*, **17**, 699-707.

[7] Lu, J.C., Huang, Y.F. and Lü, N.Q. (2014) Computer-Aided Sperm Analysis: Past, Present and Future. *Andrologia*, **46**, 329-338. https://doi.org/10.1111/and.12093

[8] Lammers, J., Splingart, C., Barriere, P., Jean, M. and Fréour, T. (2014) Double-Blind Prospective Study Comparing Two Automated Sperm Analyzers versus Manual Semen Assessment. *Journal of Assisted Reproduction and Genetics*, **31**, 35-43. https://doi.org/10.1007/s10815-013-0139-2

[9] Zini, A. (2011) Are Sperm Chromatin and DNA Defects Relevant in the Clinic? *Systems Biology in Reproductive Medicine*, **57**, 78-85. https://doi.org/10.3109/19396368.2010.515704

[10] Zini, A., Kamal, K., Phang, D., Willis, J. and Jarvi, K. (2001) Biologic Variability of Sperm DNA Denaturation in Infertile Men. *Urology*, **58**, 258-261. https://doi.org/10.1016/S0090-4295(01)01180-3

[11] Mohammed, E.M., Mosad, E., Zahran, A.M., *et al.* (2015) Acridine Orange and Flow Cytometry: Which Is Better to Measure the Effect of Varicocele on Sperm DNA Integrity? *Advances in Urology*, **2015**, Article ID: 814150. https://doi.org/10.1155/2015/814150

[12] Egozcue, S., Blanco, J., Vendrell, J.M., *et al.* (2000) Human Male Infertility: Chromosome Anomalies, Meiotic Disorders, Abnormal Spermatozoa and Recurrent Abortion. *Human Reproduction Update*, **6**, 93-105. https://doi.org/10.1093/humupd/6.1.93

[13] Sarrate, Z., Blanco, J., Anton, E., Egozcue, S., Egozcue, J. and Vidal, F. (2005) FISH Studies of Chromosome Abnormalities in Germ Cells and Its Relevance in Reproductive Counseling. *Asian Journal of Andrology*, **7**, 227-236. https://doi.org/10.1111/j.1745-7262.2005.00061.x

[14] World Health Organization (1999) WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th Edition, Cambridge University Press, Cambridge.

[15] Politch, J.A., Wolff, H., Hill, J.A. and Anderson, D.J. (1993) Comparison of Methods to Enumerate White Blood Cells in Semen. *Fertility and Sterility*, **60**, 372-375. https://doi.org/10.1016/S0015-0282(16)56116-0

[16] Hoshi, K., Katayose, H., Yanagida, K., Kimura, Y. and Sato, A. (1996) The Relationship between Acridine Orange Fluorescence of Sperm Nuclei and the Fertilizing Ability of Human Sperm. *Fertility and Sterility*, **66**, 634-639. https://doi.org/10.1016/S0015-0282(16)58581-1

[17] Muriel, L., Goyanes, V., Segrelles, E., Gosálvez, J., Alvarez, J.G. and Fernández, J.L. (2007) Increased Aneuploidy Rate in Sperm with Fragmented DNA as Determined by the Sperm Chromatin Dispersion (SCD) Test and FISH Analysis. *Journal of Andrology*, **28**, 38-49. https://doi.org/10.2164/jandrol.106.000067

[18] Jarvi, K. and Noss, M.B. (1994) Pyospermia and Male Infertility. *The Canadian Journal of Urology*, **1**, 25-30.

[19] Singer, G. and Granger, D.N. (2007) Inflammatory Responses Underlying the Microvascular Dysfunction Associated with Obesity and Insulin Resistance. *Microcirculation*, **14**, 375-387. https://doi.org/10.1080/10739680701283158

[20] La Vignera, S., Vicari, E., Condorelli, R.A., D'Agata, R. and Calogero, A.E. (2011) Male Accessory Gland Infection and Sperm Parameters (Review). *International Journal of Andrology*, **34**, 330-347. https://doi.org/10.1111/j.1365-2605.2011.01200.x