# Assessment of Sperm DNA Fragmentation for Patients Suffering from Varicocele

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## Abstract:

### Background:

Varicocele is an abnormal dilation of the testicular veins in the pampiniform plexus and characterized by retrograde blood flow in the internal spermatic veins due to incompetent or absent valves. It is considered one of the main causes of male factor infertility. Sperm DNA integrity is essential for *in vivo* and *in vitro* fertilization, and varicocele causes a progressive decrease in sperm quality .

### Objective:

To study the impact of varicocele on semen quality and sperm DNA integrity.

### Patients, Materials and Methods:

Twenty patients with varicocele and 20 men without varicocele were assigned into two groups in the Out-Patient Clinic of Urology in Al-Kadhimya Teaching Hospital . Semen was collected from both groups and the rate of sperm DNA fragmentation assessed by the "Comet assay" and categorized to 4 classes. Class I (no DNA fragmentation), class II (little DNA fragmentation), class III (meaningful DNA fragmentation), and class IV (high DNA fragmentation) .

**Results:** No statistically significant differences were found between the varicocele and control groups with respect to age. Men with varicocele had significantly higher ejaculate volume, lower sperm concentration, and lower progressive motility, and showed more abnormal morphology than men in the control group. The Varicocele group showed a lower percentage of sperm with little DNA fragmentation (class II) and a higher percentage of sperm with DNA fragmentation (class IV), than the control group.

### Conclusions:

Compared with men without varicocele, men with varicocele had a higher percentage of cells with DNA fragmentation .

**Key words:** Varicocele ; infertility; sperm DNA damage; Comet assay.

## Introduction:

Varicocele is an abnormal dilation of the testicular veins in the pampiniform plexus and characterized by retrograde blood flow in the internal spermatic veins due to incompetent or absent valves [1]. It is considered one of the main causes of male factor infertility affecting 15%–25% of the adult male population, including 35% of men with primary infertility and up to 80% of men with secondary infertility [2].Patients with varicocele have altered spermatogenesis, which has been attributed to several factors, including reflux of renal/adrenal toxic metabolites, testicular hypoxia due to venous stasis, hormonal dysfunction, hypertension in the internal spermatic veins, and increase in testicular temperature [3]. In addition, the semen of these patients has high levels of oxidative stress, as evidenced by increased levels of reactive oxygen species (ROS) and reduced total antioxidant capacity [2, 4]. Sperm DNA integrity is essential for the accurate transmission of genetic code [2], and it is considered an indicator of integrity of spermatogenesis and male fertility potential. About 10% of spermatozoa from fertile men and 20%–25% of spermatozoa from infertile men possess measurable levels of DNA damage [5].

In men with varicocele, sperm DNA damage is correlated with an abnormal retention of sperm cytoplasmic droplets in the mid piece region of the spermatozoa. These sperm cytoplasmic droplets are associated with high levels of ROS[5,6]. During spermatogenesis, sperm chromatin is deeply rearranged, and DNA -linked histones are replaced by protamines. Errors in this process may render DNA more susceptible to damage and, especially, to the action of ROS[7,8]. Varicocele leads to venous stasis of the testis, which causes heat stress, hypoxia, and accumulation of toxic metabolites. In the hypoxia stage, the production of ROS is increased, and, without blood renewal, the concentration of antioxidant enzymes is reduced in the testis. The lack of balance between ROS production and antioxidant protection leads to oxidative stress. Thus, overproduction of ROS causes lipid peroxidation, which is responsible for many deleterious events that affect sperm chromatin and induce frequent DNA strand breaks [2, 4, 9] . Sperm functional integrity is essential for *in vivo* and *in vitro* fertilization[10], and

varicocele causes a progressive decrease in sperm quality[11]. Thus, the purpose of this study was to assess the impact of varicocele on semen quality and sperm DNA integrity.

## Patients, Materials and Methods:

A total of 40 males aged 20-40 years were included in this prospective study which was conducted in Al-Kadhmyia Teaching Hospital during the period between July 2010 and December 2011.

Twenty males with varicocele were assigned in the (varicocele group). The other 20 males, without varicocele were assigned in the (control group). Male with evidence of urogenital infection, fever occurring within 90 days before semen analysis, Leukocytospermia, systemic diseases such as cancer, endocrinopathies, or history of smoking were excluded from the study. Varicocele diagnosis was performed by physical examination of the scrotum in a room temperature clinic. The varicocele group consist of 4 men with grade I left varicocele; 8 men with grade II left varicocele and 8 men with grade III left varicocele. Semen samples were obtained by masturbation after 2-4 days of ejaculatory abstinence and were analyzed within 1 hour of collection at The High Institute for Infertility

diagnosis and Assisted Reproductive Technologies. After semen liquefaction, seminal analysis was performed according to World Health Organization laboratory manual.[12] Sperm nuclear DNA integrity was evaluated by a modified alkaline single-cell gel electrophoresis, or Comet assay, adapted from Donnelly et al [13].Damaged DNA migrates during electrophoresis from the nucleus toward the anode , forming the shape of comet with a head (cell nucleus with intact DNA) and a tail (relaxed and broken DNA). Using submarine Electrophoresis system, (Mupid-exu , Japan).DNA damage as assessed by comet tail and nuclear intensity and visually classified as class I (high DNA integrity) to class IV (high DNA fragmentation) . Class I cells showed a nucleus with intense fluorescence and did not show a comet tail. Class II cells still presented an evident nucleus but also a comet tail. Class III cells presented a weak nucleus and a strong tail, and class IV cells did not present a nucleus, only a comet tail (Figure 1)

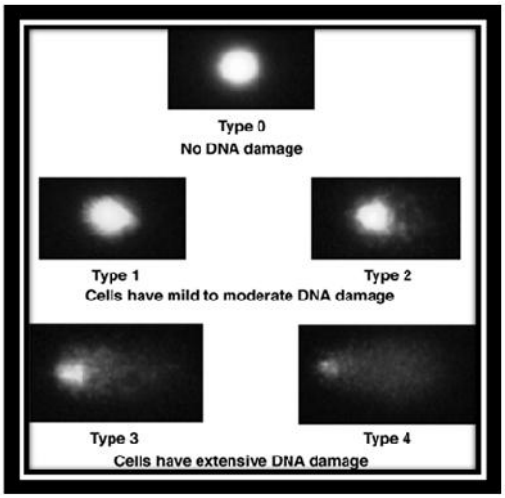


Figure 1 DNA fragmentation using comet assay [14]

## Statistical Analysis:

Data are reported as mean ± SD. The comparison between both groups were tested by student t-test using statistical packages for social sciences (SPSS) version(14). Correlation between two outcomes were evaluated using Pearson correlation coefficients. P-value<0.05 was considered statistically significant.

## Results:

In this study the mean age was (34.8) year in the control group and (36.2) year in the varicocele group. The mean semen volume in the control group was (2.6) ml, and (3.8) ml in the varicocele group which is statistically significant (p=0.016) The mean sperm concentration was ($135.5x10^6$) & ($62.8x10^6$) for the control group and varicocele group respectively which is also statistically significant (p=0.005). The mean progressive motility was (60.2%) for the control group and (46.5%) for the varicocele group which is statistically significant (p=0.035). The mean value for non-progressive & immotile sperms was (35.4%) in the control group and (49.8%) for the varicocele group which is a significant value (p = 0.014).
The mean normal sperm morphology was (35.4%) in the control group and (15.2%) in the varicocele group which is statistically significant (p = 0.001).
No statistically significant difference was found between the varicocele and control group with respect to age.

Table -1 summarizes the results of this study.

**Table - 1. Comparison of the results of semen analysis between the varicocele and control group.**

| Parameters | Control group (20 patients) Mean ± SD | Varicocele group (20 patients) Mean ± SD | P-*value* |
|---|---|---|---|
| Age, years | 34.8 ± 3.6 | 36.2 ± 3.2 | 0.210 |
| Abstinence, days | 3.3 ± 3.3 | 3.6 ± 2.6 | 0.455 |
| Volume, mL | 2.6 ± 0.8 | 3.8 ± 2.0 | 0.016 |
| Sperm concentration, $\times 10^6$/mL | 133.5 ± 81.5 | 62.8 ± 58.5 | 0.005 |
| Progressive sperm motility, a+b, % | 60.2 ± 8.7 | 46.5 ± 21.9 | 0.035 |
| Non progressive & Immotile sperm, c,d, % | 35.4 ± 9.0 | 49.8 ± 21.8 | 0.014 |
| Normal Sperm morphology, % | 35.4 ± 3.4 | 15.2 ± 3.1 | 0.001 |

Comparison of Comet assay results between the control group and varicocele group are shown in Table - 2. The varicocele group presented a significantly lower percentage of sperm with intact DNA (class II) and a higher percentage of sperm nuclear DNA fragmentation (class IV).

**Table 2. Comparison of Comet assay between varicocele and control group**

| Class | Control group (mean ± SD) | Varicocele group (mean ± SD) | P-value |
|---|---|---|---|
| Comet class I | 23.5 ± 16.5 | 32.5 ± 17.3 | 0.117 |
| Comet class II | 62.8 ± 17.3 | 48.7 ± 13.6 | 0.011 |
| Comet class III | 10.8 ± 5.5 | 12.5 ± 7.7 | 0.455 |
| Comet class IV | 2.9 ± 2.0 | 6.3 ± 4.9 | 0.018 |

## Discussion:

Despite the high frequency of men with varicocele within the infertile population, the exact mechanism by which varicocele negatively affects sperm function has not yet been resolved [15].

In the present study, we observed that men with varicocele exhibited an increase in ejaculate volume and a reduction in normal sperm morphology, progressive motility, and concentration. These findings corroborate literature data that show reduced semen quality associated with varicocele [16,17]. Moreover, we demonstrated that sperm from men with varicocele showed higher levels of DNA fragmentation than sperm from the control group. In the varicocele group, there were more cells with highly fragmented DNA (class IV), while in the control group there were more cells with low or no DNA fragmentation

Three possible mechanisms that might lead to DNA fragmentation. These are abortive apoptosis, abnormal chromatin packaging, and oxidative stress [18]. In mammalian testes, germ cells expand clonally through many rounds of mitosis before undergoing meiosis and the differentiation steps that result in mature spermatozoa. This clonal expansion is excessive, requiring that a mechanism exist—apoptosis—to match the number of germ cells with the supportive capacity of Sertoli cells [19]. As much as 25%–75% of testicular germ cells are lost because of apoptosis during normal spermatogenesis [20]. Therefore, in testes, apoptosis is responsible for populational control and germ cell selection[19]. However, in some cases of testes with hypospermatogenesis, testicular germ cell apoptosis is initiated but several cells may escape this process and continue the maturation, contributing to poor sperm quality and ejaculation of sperm with apoptotic traits, such as externalized phosphatidyl serine and nuclear DNA fragmentation [18,21].

Since varicocele induces an increase in ROS production both in the testicle and in seminal plasma [2, 22], it is likely that the sperm DNA fragmentation observed in men with varicocele is caused by oxidative stress, which is derived from ROS overproduction.

In this study, we observed alterations in one functional aspects of spermatozoa, probably due to the same mechanism—oxidative stress. It is important to verify whether a decrease in ROS concentration or an increase in antioxidative levels, both in testicle and in seminal plasma, could improve these functional aspects.

## Conclusions

Compared with men without varicocele, men with varicocele had a higher percentage of cells with DNA fragmentation . Indeed, varicocele causes a decrease in motility, concentration and sperm normal morphology . The sperm DNA fragmentation could be an important factor in deciding treatment options for men with varicocele.

## References


1. Gat Y, Bachar G N, Zukerman Z, Belenky A and Gornish M. Varicocele: a bilateral disease, Fertil Steril 2004;81: 424–429.
2. Smith R, Kaune H, Parodi D, Madariaga M. et al. Increased sperm DNA damage in patients with varicocele: relationship with seminal oxidative stress, Hum Reprod 2006;21: 986–993.
3. Naughton C. K, Nangia A. K and Agarwal A. Varicocele and male infertility. Part II. Pathophysiology of varicoceles in male infertility, Hum Reprod Update 2001;7: 473–481.
4. Hendin B, Kolettis P, Sharma R.K, Thomas A. J Jr. and Agarwal A. Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity, J Urol 1999;161: 1831–1834.
5. O'Brien J and Zini A. Sperm DNA integrity and male infertility: a review, Urology 2005;65: 16–22.
6. Fisher M.A, Willis J and Zini A. Human sperm DNA integrity: correlation with sperm cytoplasmic droplets, Urology 2003; 61: pp. 207–211.
7. Sakkas D, Moffatt O, Manicardi G.C, Mariethoz E, Tarozzi N and Bizzaro D. Nature of DNA damage in ejaculated human spermatozoa and the possible involvement of apoptosis, Biol Reprod 2002;66: 1061–1067.
8. Gardiner-Garden M, Ballesteros M, Gordon M and Tam P.P. Histone- and protamine- DNA association: conservation of different patterns within the beta-globin domain in human sperm, Mol Cell Biol 1998; 18: 3350–3356.
9. Aitken R. J and Krausz C. Oxidative stress, DNA damage and Y chromosome, Reproduction 2001;122: 497–506.
10. Amann R.P. Can the fertility potential of a seminal sample be predicted accurately. J Androl 1989;10: 89–98.
11. Zini A, Blumenfeld A, Libman J and Willis J. Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity, Hum Reprod 2005;20: 1018–1021.
12. World Health Organization, Laboratory manual for the examination of human semen and sperm-cervical mucus interaction (4th ed.), Cambridge University Press, Cambridge (1999).
13. Donnelly E.T, O'Connell M, Mcclure N and Lewis S.E. Differences in nuclear DNA fragmentation and human mitochondrial integrity of semen and prepared spermatozoa, Hum Reprod 2000;15: 1552–1561.
14. Blumer CG, Fariello RM, Restelli AE, et al. Sperm nuclear DNA fragmentation and mitochondrial activity in men with varicocele.

Fertil Steril, 2008; 90(5): 1716-1722.
15. Marijsmit, Johannes C. Romijn, Mark F. Wildhagen, Joke L. M. Veldhoven, Robertus F.A. Decreased Sperm DNA Fragmentation After Surgical Varicocelectomy Associated With Increased Pregnancy Rate, Jurol 2013; 189: 146-150.
16. World Health Organization, The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. Fertil Steril 1992;57: 1289–1293.
17. MacLeod J, Seminal cytology in the presence of varicocele, Fertil Steril 1995;16: 735–757.
18. Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi P.G and Bianchi U. Origin of DNA damage in ejaculated human spermatozoa. Rev Reprod 1999;4: 31–37.
19. Furuchi T, Masuko K, Nishimune Y, Obinata M and Matsui Y. Inhibition of testicular germ cell apoptosis and differentiation in mice misexpressing Bcl-2 in spermatogonia. Development 1996;122: 1703–1709.
20. Fujisawa M, Hiramine T and Kamidono S. Decrease of apoptosis of germ cells in the testes of infertile men with varicocele, World J Urol 1999;17: 296–300.
21. Said T.M, Paasch U, Glander H. J and Agarwal A. Role of caspases in male infertility, Hum Reprod Update 2004;10: 39–51.
22. Turkyilmaz Z, Gulen S, Sonmez K, Karabulut R, et al. Increased nitric oxide is accompanied by lipid oxidation in adolescent varicocele. Int J Androl 2004;27: 183–187.