SPERM DNA DAMAGE RATE AMONG INFERTILE PATIENTS WITH VARICOCELE

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ABSTRACT

Background: Varicocele has a common association with male hypofertility. The prevalence of clinical varicocele is about 15% among adults and adolescents. Varicocele patients are at risk of infertility. Sperm deoxyribonucleic acid (DNA) fragmentation rate (SDFR) is considered a major factor in decreased fertilization ability in males with varicocele. Varicocele increases SDFR through heat stress, increased reactive oxygen species, and reduction in the level of total antioxidants.

Objectives: To study the rate of sperm DNA fragmentation in patients with different grades of varicocele.

Patients and methods: A prospective, case-control study was conducted at Azadi Teaching hospital in Duhok city / Iraq from March 2020 to December 2021. The study included 34 infertile patients with varicocele of different grades and 30 infertile patients without varicocele. Seminal fluid analysis was performed followed by sperm DNA analysis by Alkaline Comet Assay. The obtained data were analyzed using a Prism-GraphPad to compare the mean of parameters in patients and control subjects.

Results: Sixty-four infertile patients were included. The mean of sperm immobility and abnormal morphology rates were statistically higher in infertile patients with varicocele compared with those without varicocele (39.56±12.63 Vs 28.07±4.541) and (51.59±23.00 Vs 34.79±15.72) respectively, (P<0.0001). The fluorescence microscopic images of sperm DNA of infertile patients with varicocele showed a clear migration to DNA tail (DNA damage) compared with sperm DNA of those without varicocele (8.195 ± 0.3799 Vs. 4.794 ± 0.2186) respectively (P<0.0001). The degree of sperm DNA damage was directly related to the degree of varicocele and was significant (P<0.0001).

Conclusion: Sperm DNA damage rate was higher in infertile male patients with varicocele in comparison to those without varicocele and directly related to the grade of varicocele.

Keywords: Infertility, SDI, Sperm DNA, Varicocele

Infertility is regarded as a global public health issue as it affects around 15% of young age-group1. Up to 50% of infertile couples are related to male factor2. Unexplained infertility is commonly observed in males and is characterized by normal spermograms and no identifiable cause3. Varicocele is one of the associations with infertility in males. Reports about testicular varicocele go back to the Greek age4. The effect of testis varicocele was first noticed at the beginning of the 20th century after improvement in the sperm quality following varicocelectomy4. Varicocele is categorized clinically as grade 1: the
venous plexus of the spermatic cord is only palpable during the Valsalva maneuver, grade 2: the veins are palpable at the upright position, and grade 3: the veins are visible. Sub-clinical varicocele is diagnosed by ultrasound or angiography only.\(^5,\!^6\)

Fertile sperms should have stable DNA which is a crucial factor for successful fertilization and embryo development and growth in natural and assisted reproductive techniques. The integrity of our DNA is continuously challenged by many known and unknown endogenous and exogenous factors (some are preventable or correctable while others are not).\(^7\)

The DNA damage types include 1-single-strand breaks (SSBs), 2-double-strand breaks (DSBs) 3-nitrogen base mismatch, 3-loss of DNA base (abasic site), 4-(DNA) base modification, and 5- cross-link and pyrimidine dimer.\(^8\)

The type and degree of DNA damage or fragmentation depend on cell type and cell cycle stage. The cell has several ways to repair the already damaged (DNA), and inaccurate repair may have different consequences. While our somatic cells inevitably die by age or disease, the germ cell lines can maintain sufficient DNA integrity to pass on our genome to the next generations.

Damage to the DNA is caused by many endogenous and exogenous factors, intratesticular through defective apoptosis or epididymal through excessive reactive oxygen species (ROS) production.\(^9,\!^11\)

Varicocele, high scrotal temperature, drugs, xenobiotics, smoking, pollution, and aging have been associated with increased sperm DNA damage rates.\(^12,\!^13\)

Venous stasis due to varicocele in testis leads to hypoxia, heat stress, and accumulation of metabolic waste; all of these will lead to an increase in ROS production in seminal fluid.\(^14\) In addition, venous stasis prevents blood renewal which in turn reduces the delivery of antioxidants to the affected part of the testis. An imbalance between antioxidants and ROS production leads to oxidative stress that can damage the sperm DNA.\(^15\)

Poor assisted reproductive outcomes such as reduction in fertilization rate and high rates of spontaneous miscarriage are related to sperm DNA integrity. Most studies depend on routine seminal fluid analysis for the evaluation of varicocele-related infertility.\(^10,\!^16\)

Many different techniques are available to measure the rate of sperm DNA damage levels with different sensitivity and specificity. These techniques include sperm chromatin structure assay (SCSA), sperm chromatin dispersion test (SCD), terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay, and single-cell gel electrophoresis (Alkaline comet Assay) assay.\(^17,\!^19\) The TUNEL assay and the ACA are assessing DNA damage directly and their accuracy for detection of even minimum level of sperm DNA damage is high, whereas other techniques are prone to artifacts due to the effect of acid solution.\(^20\)

Although the technique of ACA needs more care for its standardization and optimization for the measurement of sperm DNA damage, it is characterized by its high sensitivity and reliability for the detection of even low levels of DNA damage in sperms.\(^21\)

**STUDY DESIGN & METHODS**

This study included 64 participants. Thirty-four infertile patients with different severity of varicocele and 30 infertile patients without varicocele as a control.
group. Classifications of varicocele grades according to vein diameter were categorized into three grades by color Doppler ultrasound. Any patient with a history of radiation, chemotherapy, or local surgery was excluded from the study. After filling out the questionnaire form, spermograms were performed and analyzed according to World Health Organisation (WHO) after three days of abstinence. The sample was then labelled and kept in the freeze (-20 c) for later sperm DNA damage measurement by Alkaline Comet Assay.

Statistical analysis

The data and participant information were analyzed using Prism-GraphPad version 5. P value $\leq 0.05$ are statistically significant. Continuous variables are expressed by mean $\pm$ standard deviation. Categorical data were summarized as percentages.

RESULTS

From the total study group of 64 infertile patients, the age of the patients ranges between 20 and 45 years, and the duration of infertility was between 1 to 5 years, BMI was between 18 to 30. Results of age, BMI, and seminal fluid parameters including sperm morphology, motility, and others parameter were analyzed and shown in Table 1. The mean of age between patients with varicocele and those without varicocele was not significant statistically; (31.71±0.7711 vs. 34.43±1.413) (p 0.2418) respectively. No statistically significant difference was found between the mean BMI in patients with varicocele and those without varicocele; (25.76±1.038 vs. 26.04±1.212) (P 0.1369) respectively. There was no difference statistically in the family history of infertility between patients with or without varicocele; (3.147±0.239 vs. 3.759 ±0.485) (P 0.2413) respectively. The mean of low motility and abnormal morphology rates were significantly higher in patients with varicocele compared with those without varicocele. The mean abnormal morphology rate in patients with varicocele was (39.56±12.63) while in those without varicocele was (28.07±4.541) (p 0.0001). Similarly, the mean rate of immotile sperms in patients with varicocele was significantly higher than in those without varicocele; (51.59±23.00 Vs. 34.79±15.72) respectively (P 0.0001). Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>With Varicocele (n 34)</th>
<th>Without Varicocele (n 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.71±0.7711</td>
<td>34.43±1.413</td>
<td>0.2418</td>
</tr>
<tr>
<td>BMI</td>
<td>25.76±1.038</td>
<td>26.04±1.212</td>
<td>0.1369</td>
</tr>
<tr>
<td>History of infertility (years)</td>
<td>3.147±0.239</td>
<td>3.759±0.485</td>
<td>0.2413</td>
</tr>
<tr>
<td>Abnormal morphology (%)</td>
<td>39.56±12.63</td>
<td>28.07±4.541</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Motility:Immotile (%)</td>
<td>51.59±23.00</td>
<td>34.79±15.72</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

The fluorescence microscopic images of sperm (DNA) of infertile patients with varicocele showed a clear and more DNA migration to the tail (DNA damage), compared with sperm DNA quality of those without varicocele (Figure-1).
SPERM DNA DAMAGE RATE AMONG INFERTILE PATIENTS

Figure 1 A fluorescence microscopic image of sperm DNA of the infertile patient with varicocele (A) and without varicocele (B). Magnified picture of intact sperm DNA (C). Magnified picture of damaged sperm DNA (D).

Further analysis of data using CASP Lab software revealed that both groups of infertile patients have SDF, however, the damage rate in patients with varicocele was significantly higher in varicocele patients compared with those without varicocele, 8.195 ± 0.3799 Vs. 4.794 ± 0.2186, respectively (P<0.0001) table-2.

<table>
<thead>
<tr>
<th>Patients</th>
<th>With varicocele</th>
<th>Without Varicocele</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDFR (%)</td>
<td>8.195 ± 0.3799</td>
<td>4.794 ± 0.2186</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

The statistical analysis showed a higher rate of SDF in patients with varicocele with more sperm morphological abnormalities and low sperm motilities in contrast with that in patients without varicocele (Figure-2A & B).

Figure 2 Comparisons between morphological parameters of seminal fluid and the SDF rate in infertile patients without varicocele (A) and with varicocele (B).
The SDF rate with the grades of the varicocele, data showed that patients with grade-1 varicocele have the lowest level of SDF rates compared with those with grade 2 and grade-3 varicocele. The SDF rate in grade-3 patients was 1.3-fold higher than those of grade-2 and 2-fold than those of grade-1 varicocele. Table 3.

**Table 3. The rate of SDF with grades of varicocele**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Sperm DNA damage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9211 ± 4.703</td>
</tr>
<tr>
<td>2</td>
<td>0.8365 ± 7.956</td>
</tr>
<tr>
<td>3</td>
<td>1.001 ± 10.46</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Varicocele is considered an andrological disease with a high incidence (15%) in the general population and is positively associated with reduced male fertility rates\(^{11,22,23}\). The effect of clinical varicocele on the conventional semen quality and male fertility potential is controversial, and whether surgical ablation (varicocelectomy) could improve seminal fluid quality still needs further evaluation and studies to explore. Sperm genomic integrity is important for sperm cell function both in vivo and in vitro fertilization\(^{12}\). Over the past decades, many clinical studies were conducted to demonstrate 1.6% higher SDF rates in infertile patients than in fertile men (95% CI: 1.2-2.1: \(P<0.001\))\(^{24}\).

Despite the high rates of varicocele among infertile cases, the mechanism behind its negative impact on sperm quality and sperm activities is still not very clear\(^{11}\). Based on the studies by World Health Organization (WHO), the relationship between varicocele occurrence and testicular dysfunction is strong. It has been shown that 25% of those with varicocele have alteration in seminal fluid parameters\(^{25}\). Factors associated with varicocele include heat stress, androgen deprivation, exposure to toxic agents and testicular hypoxia lead to an increase in oxidative stress which in turn induces the pathways that lead to increased SDF rates. The seminal plasma normally contains antioxidant agents that protect human gametes from damaging attacks of ROS. Studies have observed that there is a high level of ROS and a reduced amount of total antioxidant in the semen of patients with varicocele\(^{26,27}\). Experimentally, exposing human sperms to exogenous ROS has resulted in increased DNA fragmentation after the exposure\(^{13}\). Although the rate of SDF rate was low in our study, we have observed statistically significant (\(P<0.0001\)) more sperm DNA damage levels in infertile patients with varicocele than in those without varicocele. A recent study done by Finelli et al.,(2021 using a TUNEL assay, have found significant and more levels of SDF in patients with varicocele compared to those without varicocele (20.8% vs 10.1%), \(P<0.01\), respectively\(^{28}\).

Zini and Dohle (2011) in their meta-analysis found that in five studies, the SDF levels were similar in infertile patients with or without having varicocele, while in other four studies higher SDF levels were found in infertile men with varicocele than those without varicocele\(^{29}\). Another meta-analysis study by Zhang et al.,(2021) concluded that patients with clinical varicocele have a higher SDF index than healthy controls\(^{30}\).

Moreover, we have found a direct relation between SDF level and the grades of the varicocele. Patients with grade-1 varicocele have the lowest level of SDF level compared to those with grade-2 and grade-3 varicocele. The differences in the
means of DNA damage in the sperms of patients with grade-1 and grade-2 compared with those of grade-2 and grade-3 varicocele collectively was significant (P<0.0001), a similar result is seen by Jellad et al., (2020) concluded that sperm DFI was directly and significantly related with the severity of varicocele31.

Blumer et al, (2008) used classes of damage to measure the level of genomic damage by Comet Assay in sperm and they found a higher level of SDF patients with advanced grades (Grade 2 and Grade 3) of varicocele compared with that in patients without varicocele; (6.1 ± 4.5 Vs. 5.5 ± 4.6) respectively, P=0.55732. A meta-analysis study performed by Wang et al., (2012) showed that patients with varicocele have significantly higher SDF rates than the control group (P<0.00001) and varicocelectomy improved sperm DNA integrity (P<0.00001)33.

Another meta-analysis performed by Roque et al., (2018) involved 1,153 men with clinical varicocele and SDF measurements. Overall, there was a significant decrease in the rates of SDF after repair of their varicoceles (MD - 8.31%, 95% CI -10.27%, -6.36%; P <0.00001)34.

Varicocele has a huge impact on sperm’s physical activities and morphological characteristics. Previous studies showed high rates of sperm abnormalities among those with varicocele32,35. Blumer et al,(2012) showed no differences in the total motile sperms in patients without varicocele compared to those with varicocele (140.6 ± 160.1 Vs. 99.8 ± 119.0; p 0.262, respectively)36. The data of this study revealed more levels of sperm abnormalities and decreased activities in those with varicocele compared with those without varicocele, which corroborates the findings of other recent studies31,37.

CONCLUSION
The results of this study showed that infertile men with clinical varicocele have a higher rate of SDF than those without varicocele, and the rate of SDF is directly related to the grade of varicocele.

Conflict of interest: No conflict of interest.

REFERENCES


29. Zini A , Dohle G. Are varicoceles associated with increased


پژوهش

بررسی زکارکافنتان

بنیانهای نخوششیت نازوک در بیماران بیماری کروی

پیشنهادی و نمونه‌گیری: قاریسکیل نیبک سیستم زیست‌شناسی گالاکسی با ورش لغت ندوزکی زالاما (نیزه). مشاهده‌گران‌فیالیا

کلینیکی نیز کیک 15% دلی شان و ترکیب. سیستم نخوششیتی کارکنری هافه کو برکارگرفی نخوششی

کو گونیت وی ز کارکنری و پیشنهاد ندوزکی.

زکارکافنتان DNA سپریم و پرت بونا و گروگرین نگه‌داری و فاکته به ندوزکی لنز وان زالاما کو

قاریسکیل همین. هدنه‌ی زکارکافنتان گرند و کم سئرلا گارمانتی و زندونا جوزیت نمکسگی تکارلیکی و کوک

یان دوزموکسیدینا زیک.

نمونه‌گیری: فکولینا یا پری برونا (DNA) سپریمی یان نخوششیتی کارکنری سپریمی د قوناقیت جدا جدا د.

ریک و دیزاین‌کنونی: فکولینا پروتیک‌تی‌کونترول در کروس سیکشنال به هزینه نخوانیان لزانکیا دهه


لک‌کچون‌ی 34 نخوششیتی نازوکیت و قاریسکیل همی و 30 نخوششیت ندوزکی بیماری کروی کارکنری و مکا کارکنری

بی‌خون‌گره. شرکه و شرکت‌نیا شی ناقا زالاما هنیه نخوانیان و همروسا شرفه‌کن‌ری به نین سپیمری زی.

Alkaline Comet Assay

زماره و نخوانیان هاتینه و هم‌مرگن نیهه شروفه‌کنیا بریکارکلی

نخونی: 64 نخوششیتی ندوزکی زیک نخونیلیا سپریمی بارا پیر یا پری برای بون لک نخوششیت ندوزکی کو بیت

تووشقیکارسیل بیون وی بیان ون کیسانیت ندوزکی و نخوششیتی کارکنری نامیت 39.56 ± 56

بیرامربار 28.21 ± 5.45 و هموسا مورفولوژیا نخواردستیا سپیمری 51.56 ± 23.79

P<0.0001

ونیت مایکروکسیا فلورسینی

با سایری‌گنج نخوششیتی ندوزکی کو تووشقیکارسیل بویین دیارک.

فوم زنیزیواریا دیارو زدنال هاته دین لک کوریا

نیز کارکافنتان لک بیاراکان نخوششی

بیماربار 8.195 ± 3.799 ± بیرامربار 4.794 ± 0.2186

یپایند هیپو لگل پرها ایرلیکسیل کو با برجام بو

P<0.0001

در نظریات: پیا زکارکافنتان DNA بی سپریمی بند تربو لک نخوششیت ندوزکیت کو تووشقیکارسیل همه لک

بیاراکان بویین نخوششیت بیماری کارکنری و سیستمیت گرندای ب قوناگیت جدا جدا بیماری کارکنری.
الخلاصة
معدل تلف الحمض النووي للحيوانات المنوية بين مرضى العقم المصابين بدوالي الخصية

الخلفية والأهداف: دوالي الخصية هي أحد الأسباب الرئيسية للعقم عند الذكور. تبلغ نسبة انتشار دوالي الخصية السريرية حوالي 15% من البالغين والمراهقين. مرضى دوالي الخصية عرضون خطر الإصابة بضعف الخصوبة والعقم. يعتبر تلف الحمض النووي للحيوانات المنوية وتفتتتها عاملًا رئيسيًا في العقم عند الذكور المصابين بدوالي الخصية. المراجع المرتبطة بدوالي الخصية تزيد من تلف الحمض النووي للحيوانات المنوية. النتائج والمشاركة الكمية.

الأهداف: دراسة العلاقة بين درجة تلف الحمض النووي للحيوانات المنوية ومعامل الحيوانات المنوية (النشاط والتشكل) في المرضى الذين يعانون من درجات مختلفة من دوالي الخصية.

طرق البحث: أجريت دراسة مقطعيه مقارنة مستقبليه في جامعة دهوك ومستشفى آزاد التعليمي في مدينة دهوك، العراق من آذار 2020 إلى كانون الأول 2021. وشملت الدراسة 34 مريضا يعانون من العقم مع درجات مختلفة من دوالي الخصية و30 مريضا يعانون من العقم بدون دوالي الخصية. تم تحليل السائل المنوي ودراسة نسبة تلف الحمض النووي للحيوانات المنوية بواسطة فحص المذنب القلبي. تم تحليل البيانات التي تم الحصول عليها باستخدام لوحة الرسم المتندوري لمقارنة متوسط المعلمات في المرضى والعينة الضابطة.

النتائج: تم تضمين أربعة وستين مريضا يعانون من العقم. كان متوسط عدم حركة الحيوانات المنوية ومعادلة التشكل غير الطبيعي أعلى بشكل ملحوظ في مرضى العقم الذين يعانون من دوالي الخصية مقارنة مع أولئك الذين لا لديهم دوالي الخصية، (5.63 ± 28.07 مقابل 39.72 ± 4.54) و (23.00 ± 15.72 مقابل 34.79 ± 5.59). على التوالي (P<0.0001) مما يتعلق بمستويات تلف الحمض النووي للحيوانات المنوية، أظهرت الصور المجهرية للخلايا الوراثية للمرضى المصابين بدوالي الخصية تغيرًا واضحاً إلى ذيل الحمض النووي (لف الحمض النووي) مصدر من الحيوانات المنوية لأولئك الذين لا لديهم دوالي الخصية (8.195 ± 0.379 مقابل 4.79 ± 0.218)على التوالي. ترتبط درجة تلف الحمض النووي للحيوانات المنوية (P<0.0001) ارتباطًا مباشرًا بدرجة دوالي الخصية وكانت معنوية.

الاستنتاجات: يكون تلف الحمض النووي للحيوانات المنوية وقلة التشكل والحركة أعلى في مرضى العقم المصابين بدوالي الخصية مقارنة مع من لا يعانون من دوالي الخصية ودرجة تلف الحمض النووي ترتبط مباشرة بدرجة دوالي الخصية.