SPERM DNA CONTENT AND SPERM MOTILITY AMONG SUBFERTILE INDIVIDUALS : A CORRELATION STUDY[+]

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Abstract

The aim of the present study is to determine the sperm DNA content in subfertile persons and correlate it with sperm motility.

A random sample of 64 subfertile males attending the Institute for Embryo Research and Infertility Treatment, University of Baghdad , were enrolled in this study through years 2004-2005 . The patients age ranged 20-45 years and their samples were assessed for sperm motility by direct light microscopy. DNA content (µg / ml) in sperm was estimated using a microchemical spectrophotometric method and data were classified into three groups according to sperm motility percent (>60% , 20-60% ,and <20 %).

The results show that data of percent motility of the three groups were statistically of significant difference (P<0.01) . The sperm DNA content (µg / ml) showed a non significant positive correlation (P>0.05), in the normal and severe asthenospermic groups and negative in the asthenospermic group. No statistically significant difference (P>0.05) of sperm DNA content between the three groups was obtained .It can be concluded that sperm motility is not related to D N A level in subfertile males.

المستخلص:

ان الهدف من هذه الدراسة هو تعيين مستوىحامض الـــ D N A في نطف مرضى ضعيفي الخصوبة وعلاقته مع حركةالنطف في السائل المنوي. ادرج اربعة وستون نموذجا عشوائيا من المرضى الذكور المراجعين لمعهدابحاث الاجنة وعلاج العقم – جامعة بغداد خلال عامي ٢٠٠٤-٢٠٠٥ تراوحت اعمارهم من ٢٠ الى ٤٥سنة. تم تقييم حركة النطف بوساطة المجهر الضوئي كما تم قياس مستوى حامض الـــ D N A (مايكروغرام في المللتر الواحد) بواسطة طريقة طيفية ضوئية كيمياوية دقيقة. صنفت البيانات الى ثلاث مجاميع وحسبت النسبة المئويه لحركه النطاف (> ٦٠ % و ٢٠-٦٠% و<٢٠ %) . اظهرت النتائج ان البيانات للنسب المئوية لحركة نطاف المني في المجاميع الثلاثة كانت بفروقات احصائية معنوية(P <0.01) . لوحظ ان مستوى حامض الـــ D N A مع حركة النطف كان على علاقة موجبة غير معنوية(P > 0.05) في مجموعتي الاسوياء normal وشديدي الضعف severe asthenospermicلحركة النطف وسالبة في مجموعة ضعيفي حركة النطفasthenospermic . لم تظهرفروقات معنوية في مستوى الحامض(P > 0.05) بين المجاميع الثلاثة. يمكن الاستنتاج ان حركة النطف ليس لها علاقة مع مستوى حامض الـــ D N A في الذكور ضعيفي الخصوبة.

[+]Received on 4/3/2006 , Accepted on 2/5/2007 .

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Introduction:

The sperm motility is one of the essential sperm parameters (count, motility and morphology) used to evaluate semen quality in normal and abnormal males .Asthenospermia is a term given to low levels of motility or forward progression or both. It has been caused by many factors [1] and could be managed by activation of spermatozoa using some compounds [2].

The motility of human semen was studied extensively associated with semen quality
function [3,4,5] .Those studies included the effects of cigarette smoking [6],hydroxyl radical and lipid peroxidation[7], oxidative stress and reactive oxygen species (ROS) [8,9],cryopreservation [10] on the DNA structural integrity .

The motility can be measured microscopically according to WHO [11] and by a computer-assisted sperm analysis (CASA) [12] . Most of the studies on the sperm DNA structure have considered different points like DNA adducts [13] , DNA damage lesions [9,12,14] , DNA integrity like denaturation and fragmentation (DD and DF) [15,16,17,18] and mitochondrial DNA (mt.DNA)deletion[19].

The deoxyribonucleic acid (DNA) of spermatozoa from human with reduced fertility has been investigated with different methods .These were :colorimetric [20],fluorimetric [21],cytometric [22].The research studies on the sperm DNA content were often contradictory favoring in turn a lower,equal or higher DNA values in infertile men [20,23].

The objective of this study is to evaluate the level of DNA content of spermatozoa and correlate it with sperm motility in a heterogenous group of subfertile men . DNA content was estimated by a Spectrophotometric method [20] which was elaborately mentioned in a former study [24].

Materials and Methods

The individuals were 64 subfertile male patients with different semen quality ranging in age from 20-45 years . Seminal fluid from each individual was obtained by masturbation after at least 3 days of sexual abstinence and examined microscopically within one hour of ejaculation. Liquefaction time ranges from 30-60 minutes. Ninty five percent of patients were of primary infertile type.

The sperm motility percent was determined microscopically.One drop (10µl) of the sample was mounted between a warm slide and covered with a coverslip . Overall motility was calculated regardless of the movement by counting motile and immotile sperm in 10 separate randomly selected (40×) fields away from the edge of the coverslip and then divided by total number of spermatozoa counted (motile + immotile)

$$\text{Percent sperm motility} = \frac{\text{number of motile sperms}}{\text{total number of sperms}} \times 100$$

The normal motility percent is (> 60 %) according to WHO [11]. Samples were deep frozen till the day of analysis . Frozen-thawed samples were used for DNA content estimation by a spectrophotometric method [20].This method was based on the formation of color reaction of deoxyribose with indole when heated in the presence of HCl .An Aqueous phase containing the stable colored indole-deoxyribose

complex was read at 480 nm using a spectrophotometer .The standard DNA solution (300 µg / ml) was obtained from Institute of Genetic Engineering ,University of Baghdad. It was prepared from human leukocytes in Tris-EDTA (TE)buffer pH 8 and was used to plot a The results are categorized into three groups:

Group I . (normal, >60 %) n=5
Group II. (asthenospermic , 20-60 %) n=27
Group III. (severe asthenospermic, <20 %) n=32

Statistical Analysis

Computerized Statistical Analysis was performed using SPSS (statistical package of social science) version 10.5 (INC.,Chicago , IL. ,USA) computer software .

Mean ± Standard Error of Mean of each parameter and Pearson's correlation coefficients (r - values) between the two different parameters were done .The (P-value) of this (r-value) was also calculated at ($P < 0.05$).

ANOVA statistical analysis which was based on calculation of percentage points of F- distribution and LSD0.05 was applied to obtain the(P-values) between the two groups [25].

Results:

Table 1, show the values of DNA content versus sperm motility (%) among the three groups represented as Mean ± SEM. The statistical mean values among these groups were of significant difference ($P<0.01$) .The correlation coefficients (r-values) between DNA count and sperm motility were also shown. A weak positive values (+0.45) and (+ 0.04) were recorded for Gr. I and Gr. III respectively .

However , a weak negative value(- 0.13) was obtained for Gr. II . The r-values were calculated from (Fig I, II & III) for Gr. I, Gr. II and Gr. III respectively. These,r-values were of statistically non significant ($P> 0.05$). Table 2, shows the statistical summary analysis of DNA content between the groups of patients. LSD0.05 measurement of Mean motility (%) was of statistically non significant difference among all groups ($P>0.05$).

Table 1: DNA content vs. sperm motility percent among groups ; Gr.I (>60%), Gr.II (20-60 %) and Gr.III (<20 %) and their correlation coefficients values (r-values).

Group	n	Motility(%)	µg DNA /ml	r-values
Gr.I	5	70.0 ± 3.84*	17.5 ± 1.29	+0.45
Gr.II	27	35.7 ± 1.8 *	15.3 ± 0.62	-0.13†
Gr.III	32	11.1 ± 1.26*	15.4 ± 0.88	+0.04†

Values are Mean ± SEM
*** Statistically significant (P<0.01)**
† Statistically non significant (P>0.05)

Table 2: Statistical summary analysis of sperm DNA content between the three groups of patients .

Group	n	Mean µgDNA/ml	LSD0.05
Gr.I	5	17.5	Gr.I vs Gr.II = 15.4†
Gr.II	27	15.3	Gr.I vs Gr.III = 15.2†
Gr.III	32	15.4	Gr. II vs. Gr.III = 8.3†

† **Statistically non significant (P>0.05)**

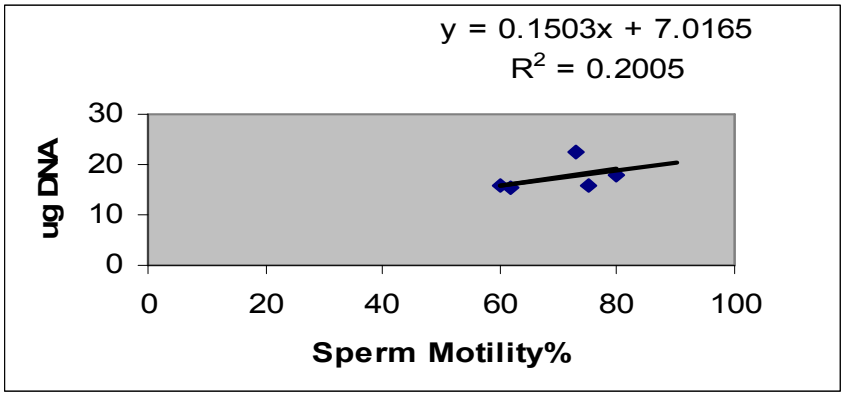

Fig.I: Correlation of sperm DNA content (µg/ml) vs. sperm motility (%) of Gr.I (>60%)

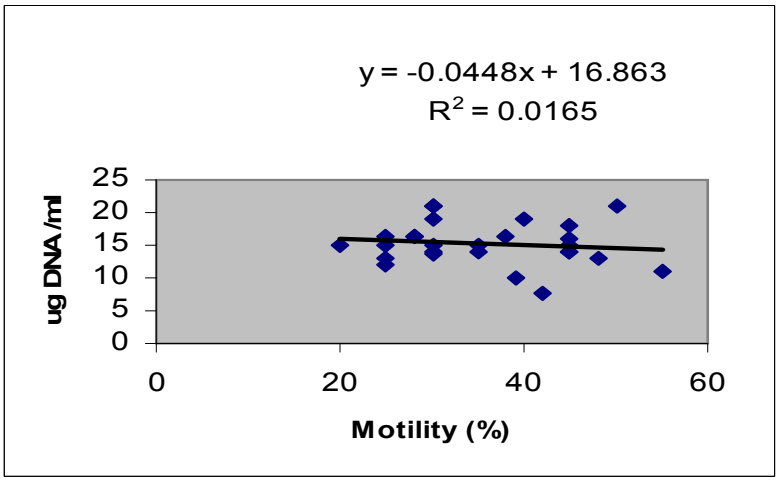

Fig.II Correlation of DNA content (µg ml) vs. sperm motility (%) of Gr.II (20-60).

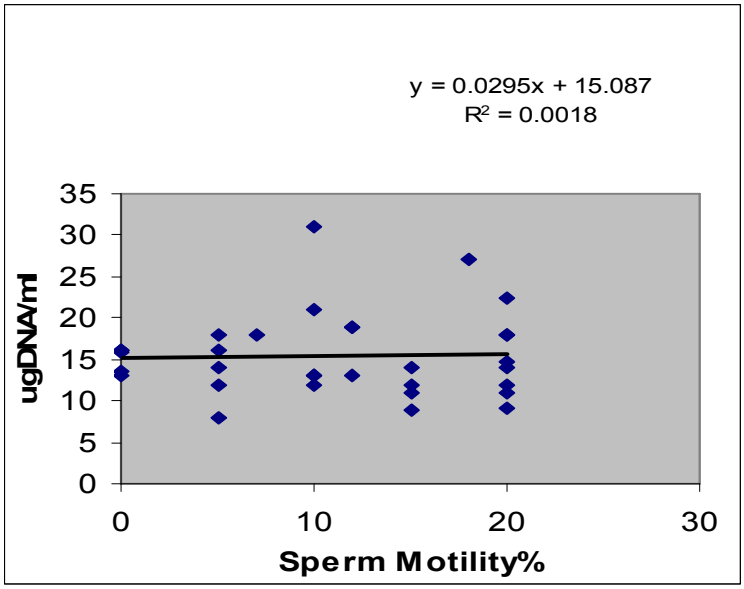

Fig.III Correlation of DNA content (ug/ml) vs. sperm motility (%) of Gr.III (< 20 %).

Discussion

The motility characteristics was used to assess and differentiate between semen of fertile and subfertile men [4].As mentioned before, various aspects of sperm DNA and the motility were studied. The results presented in this study indicate a weak non significant variable (positive or negative) correlation between the level of DNA content and sperm motility percent.This finding might be discussed with observations made by other workers.One observation was made on the association between semen parameters and the number of sperms with high DNA damage .It was concluded that the magnitudes of those associations were weak [12]. Another observation on high levels of bulky DNA adducts in human sperm correlate with impaired fertility, concluded a significant negative correlation between the levels of DNA adducts and measures of semen quality(sperm concentration and motility)[13].

A study on frequent chromosome aberrations in immotile human sperm exposed to culture media, indicate that immotile sperm does not have significantly more DNA lesions than motile sperms[14]. In another study on functional and ultrastructural features of DNA-fragmented human sperm, a negative correlation was found between DNA breakage and progressive motility indicating an impaired motility [17].

The mitochondrial DNA (mt.DNA) , found in the midpiece of the spermatozoa has a great impact on motility[26]. Its function is on respiration, oxidative phosphorylation and energy production needed for flagellar movement. The effect of mt DNA base pair deletions is associated with a decline in infertility and motility [19,27]. A conclusion was reached that those base pair deletions are negatively correlated with motility and fertility.Clearly , that significant association between mtDNA and motility could not be argued with the finding of this research work which dealt with the total DNA level (i.e. nuclear and mitochondrial DNA) .A non significant difference among normal ,asthenospermic and severe asthenospermic groups of subfertile men could be concluded.

Conclusion:

There is no significant correlation between sperm level of DNA content with the motility .

References:

1. Schill ,B. W. and Haidl, G. " Medical treatment of male infertility . In: Male and female .Insler,V. and Lunenfeld, B. (eds)" *Churchill Livingstone, U.K.*,pp.575-622,1993.
2. Micic, S. ; Tulic, C and Dotlic,R. " Kallikrein theory of infertile men with varicocele and impaired sperm motility " *Andrologia* ,22 (2) :179-183,1990.
3. Giblin ,P.T. ; Poland M.L.; Moghissi, K.S." Effects of stress and characteristic adaptability on semen Quality in healthy men." *Fertil. Steril. ,;*49 : 127,1988.
4. Hinting,A. ; Comhaire , F. ; Schoonjans, F. " Capacity of objectively assessed sperm motility characteristics in differentiating between semen of fertile and subfertile men ". *Fertil. Steril.* ;50,635,1988.
5. Farrell, P.B. ; Foote, RH. ; Zinaman, M.J. " Motility and other characteristics of human sperm can be measured by computer-assisted sperm analysis of samples stained with Hoechst 33342"*Fertil. Steril. ;* 66(3) : 446-53, 1996.
6. Shen, H.M. ; Chia .SE ; Ni,ZY. ;New, AL. ;Lee, BL. ; Ong, GN. " Detection of oxidative DNA damage in human sperm and the association with cigarette smoking" *Reprod.-Toxicol. ;*11 (5) : 675-80 ,1997.
7. Chen, C.S. ; Chao, H.T. ; Pan, R.L. ; Wei, Y.h." Hydroxyl radical-induced decline in motility and increase in lipid peroxidation and DNA modification in human sperm" *Biochem. Mol. Biol. Int .;* 43 (2) : 291-303, 1997.
8. Henkel, R. ; Kierspel, E. ; Hajimohammed ,M. ; Stalf, T. ; Hoogendij, K.C. ; "fragmentation of spermatozoa andassisted reproduction technology" ; *Reprod. Biochem. Online* ; 7 (4) : 477-83 ,2003.
9. Aitken,R.J. ;Baker, MA. and Sawyer, D. " Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease " *Reprod. Biomed online [medline www.pubmed.gov]* ; 7(1) : 65-70,2003.
10. Donnelly, E.T. ; Mc Clure, N. and Lewis, S.E. " Cryopreservation of human semen and prepared sperm : effects on motility parameters and DNA integrity" *" Feril. Steril.* ,76 (5) : 892-900, 2001.
11. World Health Organization "*Laboratory Manual for the examination of human semen and sperm cervical mucus interaction* " Cambridge , pp: 8-11 ,1999.
12. Trisini , A.T.: Singh, N.P. ; Duty, S.M. ; and Hanser, R> " Relationship between human semen parameters and deoxyribonucleic acid damage assessed by the neutral comet assay " , *Fertil. Steril.* ,82(6) : 1623-32, 2004.
13. Horak, S. ; Polanska, J. ; and Wid-lak, P. " High levels of bulky DNA adducts in human sperm correlate with impaired fertility " , *Acta. Biochem. Pol*. ,50 (1) : 197-203,2003.

14. Watanabe, S. " Frequent structural chromosome aberrations in immotile human sperm exposed to culture media " *Hum. Reprod.* ;19 (4) : 940-7 , 2004 .
15. Donnelly, E.T.; Mc Clure, N. and Lewis, S.E. " glutathione and hypotaurine in vitro : effects on human sperm motility , DNA integrity and production of reactive oxygen species. " *Mutagenesis* ,15 (1), 61-8,2000.
16. Rames,L. ; and Wetzels, A.M. " low rates of DNA fragmentation in selected motile human spermatozoa assessed by the TUNEL assay." ,*Hum. Reprod.* ;16(8) : 1703-7 , 2001.
17. Muratori, M. ; Piomboni, P. ; Baldi, E. ; Filinberti, E., Pecchiioli, P. ; Moretti, E. ; Gambera, L. ; Bacceti,B. ; Biagitti, R. Forti, G. and Maggi, M. "Functional and ultrastructural features of DNA-fragmented human sperm ", *J. Androl.* ; 21(6) : 903-12, 2000
18. Zini, A. ; Nam, R. K. ; Mak, V. ; Phang, D. and Jarvi, K. " Influence of initial sperm quality on the integrity of human sperm DNA following semen processing " , *Fertil . Steril.* ;74 (4) : 824-7 , 2000.
19. Kao, S.H. ; Chao, H.T. and Wei, Y.H. " Multiple deletions of mitochondrial DNA are associated with the decline of motility and fertility of human spermatozoa." ,; *Mol. Hum. Reprod.* ;4 (7) : 657-66 , 1998.
20. Frajese, G. Silverstroni, L., Malandrino, F. and Isidori, A. " High deoxyribonucleic acid content of spermatozoa from infertile , oligospermic human males." ; *Fertil. Steril.* ; 27 (1) : 14-20, 1976.
21. Badenoch,D.F.;Fowler,C.G.;Evans,P.R. and Lowery,P.A."DNA content of human semen-an objective measurement of sperm density" *Br.J.Urol.* 57(2):230-232,1985.
22. Molina,J.;Castilla,J.A.;Castano,J.L.;Fontes,J.;Mendozoa,N.andMartinez,L .“Chro matin status in human ejaculated spermatozoa from infertile patients and relationship to seminal parameters ”*Human Reprod;16(3):534-539,2001*.
23. Leuchtenberger,C.;Weir,D.R.and Schrader,F."The DNA content in spermatozoa of repeated seminal fluids from fertile and infertile men"*J.LabClinMed;6:851,1955*.
24. Daoud, M. S. and Al-Chalabi, F. A. " DNA content of spermatozoa with respect to seminal sperm concentration using a microchemical method " ;(*in press).*
25. Gill, J. " Design and analysis of Experiments in the animals and science ." 3rd (ed). *Iowa , The Iowa State University Press*, ;pp : 1-133, 1978.
26. Rao,J.C.B. and Martin ,S.M."Lipid peroxidation in human spermatozoa are related to midpiece abnormalities and motility" *Gamete Res*.,24,127-134,1989.
27. Kao, S.H. ; Chao H.T. and Wei, YH. " Mitochondrial deoxyribonucleic acid 4977-bp deletion is associated with diminished fertility and motility of human sperm " *Biol. Reprod.* ; 52 (4) : 729-36 ,1995.