Original Article

Sperm DNA content: An overall correlation study with sperm count, motility and morphology

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Summary:

Background: *To determine the DNA content in subfertile patients and correlate it with seminal sperm parameters, (count, motility and morphology).*
Design: *Prospective observational study.*
Setting: *College of Medicine, Department of physiological chemistry and Institute for Embryo research and Infertility treatment, University of Baghdad.*
Methods: *A random sample of 58 subfertile male patients undergoing semen evaluation and their age ranged from 20-45 years were studied. Semen samples were assessed for seminal sperms (count , motility and morphology) by direct light microscopy. Sperm DNA content was estimated using a microchemical spectrophotometric method .The data were categorized into four groups according to normal and abnormal sperms count (million / ml),percent motility and percent morphology. The normal limits of those sperm function parameters were according to WHO criteria. The four groups were named as Normozoospermic (NZS) (control) , Normoasthenozoospermic (NAZS) ,Normoasthenoteratozoospermic (NATZS) and oligoasthenoteratozoospermic (OATZS) groups.*
Results: *The Mean ± SD of the four groups were of significant difference (P < 0.05) with respect to sperm count, sperm percent motility and morphology with exceptions of certain groups. Moreover, a non significant difference (P >0.05) was found with respect to DNA content (µg /ml) and (µg/sperm) except for Gr. IV which showed a significant difference when compared to others.The correlation coefficients (r values) between sperm count and sperms motility, morphology and DNA content (µg/ml) were non significant in the four groups. Noticeably, the DNA content (µg/sperm) was statistically of significant (P <0.05) negative correlation with sperm count in all groups.*
Conclusion: *All groups were of significant difference (P<0.05) among their Mean ±SD values of their count, motility and morphology. There were few exceptions. No significant differences (P>0.05) were found with respect to DNA content (µg/ml or µg/sperm) except for oligoasthenoteratozoospermic group compared to others. No significant correlation was found between sperm count and each of sperms motility , morphology and DNA content (µg/ml) in the four groups .However, the DNA content (µg/sperm) had a statistically significant (P<0.05) negative correlation with sperm count in all groups.*
Keywords:*DNA, sperm concentration(count million/ml), Normozoospermic(NZS),Normoasthenoszoopermic(NAZS),Normoasthenoteratozoospermic(NATZS), Oligoasthenoteratozoospermic (OATZS) .*

Introduction:

The sperm essential parameters (count, motility and morphology) were used to evaluate semen quality in both normal fertile and abnormal subfertile men (1). The DNA was also studied extensively with regards to sperm count .A study employed the estimation of DNA content in sperms as a measure of sperm density (2). The DNA content of human spermatozoa with reduced fertility had been studied with spectrophotometric method (3,4).An increased sperm nuclear DNA damage was found in normozoospermic infertile men(5). Moreover, a negative correlation was observed between DNA fragmentation (DF) and sperm concentration (6).The role of the sperm chromatin abnormalities and DNA damage in male infertility was also reported (7).

The DNA was also studied with regards to sperms percent motility and morphology .The effects on DNA structural integrity (8 , 9) , mitochondrial DNA (mt. DNA) deletion (10) and recently sperm DNA content in subfertile asthenozoospermic men (11) was reported .

The DNA content of morphologically different sperm types for normal and subfertile

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men was recorded (12,13). DNA´s damage (denaturation(DD),fragmentation (DF)) and DNA content and their relation to sperm morphology were reported in fertile and subfertile men (14,15,16) and recently sperm DNA content in subfertile men (17) was reported.

The objective of the present study was to find a correlation between the level of DNA content and collectively the seminal sperm parameters (count, motility and morphology) in heterogenous groups of subfertile men. Four groups (normozoospermic(NZS) ,normoasthenozoospermic(NAZS), normoasthenoteratozoospermic (NATZS) and oligoasthenoteratozoospermic(OATZS) groups) were categorized for that purpose.

Materials & Methods:

The individuals were 58 subfertile male patients with different semen quality ranging in age from 20-45 years. Seminal fluid from each one 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 those men were of primary subfertile type.

One drop (10µl) of the sample was mounted between a warm slide and covered with a coverslip. Sperms concentration, motility and morphology were estimated in 10 separate randomly selected (40x) fields. Sperm counts (million per milliliter) was obtained by multiplying the mean numbers of sperms in those 10 fields by a factor of one million. Percent motility for motile sperms and percent morphology for abnormal sperms were calculated. The following equations were used:

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

$$\text{Percent abnormal sperm morphology} = \frac{\text{number of abnormal sperm}}{\text{total number of sperms}} \times 100$$

The normal limits for each parameters according to WHO (18) were:

sperm concentration (counts) = > 20 (million/ml)
sperm motility percent = > 60 %
sperm morphology percent = < 50 %

Semen samples were deep frozen till the day of analysis .Frozen – thawed samples were used for the estimation of DNA content estimation by the method based on the formation of color reaction of the deoxyribose with the indole (3 ,4) .

The standard DNA solution (300µg/ml) was obtained from Institute of Genetic Engineering, University of Baghdad.

The values of calculated DNA content expressed in (µg/ml) were categorized into four groups according to the three sperm function parameters:

Gr.I Normozoospermic group [normal sperms count, motility and morphology i.e.control, n = 6].

Gr.II Normoasthenozoospermic [normal sperms, count and morphology with abnormal motility, n =8].

Gr.III Normoasthenoteratozoospermic [normal sperms count with abnormal motility and morphology, n =20].

Gr.IV Oligoasthenoteratozoospermic group [abnormal sperms count, motility and morphology, n =24].

The values of DNA content per spermatozoon could be calculated and expressed in (µg DNA x $10^{-}6$/ sperm).

Statistical Analysis:

Mean ± Standard Error of Mean of each parameter was done. In order to test differences among group means, analysis of variance was determined for each parameter at probability level of (0.05). Correlation coefficients (r -values) were calculated between sperm concentration (count million per ml) and each of the other parameters.(19).

Results:

The data presented in the table, showed that the Mean values ± SD of the four groups of variable statistical differences. Measurement of LSD 0.05 differences among the groups was considered significant .Groups with respect to:

1) sperm count :were all of significant ($P<0.05$) difference except between Gr.II & Gr.III.
2) sperm (%)motility: were all of significant difference ($P<0.05$).
3) sperm (%) morphology : were all of significant difference ($P<0.05$). except between Gr.I & Gr.II and Gr.III & Gr. IV .
4) µg DNA /ml: were all of non significant difference ($P>0.05$).
5) µg DNA / sperm: were of non significant difference ($P>0.05$) except between Gr.IV and the other three groups.

The correlation coefficients (r -values) between sperm count (million/ml) and each of the other parameters (% motility, % morphology, µg DNA / ml , µg DNA x $10^{-}6$ / sperm) were calculated and presented in the table .The data indicated that the sperms motility percent and morphology percent had a positive and negative non significant ($P>0.05$) correlation with sperm count, respectively.

The DNA content (µg DNA /ml) in all groups showed a negative non significant correlation ($P>0.05$) with sperm count.

Noticeably, the DNA content per sperm in all groups showed a statistically negative significant correlation ($P<0.05$) with sperm count.

Table . Statistical analysis of DNA content in Normozoospermic Group (Gr.I. control , n=6 , Normoasthenozoospermic group (Gr.II.n=8) , Normoasthenoteratozoospermic group (Gr.III ,n=20) and Oligoasthenoteratozoospermic group (Gr.IV., n =24).

Groups	Mean ± SEM				
	Count million/ml	Motility%	Morphology%	µg DNA ml	µg DNA x 10^{-6}/ sperm
Gr.I	72.5 ± 7.67	69.2 ± 3.26	44.7 ± 2.11	16.4 ± 1.58	0.248 ± 0.05
		r = 0.196 P > 0.05	r= − 0.291 P > 0.05	r= − 0.494 P > 0.05	r= − 0.853 P < 0.05
Gr.II	51.1 ± 6.83	40.3 ± 2.3	46.5 ± 1.24	14.33 ± 1.568	0.34 9±0.097
		r= 0.30 P > 0.05	r= − 0.281 P > 0.05	r= − 0.028 P > 0.05	r= − 0.77 P< 0.05
Gr.III	51.9 ± 3.96	20.7 ± 3.32	80.7 ± 2.99	14.25 ± 1.131	0.333 ± 0.051
		r= 0.225 P > 0.05	r= − 0.211 P > 0.05	= − 0.016 P > 0.05	r= − 0.628 P< 0.01
Gr.IV	6.33 ± 1.236	12.75 ± 1.888	83.13 ± 4.586	13.69 ±0.613	5.40 ± 1.012
		r= 0.332 P > 0.05	r= − 0.213 P > 0.05	r= − 0.249 P > 0.05	r= −0.719 P < 0.01

Discussion:

The results presented in this study indicated that there were significant differences (P<0.05) between the groups with respect to each of the parameter considered, as shown in the table . The exceptions were in the NAZS and NATZS groups which had nearly equal and within normal sperm counts (51.1 & 51.9 million / ml, respectively).This can also be argued for the normal and abnormal percent abnormal morphology in the NZS and NAZS groups (44.7 % & 46.5 % ,respectively) and for the NATZS and OATZS groups (80.7 % & 83.13 % , respectively).

The non significant differences (P>0.05) in the DNA content (µg / ml) indicated that the level of the DNA was irrelevant of the semen parameters (count , motility and morphology) which were the criteria for the four groups. However, when the DNA content was calculated per sperm , the values showed a remarkable difference between the OATZS group and the other three groups. This could be attributed to the much lower values of the sperm count (Mean value = 6.3 million / ml) in this group compared to the other three groups.

The relation of sperm (percent motility & morphology) and DNA content (µg / ml) with sperm count indicated that these parameters were either positively or negatively correlated,but of non significant value. In contrast, the DNA content per sperm (obtained depending on the values of sperm count) were significant (P< 0.05) and negatively correlated with the sperm count (concentration).

The observations made on the high DNA content in spermatozoa (5.4 µg DNA / sperm) in the subfertile oligoasthenoteratozoospermic men than the other three groups was in agreement to that of Oforofuo et al (20) and Parez et al (21) . However, it was contradictory with that reported by Leuchtenberger et al (22). They found wide variations in the DNA content of spermatozoa from infertile patients compared with that of normal men and a lower DNA content in the patients with oligospermia. The results were also in full agreement with those of Frajese et al (3) worked on oligospermic patients affected by idiopatheic spermatic arrest and Daoud & Al-Chalabi (4) worked on a random sample of subfertile male patients.

Up to our knowledge very few reports in the literature were found dealing with the relation of spermatic level of DNA with the relation of spermatic level of DNA with their motility and morphology.Most of them dealt with the study on DNA structure (integrity or damage), mt.DNA deletion ,DD.,DF.In previous reports (11,17), a correlation study of DNA content with (%motility)

& (% morphology) respectively, was achieved . Random samples of infertile males were grouped into normal, asthenospermic (moderate & severe) and teratospermic groups. It was concluded that non significant difference of DNA content was found among the asthenospermic & their normal control groups .That was in agreement with conclusion made in previous reports (8,23, 24) on quite similar studies . Moreover, a non significant negative correlation was also found among the teratospermic and its normal control group. That was in consistent with similar studies on DF and morphology of the ejaculated sperm (6 ,14) and also on two markers of sperm integrity (DD& DF) in infertile men (9 , 25) .

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