Demonstration of the DNA Integrity in Semen Samples with Normospermia, Oligozoospermia and Azoospermia by Comet Method and Immunohistochemical Determination of EGF-R and TGF-α

Normospermi, Oligospermi ve Azospermik Olgularda DNA Bütünlüğünün Comet Yöntemiyle Belirlenmesi ve EGF-R ve TGF-α'nın İmmunohistokimyasal Olarak Gösterilmesi

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ABSTRACT

Objective: The aim of this study was to compare the rate of sperm cells with DNA damage by the comet assay, and to determine EGF-R and TGF-α localization by using immunohistochemical methods in normospermic, oligozoospermic and azoospermic events.
Methods: Semen samples were obtained from 30 men attending the Infertility Clinic of Baskent University Hospital. We used the comet assay to determine the DNA damage of sperm cells. Indirect immunohistochemical methods were used with EGF-R and TGF-α polyclonal antibodies.
Results: To determine the DNA damage of sperm cells, the comet assay was used in normospermic, oligozoospermic and azoospermic events and a statistically significant difference was found among the treatment groups (p=0.038). The differences between normospermic and azoospermic events is greater than normospermic and oligozoospermic events (p=0.05). Furthermore, EGF-R and TGF-α immunoreaction on the sperm cell surface of normospermic and oligozoospermic events were stronger than azoospermic sperm cells. Reactivity of these growth factors was related with sperm morphology.
Conclusion: In azoospermic semen samples, DNA damage was higher, and also the reactions for EGF-R and TGF-α were weaker when compared to normal semen samples. *(Gazi Med J 2011; 22: 33-40)*

Key Words: Comet, immunohistochemistry, semen, EGF-R, TGF-α

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ÖZET

Amaç: Bu çalışmanın amacı, komet analizi ile DNA hasarı olan sperm hücrelerinin oranını belirlemek ve normospermi, oligozoospermi ve azospermi olgularında EGF-R ve TGF-α dağılımını immünohistokimyasal yöntemlerle belirlemektir.
Yöntemler: Semen örnekleri Başkent Üniversitesi Hastanesi İnfertilite Kliniğine başvuran 30 erkekten alındı. Sperm hücrelerini belirleyebilmek amacı ile komet analizi kullanıldı. EGF-R ve TGF-α poliklonal antikorları ile de indirekt immünohistokimyasal yöntem uygulandı.
Bulgular: Komet analizi sonrasında DNA hasarı incelendiğinde normospermi, oligozoospermi ve azospermi olgularında gruplar arasında istatistiksel farklar bulundu (p=0.038). Normospermi ve azospermi arasındaki fark normospermi ve oligozoospermi grupları arasındaki farktan daha büyük olarak bulundu (p=0.05). İmmünohistokimyasal değerlendirmeler sonucunda normospermi ve oligozoospermi gruplarındaki tutulumun azospermi grubuna göre daha kuvvetli olduğu belirlendi. Tutulumun sperm morfolojisi ile ilişkili olduğu saptandı.
Sonuç: İnfertilite nedeni olan azospermik olgularda DNA hasarı fazla, buna karşın EGF-R ve TGF-α immünreaksiyonunun normospermik olgulara karşın zayıf olduğu belirlendi. *(Gazi Med J 2011; 22: 33-40)*

Anahtar Sözcükler: Komet, immunohistokimya, semen, EGF-R, TGF-α

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INTRODUCTION

Spermatogenesis is a complex process in which stem spermatogonia, through a series of events involving mitosis, meiosis and cellular differentiation, become mature spermatozoa. Infertility affects approximately 15% of married couples and, of these cases; approximately 50% are due to male factors (1). During sperm maturation process, the developing sperm cell is sensitive to external stresses such as chemicals, radiation, toxicants, and heat. Testicular cells and spermatozoa may be damaged by these stresses, altering the production and integrity of mature sperm. Examples of damage include decreased sperm production and altered sperm morphology following mutagen exposure (2). Anomalies such as loosely packaged chromatin and damaged DNA have been reported in poor quality semen samples (3). Recently it has been shown that a significant proportion of motile spermatozoa from infertile donors contain fragmented DNA (4).

The factors leading to male infertility have not been explained yet but it is thought that the DNA strand breaks which is one of the fundamental elements in apoptosis also named controlled cell death affects male infertility (5). Apoptosis is under hormonal control or influence (6). According to current studies, another factor that may influence the initiation of apoptosis in the process of spermatogenesis, is the fundamental role and degree and presentation of reactive oxygen species (ROS) (7).

Growth factors have substances which stimulate cell growth and division (8, 9). EGF (Epidermal Growth Factor), which has a role in growth and division of the cell, is a 53-amino acid polypeptide, first isolated from the mouse submaxillary gland. Subsequently; it has been also detected in several tissues and body fluids of human, rat and mouse. EGF has also been detected in semen and testicular tissue and it is known that EGF and Transforming Growth Factor- alpha (TGF-α) modulate mitogenic activity on the germ cells (10). Assuming that EGF and TGF-α have played an important role on numerical regulation of sperm cells in semen, we intend to determine the existence of these growth factors in normospermic, oligozoospermic and azoospermic semen samples.

Oligozoospermia may be due to a hyperactive apoptotic process (5). In this respect; we intend to compare the rate of sperm cells with damaged DNA in normospermic, oligozoospermic and azoospermic semen samples. Our aim is to show the existence and effects of EGF and TGF-α growth factors in semen samples which have different sperm concentrations. The alkaline SCGE (single cell gel electrophoresis) assay is widely used for measuring DNA damage in somatic cells (11). Nowadays this technique has been adapted to measure DNA damage of sperm cells (12). Comet assay was compared with ELISA technique, and its confidence was demonstrated (13).

MATERIAL AND METHODS

2.1. Preparation of semen samples: Semen samples were obtained from 30 men attending the Infertility Clinic of Baskent University Hospital, Ankara, Turkey. All semen samples were obtained by masturbation into sterile containers after abstinence from sexual activity for 3-5 days. Routine semen analysis was carried out by light microscopy to provide details of concentration, motility and morphology. First, samples were classified as normospermic (sperm concentration $\geq 20 \times 10^6$) (n=10) and oligozoospermic (sperm concentration $<20 \times 10^6$) (n=10). To prepare the azoospermic events TESE was used for obtaining sperm samples Testicular biopsy was performed under local anaesthesia. Following scrototomy, testicular tissue samples were taken under microscopy from each patient and sperm cells were picked.

2.2. Semen washing: The semen samples were fractionated on a discontinuous two-step percoll gradient centrifugation. 1 ml of semen was layered on top of two gradients of percoll (90% and 40%), centrifuged at 1400 rpm for 10 min then washed in IVF-20 (IVF Science Scandinavia, lot: 2024GA10) and centrifuged at 1400 rpm for 10 min again. The washing was repeated twice and concentrated to 10 x 10^6/ml applying the swim-up technique.

2.3. Comet assay: The single cell gel electrophoresis (comet) assay was applied to normospermic, oligozoospermic and azoospermic events. In summary; high melting point agars gel (HMA: Sigma; A7174, lot: 82H0609) was pipetted onto fully frosted slides and allowed to solidify at 4°C. A top layer was then made by mixing low melting point agars (LMA: Sigma; A4018, lot: 91H0172) with 10 μl sperm suspension. This was pipetted onto the first layer and allowed to solidify. The slides were placed into lysing solution for 1h. (22.5 ml 2.5 mol/1 NaCI, 100mmol/l Na_2EDTA, 10mmol/l Tris at pH:10 with 250 μl of 1% Triton X-100 added just before use) to decondense the DNA, 2.5 ml dithiothreitol (DTT) was then added to a final concentration of 10mmol/l for 30 min at 4°C. The slides were removed from the lysing solution and placed in a horizontal electrophoresis tank, filled with freshly prepared electrophoresis solution, for 20 min to allow the DNA to unwind. Electrophoresis was then conducted for 10 min at 30 V. Subsequently, the slides were drained and flooded with neutralizing solution (0.4 M Tris: Sigma; at pH:7) to remove any alkali and detergents. After neutralization, the slides were stained with 50 μl of 20 μg/ml ethidium bromide (1239-45-8; Sigma) and covered with a coverslip.

The slides were viewed using Nikon Eclipse E 600 epiflourescence microscope. They were analyzed from front to back and from left to right to prevent reanalysis of any of the spermatozoa. Comets from broken ends of the negatively charged DNA molecule became free to migrate in the electric field towards the anode. The assay provides direct determination of the extent of DNA damage in individual cells, and the extent of DNA damage can be assessed from the length of DNA migration, which is derived by subtracting the diameter of the nucleus from the total length of the image (14).

We determined the degree of damage by grading the cells as; undamaged (no migration), low damaged (limited migration=at low damage levels, stretching of individual pieces is likely to occur), and damaged (extensive migration=with increasing numbers of breaks, DNA pieces migrate freely into the tail forming comet images)

2.4. Immunohistochemical Procedures: Each semen sample was slipped on slides covered with polylysin and fixed with PBS (phosphate buffer saline) and air dried. Endogenous peroxidase activity was blocked in 3% Hydrogen peroxidase (Fisher Scientific, Melrose Park, IL) for 10 minutes. Samples were incubated with saponin to facilitate binding of primary antibody to antigenic areas. Epitopes were stabilized by application of serum blocking solution (Goat serum, Part # A 1236-EA, Oncogene Science, Manhasset, New York, USA) for 20 minutes. Sections were incubated with antibodies of EGF-R rabbit polyclonal antibody Ab-4 (100ìg/ml Lot # DO 3571-1, Cat # PC19-100UG, Oncogene Science, Manhasset, New York, USA) and TGF- α rabbit monoclonal antibody Ab-2 (100ìg/ml Lot # 409401-5, Cat # GF10-100UG, Oncogene Science, Manhasset, New York, USA) overnight at +4°C. The secondary antibody, 1% diluted biotin la-

beled anti-rabbit total Ig (Biotinylated antibody, Part # JA 1090-EA, Oncogene Science, Manhasset, New York, USA) was applied for 30 minutes at room temperature. A negative control was done by using normal rabbit IgG (Lot # DO2131-4, Cat # NI01-100UG, Oncogene Science, Manhasset, New York, USA) instead of primary antibody. After washing with PBS, avidin-biotin-complex-peroxidase (ABC, Part # JA 1236-EA, Oncogene Science, Manhasset, New York, USA) was applied to the slides. Diaminobenzidine (DAB, Oncogene Science, Manhasset, New York, USA) was used as the chromogen. Afterwards, slides were counterstained with picric acid solution in tris-buffer saline. Slides were settled on a tank, covered with picric acid solution, heated and dried for 5 minutes, then washed with distilled water, dried in air and mounted with conventional medium (Microscopie Entellan # 740212765, Merck, Germany). The intensity of the immunoperoxidase reaction was classified under light microscope (Olympus BH-2) (15).

RESULTS

3.1. Comet's Results: Demographic data of treatment groups such as age, sperm concentration, sperm morphology and motilities were summarized in Table 1.

Evaluation of the comet study for 100 sperm cells for each sample population showed that sperm cells with morphological anomaly have DNA damage. Especially sperms with neck, head and tail anomaly have damaged DNA, but sperms with piriform head anomaly have not had DNA damage, as with sperm cells with normal morphology (Fig. 1).

Table 1. Demographic data of treatment groups such as age, sperm concentration, and sperm morphology and motilities

Number of Patient	Age	Diagnosis	Details of Spermiogram			Results of Comet		
			Quantity (million)	Morphology (kruger normal %)	Motility (%)	Damage	Low Damage	Undamaged
1	34	Normospermia	205	3	85	50	30	20
2	32	Normospermia	185	6	84	17	24	59
3	28	Normospermia	130	1	69	48	47	5
4	30	Normospermia	80	2	75	18	44	38
5	31	Normospermia	60	0	68	65	26	9
6	26	Normospermia	53	1	64	68	15	17
7	32	Normospermia	41	0	64	72	8	20
8	27	Normospermia	37	1	87	53	40	7
9	33	Normospermia	35	0	43	25	15	60
10	27	Normospermia	25	1	76	53	38	9
11	25	Oligozoospermia	18	0	78	69	30	1
12	31	Oligozoospermia	17	0	59	48	15	37
13	29	Oligozoospermia	16	0	68	40	54	6
14	34	Oligozoospermia	14	0	65	32	60	8
15	29	Oligozoospermia	13	1	58	43	50	7
16	27	Oligozoospermia	10	0	46	60	36	4
17	26	Oligozoospermia	10	0	10	72	25	3
18	32	Oligozoospermia	8	0	47	58	37	5
19	33	Oligozoospermia	5	0	54	45	42	3
20	26	Oligozoospermia	3.2	0	37	68	27	5
21	28	Azoospermia	0	0	0	43	47	10
22	29	Azoospermia	0	0	0	61	20	19
23	31	Azoospermia	0	0	0	70	29	1
24	26	Azoospermia	0	0	0	68	32	0
25	33	Azoospermia	0	0	0	72	27	1
26	25	Azoospermia	0	0	0	64	35	1
27	31	Azoospermia	0	0	0	74	26	0
28	29	Azoospermia	0	0	0	59	35	6
29	27	Azoospermia	0	0	0	72	28	0
30	34	Azoospermia	0	0	0	68	30	2

3.2. Statical Results: All data were analyzed by analysis of variance. The calculations were performed by one-way analysis of variance (ANOVA) using SPSS package computerized software. Correlation coefficients (R) with p>0.05 were considered insignificant. In these conditions; statistical differences of each parameter are shown in Table 2. There were no statistically significant differences among the ages of treatment groups (p=0.0810). According to Spearman correlation, there were statistically differences among the treatment groups for the sperm concentration, sperm morphology and motility. We have determined that the morphological differences between normospermic and azoospermic events was greater than normospermic and oligozoospermic events (p<0.05) (Table 2).

We have also compared comet scores among the treatment groups according to the Spearman correlation. The rate of sperm cells with damaged DNA in treatment groups was different from each other (p=0.038). This difference was greater between normospermic and azoospermic groups than normospermic and oligozoospermic groups (p<0.05). There was no statistically significant difference for the sperm cells with low damaged DNA among the treatment groups (p=0.0344). The rate of sperm cells with undamaged DNA is found different only between normospermic and azoospermic events (Table 3).

According to Spearman correlation, the comparison of all parameters showed that sperm morphology was related with DNA damaged of sperm. Furthermore morphological features were related with motility (Table 4).

We also evaluated our data according to Pearson correlation and results of this parametric test support the Spearman correlation (Table 5).

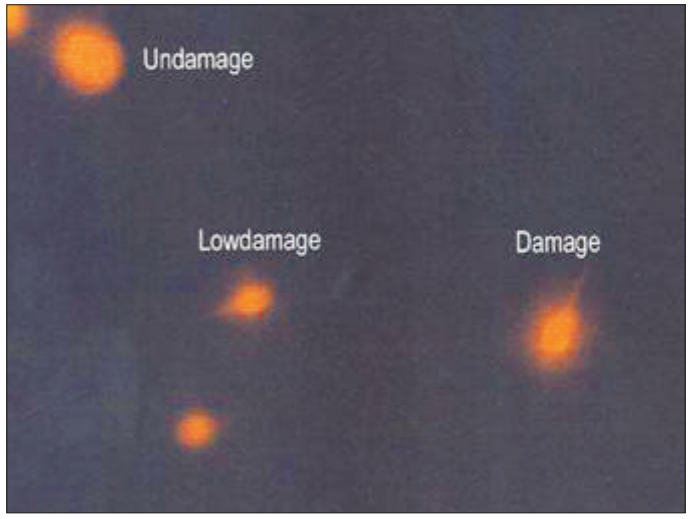

Figure 1. Normospermic semen samples (Ethidium bromide staining X1000)

Table 2. Features of semen

Parameters of Semen	Normospermia			Oligozoospermia			Azoospermia			P
	Y	M	S	Y	M	S	Y	M	S	
Sperm Concentration	85.1	56.5	65.353	11.42	11.5	5.045	–	–	–	*,**<0.05
Motility	71.5	72	13.142	52.2	56	18.972	–	–	–	*,**<0.05
Morphology	1.5	1	1.841	0.1	–	0.316	–	–	–	*,**<0.05

*: the differences between normospermic and azoospermic events
**: the differences between normospermic and oligozoospermic events
Y: Mean, M: Median, S: Standard deviation, P: Probability

Table 3. Comet scores

Comet Scores	Normospermia			Oligozoospermia			Azoospermia			P
	Y	M	S	Y	M	S	Y	M	S	
Damaged	46.9	51.5	20.267	53.5	53	13.794	65.1	68	9.183	*<0.05
Low damaged	28.7	28	12.72	37.6	36.5	13.366	30.9	29.5	6.82	–
Undamaged	17.2	18.5	19.8	7.9	5	4.656	3	1	6.115	*,**<0.05

*: the differences between normospermic and azoospermic events
**: the differences between normospermic and oligozoospermic events
Y: Mean, M: Median, S: Standard deviation, P: Probability

Table 4. Evaluation of parameters of semen and comet according to spearman correlation

	Motility		Damaged		Undamaged		Low damaged	
	R	P	R	P	R	P	R	P
Morphology	0.673	0.000	-0.444	0,0142	0.472	0.00879	0.178	0.344
Damaged	-0.482	0.0072	–	–	-0.642	–	-0.489	0.0063
Motility	–	–	–	–	0.527	0.00295	0.191	0.309
Low damaged	–	–	–	–	-0.170	0.365	–	–

R: correlation coefficient, P: Probability

Table 5. Evaluation of parameters of semen and comet according to pearson correlation

	Motility		Damaged		Undamaged		Low damaged	
	R	P	R	P	R	P	R	P
Morphology	-0.551	0.00159	–	–	0.582	0.00073	-0.0145	0.939
Concentration	0.613	0.000319	-0.460	0.0106	0.522	0.00306	-0.0568	0.765
Motility	-0.512	0.00381	–	–	0.393	0.0318	0.161	0.396

R: correlation coefficient, P: Probability

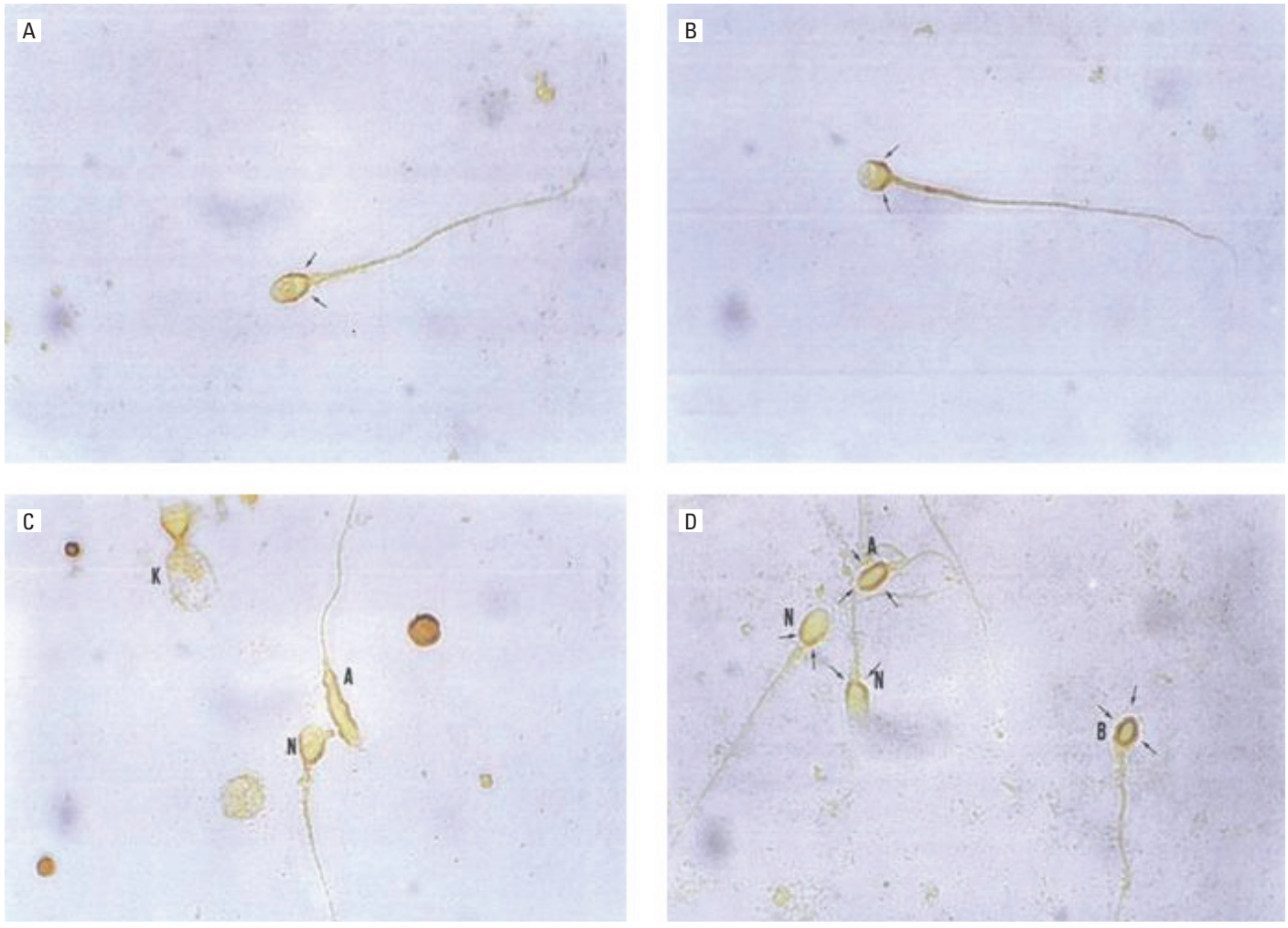

Figure 2. A) Normospermic semen samples. ↑: EGF-R immunoreactivity is seen in bottom region of acrosome of the sperm with normal morphology (Immunoperoxidase - Picric acid X1000), B) Normospermic semen samples. ↑: EGF-R immunoreactivity is seen in bottom region of acrosome of the sperm with normal morphology (Immunoperoxidase - Picric acid X1000), C) Normospermic semen samples. EGF - R immunoreactions especially around the sperm head in morphological abnormal sperm. A: sperm with amorphous head, K: sperm with tail anomaly, N: normal sperm with immunoreactivity in bottom region of acrosome (Immunoperoxidase - Picric acid X1000), D) Normospermic semen samples. TGF-α immunoreactions especially around the sperm head in morphological abnormal sperm. A: sperm with small acrosome, B: sperm with small head, N: normal sperm with immunoreactivity in bottom region of acrosome (Immunoperoxidase - Picric acid X1000)

3.3. Immunohistochemical Results: Expression of EGF-R and TGF-α in the normospermic, oligozoospermic and azoospermic semen species were studied with immunohistochemical methods and examined using a light microscope. In normospermic species; immunoreactitivity of EGF-R (Fig. 2a) and TGF-α (Fig. 2b) were determined at the bottom region of the acrosome of the sperm with normal morphology. However; the immunoreactions of EGF-R (Fig. 2c) and TGF-α (Fig. 2d) of morphologically abnormal sperm, especially midpiece, tail, head and neck anomalies were all around the sperm head.

Reaction localization of EGF-R and TGF-α was the same with oligozoospermic and azoospermic events, but reaction intensity of these growth factors on the sperm cells of normospermic and oligozoospermic semen samples was stronger than azoospermic samples. In oligozoospermic semen samples; immunoreactivity was the same as the normozoospermic samples; EGF-R (Fig. 3a) and TGF-α (Fig. 3b) were observed in the bottom regions of acrosomes in normal morphologic sperm. However, in the abnormal morphologic sperm, EGF-R (Fig. 3c) and TGF-α (Fig. 3d) immunostaining were seen all around the sperm head.

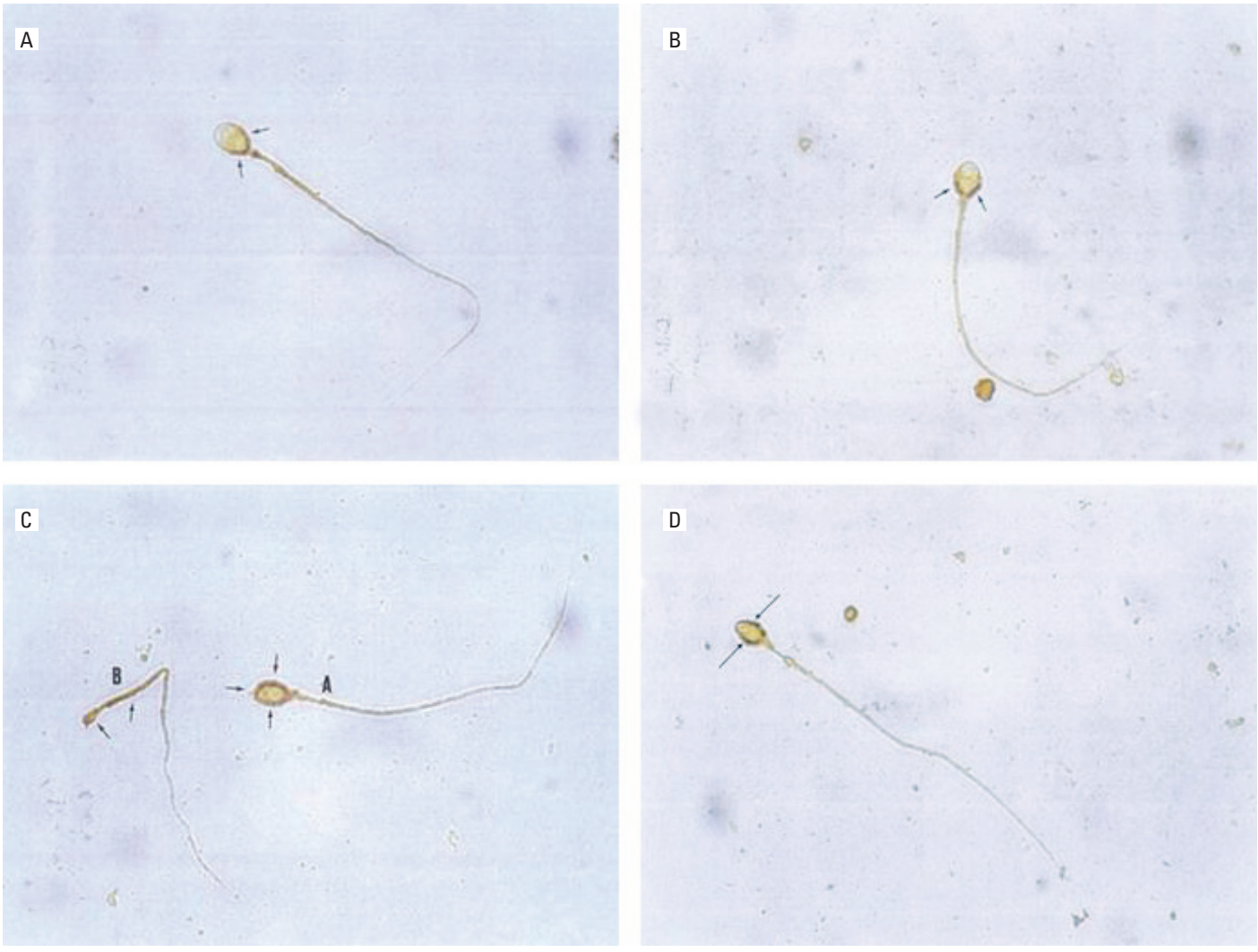

Figure 3. A) Oligozoospermic semen samples. ↑: EGF-R immunoreactivity is seen in bottom region of acrosome of the sperm with normal morphology (Immunoperoxidase - Picric acid X1000), B) Oligozoospermic semen samples. ↑: TGF-α immunoreactivity is seen in bottom region of acrosome of the sperm with normal morphology (Immunoperoxidase - Picric acid X1000), C) Oligozoospermic semen samples. ↑: EGF- R immunoreactions are seen especially around the sperm head with small acrosome (A). In small head sperm, immunoreactivity is seen in head, mid piece and tail regions (B) (Immunoperoxidase - Picric acid X1000), D) Oligozoospermic semen samples. ↑: TGF-α immunoreactivity is determined around the abnormal sperm with pointed head and vacuolar acrosome (Immunoperoxidase - Picric acid X1000)

In azoospermic semen samples, only sperm cells with morphologic abnormality were demonstrated and they showed strong EGF-R and TGF-α immunoreactions (Fig. 4a). In contrast, in azoospermic samples, sperms with a piriform head abnormality showed immunoreaction at the bottom region of the acrosome, as in sperms with normal morphology (Fig. 4b).

Briefly; reaction localization of these growth factors changes according to the sperm morphology. If the sperm cells have normal morphology, reaction was seen in the bottom region of the acrosome, but if they have a morphological anomaly, reaction was seen all around the sperm head.

DISCUSSION

Many studies indicate that growth factors influence the proliferation and biological functions of various cells in the testis. Among these growth factors, EGF and TGF-α are locally produced growth factors involved in spermatogenesis in the human testis via an autocrine and/or paracrine mechanism. EGF is produced in the testis, specifically in the more mature germ cells residing in the adluminal compartment of the blood testes barrier (16).

Immunlocalization of EGF-R in germ cells is dependent upon the cycle of the seminiferous epithelium (17). Indeed, they show preponderant expression of the receptor during the meiotic process and spermiogenesis, and at the cellular level, a preponderant staining in pachytene spermatocytes in stages before and during meiosis, as well as in newly formed spermatids until the appearance of their acrosome. In addition, EGF-R staining was high in spermatids at the end of the elongation and in maturing spermatozoa during the formation and the removal of the residual bodies. Together, these findings suggest that, in germ cells, the EGF system might be implicated in the meiotic process.

In the study by Naz (10), EGF was detected by radioimmunoassay as well as radioreceptor assay in seminal plasma of fertile men. However there were no statistical differences between the levels of EGF or EGF/TGF-α in seminal plasma of fertile and infertile men. Also

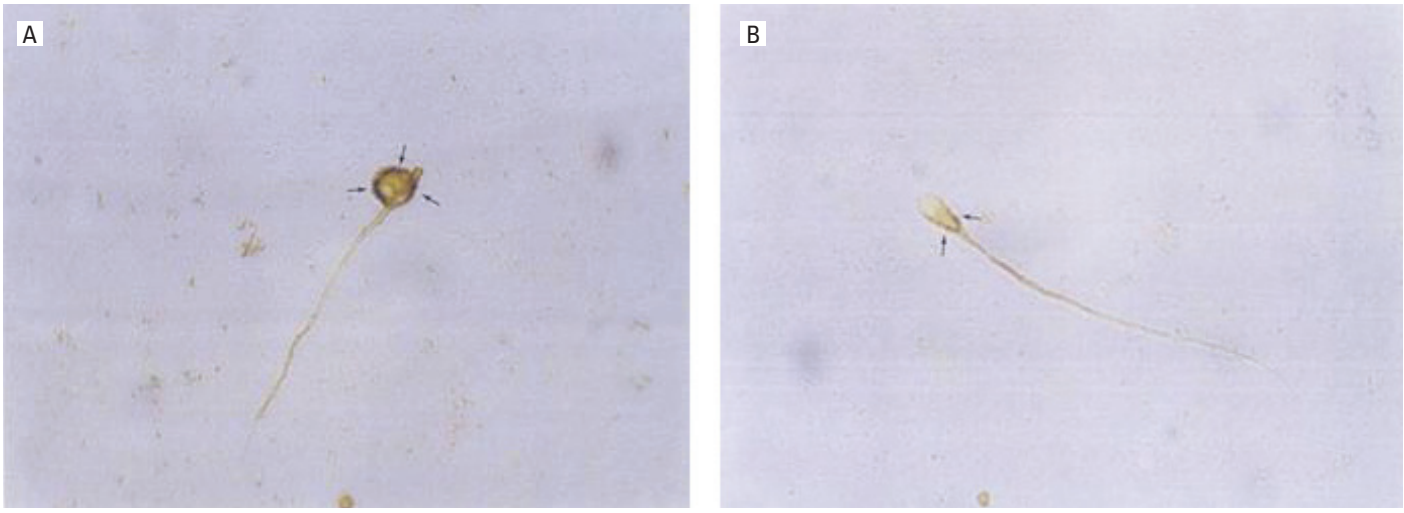

Figure 4. A) Azoospermic semen samples. ↑: Only sperm cell with morphologic anomaly are shown and strong EGF-R immunoreactivity observed (Immunoperoxidase - Picric acid X1000), B) Azoospermic semen samples. ↑: Only sperm cell with piriform head anomaly, reaction was observed in bottom region of acrosome as in sperm cells with normal morphology (Immunoperoxidase - Picric acid X1000)

there was no significant correlation of the EGF or EGF/TGF-α levels with total sperm number and sperm motility characteristics in these subject groups.

The mature sperm cells from various mammalian species, including humans, have EGF receptors on the plasma membrane in the acrosomal region, and these EGF receptors are functionally active and respond to ligand binding (17).

EGF and TGF-α exhibit different cellular distribution at different stages of testicular development. TGF-α was predominantly present in somatic cells but not in germ cells. In contrast, EGF was preponderant in tubular cells (Sertoli and germ cells). These observations are consistent with the presence of TGF-α mRNA in Sertoli cells but not in germ cells. In contrast to TGF-α, EGF was present in germ cells. Consistent with the present observation, EGF has also been described in the adult mouse germ cells, namely in pachytene spermatocytes and round spermatids. However, it must be noted that EGF mRNA has not been detected in isolated rat sertoli and germ cells with human EGF cDNA probe (18).

In the study by Hughes (13); both the ELISA and comet assays are used to assess DNA integrity in sperm samples from fertile and infertile men and these results compared. According to Hughes, DNA integrity determined by the ELISA and comet assays were found similar for both fertile and infertile men. Hughes also stressed that the comet assay is as accurate as established previous methods. The main advantage of the comet assay over the ELISA assay is that DNA damage can be assessed in individual cells. In this respect, we preferred the comet assay for the indication of DNA damage in sperm cells, in normospermic, oligozoospermic and azoospermic semen samples.

Host (5), reported a significantly higher incidence of DNA strand breaks in men with oligospermia compared to men with normal spermiograms. It has been suggested that, in men with oligozoospermia, the seminal plasma contains neutrophils which generate reactive oxygen species (ROS) on sperm. In other situations, reactive oxygen species have been shown to induce apoptosis in living cells.

Although the exact factors and mechanisms causing male infertility are still largely elusive, one of the significant developments in recent years is that the explanation of reactive oxygen species (ROS) and oxidative damage are closely associated with impaired sperm function and male infertility (19). 8-hydroxydeoxyguanosine (8-OHdG) is considered to be a precise and sensitive biomarker of oxidative DNA damage. The earlier study by Hang-Ming (20) was thus designed to evaluate the extent of oxidative DNA damage in sperm cells and their association with male infertility by assaying the 8-OHdG levels. ROS production in infertile subjects was also found to be significantly higher than that in normal subjects. It was revealed that infertile patients' sperm cells contained significantly higher levels of 8-OHdG in DNA than did those of healthy subjects, and the levels of 8-OHdG in sperm DNA were closely correlated with a number of conventional seminal parameters, such as sperm density, sperm motility, total sperm number and sperm morphology.

Apoptosis is a result of DNA damage in oligozoospermia and azospermia may therefore be due to a hyperactive apoptotic process. We wished to compare the rate of DNA damage of normospermic, oligozoospermic and azoospermic semen samples and our results supported this opinion. We used the comet assay to determine DNA damage in sperm cells. We indicate that there is significantly statistical difference among the rate of DNA damage of sperm cells of treatment groups. The difference, between normospermic and azoospermic events was greater than normospermic and oligozoospermic events. It was noticed that sperm cells with a morphologic anomaly have damaged DNA. Sperm cells with piriform anomaly do not have damaged DNA as in sperm cells with normal morphology.

In the present study, we demonstrated that DNA damage rate of semen samples, which have low sperm concentration leading to infertility like azoospermic semen samples, were greater and reaction of EGF and TGF-α is weaker when compared with the normal semen samples. When the immunolocalization of these growth factors were examined for each semen groups, it was seen that immunoreactions was on the bottom region of the sperm head of the sperm cells with normal morphology, and all around the sperm head in sperm cells with morphologic anomaly. However, immunoreactions of these growth factors were on the bottom region of the acrosome of the sperm cells with piriform head anomaly las in normal sperm cells. Furthermore, the reaction intensities of these growth factors were greater on the sperm with morphologic anomaly when compared with normal sperm cells.

In conclusion, DNA damage was higher in azoospermic semen samples, and also the reactions for EGF-R and TGF-α were weaker when compared to normal semen samples.

Conflict of Interest

No conflict of interest was declared by the authors.

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