Relation of the Morphological Alterations of Spermatozoa with Motility

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Özet

Amaç: Spermatozoadaki morfolojik bozuklukların lokalizasyonu ile motilite arasındaki ilişkinin ortaya konması ve bunun fertilizasyon üzerine etkilerinin araştırılması.
Materyal ve Metod: İnfertilite nedeniyle başvuran ve kadın faktörü normal olarak bulunan 388 erkek hastada sperm motilite ve Kruger'e göre morfoloji analizleri yapıldı. Bir kısım olguda sperm yüzeyinde antisperm antikor (ASA) (IgG ve IgA) bulunma oranları da ayrıca çalışıldı. Sonuçlar istatistiksel olarak karşılaştırıldı.
Sonuçlar: Baş anomalili olgularda a + b normal motiliteli spermatozoa oranları ortalama %65 ($p>0.05$) bulunurken, orta parça ve kuyruk anomalili olgularda bu oranlar sırasıyla %15 ($p<0.01$) ve %20 ($p<0.01$) olarak bulundular. Antisperm antikorun yüksek (>%10) bulunma oranları ise, baş, orta parça ve kuyruk anomalili olgularda sırasıyla %16 ($p<0.01$), %13 ($p<0.01$) ve %18 ($p<0.01$) şeklindeydi. Normal motilite gösteren olgularda ise ASA yüksekliğine %4 rastlanılırken, astenozoospermili olgularda bu oran %16 ($p<0.01$) oldu.
Tartışma: Bu çalışmamız, sperm morfoloji bozuklukları ile motilite arasında yakın ve anlamlı bir ilişki bulunduğunu ortaya koymuştur. Morfolojik bozukluğun lokalizasyonu motiliteyi etkilemesi bakımından önem taşımaktadır. Motilitenin normal olduğu olgularda da ASA aranması özellikle sperm baş defekti olgularında pozitif sonuç verebilir.

Anahtar kelimeler: spermatozoa, morfoloji, motilite, infertilite.

Summary

Purpose: To establish the relation between the localization of the morphological alterations of the spermatozoa and motility, as well as its effects on fertilization.
Materials and Methods: Sperm motility and morphological analysis according to the Kruger's strick criteria were done in 388 infertile men in whom female factor were found to be normal. In a group of cases antisperm antikor (ASA) (IgG and IgA) attachment ratio on the spermatozoa were also tested. Results were statistically compared.
Results: In cases with head abnormalities a + b motility rate was found approximately as 65 % ($p>0.05$). However, in cases with mid-piece and tail deformities this was found as 15% ($p<0.01$) and 20% ($p<0.01$), respectively. Antisperm antibody positivity (ASA >10%) was also high in these cases: 16% ($p<0.01$) in head-abnormality cases, 13% ($p<0.01$) in mid-piece-abnormality cases, and 18% ($p<0.01$) in tail abnormality cases. Only 4% of the men with normal spermiyogram parameters presented ASA positivity, however, in men with asthenozoospermia this rate was found to be 16% ($p<0.01$).
Discussion: Our study revaled that there is a close relation between the morphological abnormalities of the sperms and their motility. Localization of the morphological abnormality gains importance due to its effect on the motility. Investigation of the ASA in cases with normal motility, especially in cases with abnormal sperm head formation, may also give positive result.

Keywords: spermatozoa, morphology, motility, infertility.

Introduction

The attainment of fertilizing ability of spermatozoa is associated with cytological changes in the acrosome of the sperm surface, in the structural quality of the nuclear chromatin and certain tail organelles, and in the capacity for sustained progressive motility (12). The motile tail of a sperm is a long flagellum whose central axoneme emanates from a basal body situated just posterior to the nucleus. The axoneme consists of two central singlet microtubules surrounded by nine evenly spaced microtubule doublets. The usual 9+2 pattern of the axoneme is further surrounded by nine outer dense fibers composed mainly of keratin. Flagellar movement is driven by dynein motor proteins, which use the energy of ATP hydrolysis to slide the microtubules (3). The ATP is generated by highly specialized mitochondria in the midpiece.

A normal spermatozoa consists of two morphologically and functionally distinct regions: *tail* and *head*; both are enclosed by a single plasma membrane (6). Whereas the lipid bilayer determines the basic structure of biological membranes, proteins are responsible for most membrane functions, serving as specific receptors, enzymes, transport proteins, and so on. All proteins begin being syntesized on ribosomes in the cytosol, except for the few that are synthesized on the ribosomes of mitochondria. All proteins destined for *the intracellular membrane-bounded organelles, transport vesicles* and *the plasma membrane* are first imported into the endoplasmic reticulum *(ER)* from the cytosol. Proteins made in the ER are automatically delivered to the *trans Golgi network* and then to the plasma membrane by mainly the *membrane-bound protein translocators* and *transport vesicles* (1).

All organelle in the cytoplasm have the membrane structure in the same structure. Many vital biochemical processes take place in or on membrane surfaces (7). Each organelle membrane must also have a mechanism for importing, and incorporating into the organelle, the specific proteins that make the organelle unique. Also, *microtubule motor proteins* are largely responsible for the spatial organization and directed movements of organelles in the cytoplasm (10). In addition, mitochondria occupy a substantial portion of the volume of spermatozoa, and have highly specialized membranes that play a crucial part in its activities. During the development of the flagellum of the sperm tail, microtubules wind helically around the axoneme, where they are thought to help localize the mitochondria in the tail (2,4). So that, any abnormalities in protein transport and in membrane structure would cause both morphological deformities as well as functional abnormalities. For this reason, in asthenozoospermic spermatozoa, structural abnormalities of these organelle such as microtubules or plasma membrane can also be associated with functional abnormalities such as impaired motility. Regarding these observations, we decided to correlate the morphological alterations of the spermatozoa with motility patterns, clinically.

Material and Methods

388 men referring to our reproductive disorders department with infertility problems and normal female factor were included into the study. In all subjects; detailed anamnesis were taken and physical examination were performed. Serum hormon analyses and biochemical investigations were done. Testicular structure and blood flow were evaluated with color flow Doppler sonography.

Sperm density and motility were measured in Makler chamber (15). Motility was defined as the proportion of motile spermatozoa in an ejaculate. Forward progression was classified by light microscopy on an standardized scale: *a) forward progressiv - rapid; b) forward progressiv - slow; c) nonprogressive; d) immotile*. To discriminate dead and viable spermatozoa, HOS and eozin-Y vitality tests were done.

To assess sperm morphology, spermatozoa were stained with *SpermMac*. According to the so-called strict criteria, following characteristics were used to define a normal spermatozoon (11):

- Smooth oval head 5-6μm in length and 2.5-3.5μm in diameter.
- Well defined acrosome involving 40-60% of the head
- No defects of the midpiece or tail
- No cytoplasmic droplets more than half the size of the sperm head
- Borderline forms were considered abnormal.

Three different types of sperm abnormality were taken into consideration: *Head, Neck and midpiece, Tail*. In classification of the patients, the most prominent type of the abnormality was taken into consideration. Sperm surface antibodies (IgG and A) were detected by the mixed antiglobulin reaction (MAR) test (15). A value of 10% or more of motile sperm carrying latex particles constituted a positive result.

Results

We found a possitive correlation between the age and impairments in sperm motility and morphology parameters (*table 1*).

Table 1: Correlation between the age groups and seminal parameters

	Age groups		
	20 – 29	30 – 39	40 – 50
Volume (ml)	5 (2-7)	3.5 (1.5-4.5)	2.9 (1-4)
Concentration (10^6/ml)	15 (0-45)	11 (0-52)	7 (0-12)
Motility (a + b)	35 (10-60)	25 (10-45)	10 (0-40)
Normal morphology	16 (2-30)	10 (2-18)	6 (0-12)

In 34% of the cases varicocele was found as a responsible factor for infertility (*table 2*).

Table 2: Possible etiological factors faund in the infertile patients

	Patient no.	**%**
Varaicocele	131	34
Cryptorchidism	15	4
Orchitis,epididymitis	24	6
Chronic prostatitis	43	11
Leukocytospermia	63	16
Retrograde ejaculation	3	1
Erectile dysfunction	7	2
Hormonal disorders	7	2
Trauma	5	1
Testicular tumors	3	0.8
Inguinal operations	1	0.2
Ejaculatory duct obstructions	20	5
Idiopathic	66	17
Total	388	100

There was no significant correlation between the head defets of the spermatozoa and motility properties. However, morphological abnormalities of neck, midpiece and tail were significantly associated with impaired sperm motility (*table 3*). In majority of the teratozoospermic men, forward motility lack were present in their spermatozoa.

The incidence of positive antisperm antibodies was higher in men with increased abnormal sperm forms as well as

Table 3: Correlation between the morphological abnormalities and sperm motility types

	Motility types					
	Pts. No.	a	b	c	d	
Head	105	40	25	25	10	p>0.05
Neck,Midpiece	144	10	5	55	30	p<0.01
Tail	91	5	15	20	60	p<0.01
Normal form	48	40	30	20	10	

decreased sperm motility (*table 4, 5*). No significant correlation was found between the localization of the morphological defects and ASA rates.

Table 4: Correlation between the antisperm antibody rates and morphological bnormalities

	Pts. no.	ASA > 10%		
		Pts. no.	%	
Head	18	3	16	p<0.01
Neck/Midpiece	30	4	13	p<0.01
Tail	11	2	18	p<0.01
Normal forms	26	1	3	

Table 5: Correlation between the antisperm antibody rates and sperm motility types

	Pts. no	ASA > 10%		
		Pts. no	%	
Normal motility	23	1	4	
Asthenozoospermy	62	10	16	p<0.01

Discussion

Our results indicate that the sperm with abnormal morphology are more likely to be immotile, and if motile, to swim slower than normal sperm. In this study sperm motility was found to be less effected in cases with head abnormalities than in those of neck/mid piece and tail defects. When forward progression was impaired, majority of the cases had neck/midpiece or tail defects as well. However, in these cases sperm head was completely in normal shape.

Movement quality of the spermatozoa varies enormously and may give a prognosis of conception. However, motility alone is poorly correlated with pregnancy rates in vivo and fertilization rates in vitro (16). In some patients with necrozoospermia, the sperm are alive with normal morphology yet are immotile because of structural defects in the axonemal component of the flagella. Sperm samples can be classified by capacitation patterns, including forward progressive, transition phase, and hyperactivated motility, and the lateral movement of sperm heads may confer fertility. Spermatozoa are immotile in some patients with deficits in central microtubules, axoneme, or fibrous sheat. Spermatozoa with absent structures such as globozoospermia and its malformations as in tapering heads, microheads and amorphous heads, are motile, lack acrosome and postacrosomal sheat, have abnormal midpieces and mitochondria, and cannot achieve fertilization (13).

According to our findings, the conditions with neck/midpiece or tail abnormalities were associated with suppressed sperm motility. Some defects arising in number or arrangement of mitochondria, in the axoneme, or in the microtubule arrangement suppresses sperm motility (9). In a shortened midpiece anomaly, mitochondria may be defective. Coiled, broken, or double tails, tail aprotrusion and agenesis, and disorganized tails impair sperm motility and the function (8). In cases with defective tail, the fibrous sheat may be thickened, absent, or defective as well as poor development of outer dense fibers. In these cases sperm motility may be suppressed.

We observed that when the rate of cytoplasmic residues were increased sperm motility were also significantly impaired. Human spermatozoa exhibit a capacity to generate reactive oxygen species (ROS) and initiate peroxidation of the unsaturated fatty acids in *the sperm plasma membrane*, which plays a key role in the etiology of male infertility (14). It has been suggested that abnormal retention of cytoplasmic residues by human spermatozoa is associated with the generation of ROS in semen and defective sperm function (17). For example, varicocelectomy can improve the disposal of residual sperm cytoplasm by the testis and/or epididymis in infertile men with varicocele, and reduces the potential for ROS generation by human spermatozoa in these men.

Ultrastructural abnormalities of spermatozoa may impair sperm motility. However, motility alone is poorly correlated with pregnancy rates in vivo and fertilization rates in vitro. In some patients with necrozoospermia, the sperm are alive with normal morphology yet are immotile because of structural defects in the axonemal component of the flagella (5).

We believe that ultrastructural abnormalities of spermatozoa may impair sperm motility, and multiparametric evaluation of spermatozoa are needed, since considering a single parameter does not help predict suspected male infertility, and additional variables describing sperm function are also necessary. Although there is no clear proofs, in infertility cases with normal sperm motility, ASA directed against to sperm head region should also be investigated especially in the cases with sperm head defects. However, more studies on the biophysical effects of sperm plasma membrane structure and function on the sperm flagellar movement, both in fertile and infertile males, will be required to delineate fully the interrelation of sperm morphological alterations and motility characteristics.

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