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SPERM CHANGES IN CASES WITH VARICOCELE

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Abstract

The aim of this study is to determine the percentage of spermatozoa with abnormal morphology in ejaculates of men with Varicocele by morphometric measurements. Cytological and morphometric studies were performed on sperm samples according to the WHO criteria on 329 patients with vascular disease Varicocele and a control group of 20 healthy men. The results showed the highest percentage of spermatozoa with head anomalies (64%), while tail anomalies and cytoplasmic droplets were rarely observed. In conclusion, the results of the present study suggest a narrow dependence between the degree of degenerative changes in the morpho-functional characteristics of spermatozoa.

Key words: male infertility, morphometric measurements, spermatozoa, Varicocele

Introduction. One of the most common pathological causes of male infertility is the idiopathic Varicocele [1] which is characterized by venous blood stasis in the scrotum and disturbances in the testicular metabolism. The haemodynamics of the male genitals is impaired which results in fertility problems in active reproductive-aged patients [2].

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Varicocele is characterized by a high percentage of subfertility. It represents a cause of primary infertility in 30% of the cases, and it leads to a secondary infertility in 80% of the patients [3].

Changes of the sperm parameters in patients with Varicocele are first reported by McLeod [4] in 1965 and later by other authors [5,6] and they comprise of Oligozoospermia, high percentage of abnormal gametes (teratospermia) and reduced sperm motility even Astenospermia.

The morphometric measurements allow evaluation of the parameters both of normal spermatozoa and those with alterations in the size and shape of the head and tail [7,8]. These studies add new insights to the morphological characteristics of the abnormal gametes.

The aim of the present study is to determine the percentage of spermatozoa with abnormal morphology in ejaculates of men with Varicocele by morphometric measurements.

Material and methods. Cytological and morphometric studies on ejaculates of 329 patients with Varicocele were carried out according to the WHO criteria [9]. The results were compared with those of 20 healthy men.

The following methods were used:

- Medical history and physical examination.
- Light microscope studies of spermatozoa after routine staining with Yashkovski, Papanicolaou, haematoxyline-eosin, etc.
- Morphometric studies of spermatozoa for evaluation of a number of morphometric parameters characterizing the sperm cell size and shape: L = longitudinal diameter – length; W = transversal diameter – width.

  The following formulae for determining the length of both diameters were used:

  $$L = K_1 \times n_1, \quad T = K_1 \times n_1,$$

  L = length of the longitudinal diameter;
  T = length of the transversal diameter;
  K_1 = const.;
  n_1 = number of divisions of the eyepiece micrometer on the measured length;

  - Ratio length/width (L/W);
  - A – area of the head;
  - C – perimeter of the head.
- The following criteria for evaluation of the fertilizing ability in Varicocele patients were used: preserved fertilizing ability, relatively preserved fertilizing ability, poor fertilizing ability, missing fertilizing ability.

Table 1

Morphometric studies of a spermatozoid head

Head forms		Morphometric parameters ($X \pm \mathrm{SD}$)		
		L (µm)	W (µm)	L/W
Normal		4.36 ± 0.58	2.59 ± 0.03	1.49 ± 0.21
Injured configuration	Microcephalic	5.67 ± 0.31	3.25 ± 0.89	1.85 ± 0.41
	Macrocephalic	2.52 ± 0.53	$1.75 \pm 0.59^{**}$	1.52 ± 0.53
	Round	3.84 ± 0.91	3.84 ± 0.91	1
	Elongate	4.78 ± 0.30	$1.57 \pm 0.18^{**}$	$3.09 \pm 0.36^{***}$

Data are represented as mean geometrical values (95% confidence limits);

L = length; W = width; $^{**}p < 0.01$; $^{***}p < 0.001$

- Statistical analysis by Student's t-test using statistical package SPSS. Difference was considered significant at $p < 0.001$.

Results. ***Changes in the size and shape of the spermatozoid head.*** Table 1 summarizes the results for the mean values of the measured parameters (length L, width W and L/W ratio) of spermatozoa with normal and abnormal head shape (Fig. 1).

The study on microcephalic configurations shows abberation in L and W parameters, while elongated heads are characterized by significant changes only in the head width. For that reason we observe considerable aberration in the ratio L/W (3.9) for the elongated abnormality in comparison to the normal and other head shapes (except for the round shape).

Data from measurement of the area (A) and perimeter (C) of the sperm heads reveal significant aberration especially in the microcephalic and macrocephalic shapes as compared to the spermatozoa with normal-sized heads (Table 1).

The morphological studies in Varicocele show two predominant types of abnormal head: elongated (including pyriform and tapered) and microcephalic (Fig. 2). The first configuration has the highest percentage (31.50%) and the microcephalic gametes represent 25% of the abnormal shapes. Macrocephalic (9%) and round-headed (4.50%) spermatozoa are also observed. The other atypical forms (amorphous and double head) are rare.

Another morphological alterations in shape of the spermatozoa. Other anomalies in the sperm morphology such as cytoplasmic residue or flagellum malformation are rarely registered (between 3 and 5%) in comparison to the head abnormalities.

Gametes with no tail or coiled tail are most commonly observed, the short and broken tail can rarely be seen (Fig. 3*a–c*).

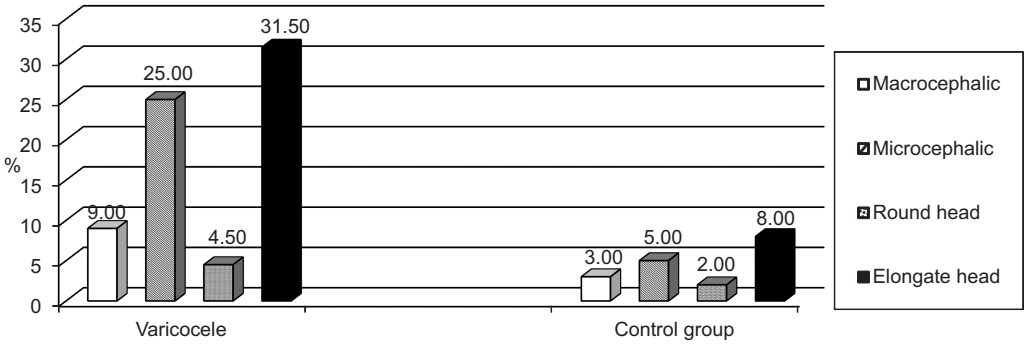

Fig. 2. Anomalies in a spermatozoid head (%)

The cytoplasmic droplet, as a particular deformation, is most frequently found around the neck, embracing the postacrosomal section of the head or the anterior part of the mitochondrial sheath (Fig. 3*e*–*f*).

Besides spermatozoa with single defects, a high percentage of gametes with two or more anomalies in the sperm head and tail (12%) accompanied by cytoplasmic droplet is observed in Varicocele (Fig. 3*d*).

The results show the highest percentage of sperm head anomalies (64%), less commonly observed tail anomalies and rarely seen cytoplasmic droplet.

Spermatological parameters. In 48.02% of the patients with Varicocele we found reduced concentration of the mature germ cells in the semen plasma – various grades of Oligozoospermia (Gr. I–III) with mean number of gametes 18.43 mln/ml (1–39 mln/ml). The reduced number of spermatozoa in Oligozoospermia (Gr. I–III) is above four times lower in comparison to the control group and the cases with Normospermia (Table 2).

On the other hand, the viability of the gametes plays an important role in the assessment of their fertilizing ability. Our results demonstrate reduced percentage of the vital gametes in ejaculate samples of men with Normo- and Oligozoospermia by 1.4-fold and 1.6-fold, respectively, as compared with the control group (Table 3).

T a b l e 2

Mean values of sperm concentration (mln/ml) in ejaculate samples from Varicocele patients and vitality of spermatozoa

Patients	Normospermia (mln/ml)	Oligozoospermia Gr. I–III (mln/ml)	Control group (mln/ml)
Varicocele	51.98%	48.02%	
($n = 329$)	73.07 ± 31.08	18.43 ± 10.6	78.73 ± 18.45
Vitality	59.84%	52.28%	83.47%

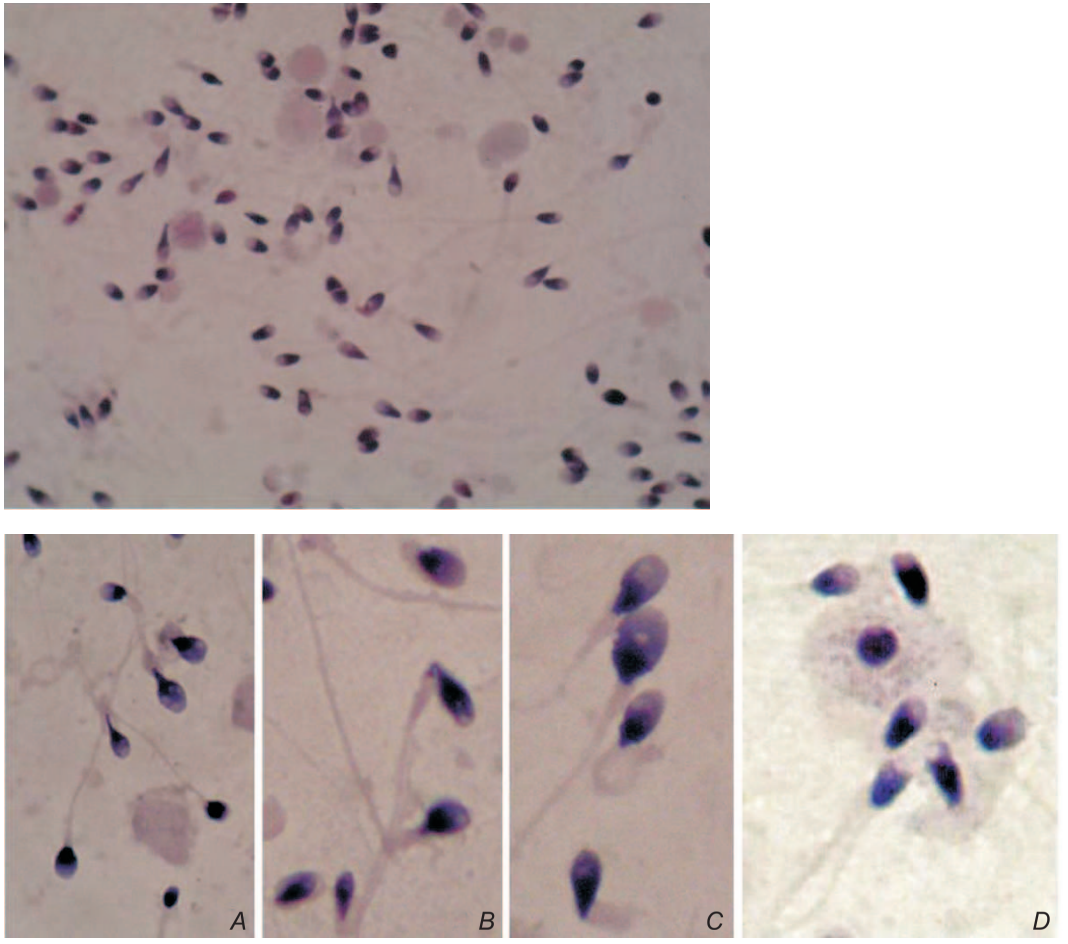

Fig. 1. Spermatozoa with abnormal head: Overview (top); *A*) small, macrocephalic, round head; *B*) elongate head; *C*) pyriform and megalohead. Papanicolaou. ×450; *D*) normal, elongate and degenerating spermatid. HE. ×600

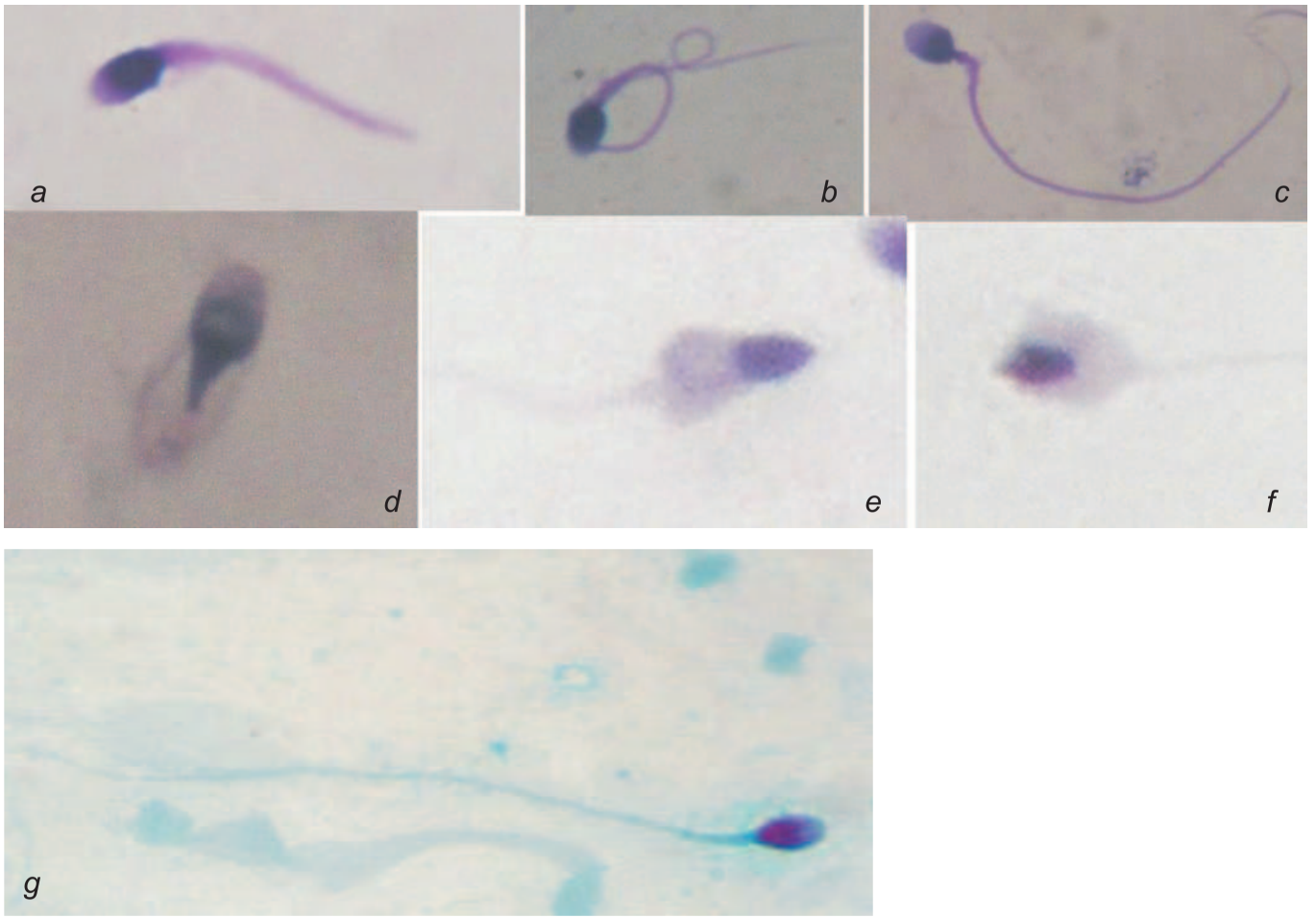

Fig. 3. Spermatozoa with tail defects, cytoplasmic droplet and mixed anomalies: *a–c*) short, broken and coiled tail; *d*) mixed type anomalies. Yashkovsky. ×600; *e–f*) cytoplasmic droplet; *g*) normal gamete. HE. ×600

Table 3

Percentage of spermatozoa with normal and impaired morphology in ejaculates from Varicocele patients

Patients	Number of spermatozoa (mln/ml)	Gametes with normal morphology (%)	Gametes with impaired morphology (%)
Varicocele ($n = 329$)	$n = 171$ Normospermia 73.07 ± 31.08 $n = 158$ Oligozoospermia Gr. I–III 18.43 ± 10.60	Normospermia 41.54 Oligozoospermia Gr. I–III 36.68	Normospermia 58.46 Oligozoospermia Gr. I–III 63.32
Control group ($n = 20$)	78.73 ± 18.45	78.63	21.37

The relationship between the number of spermatozoa and the percentage of gametes with normal and abnormal morphology in the ejaculate samples from Varicocele patients is shown on Table 3. The results demonstrate a significant increase in the number of the abnormal germ cells in the Varicocele groups with Normo- and Oligozoospermia.

The Varicocele pathology is characterized by a combination of a low cellular concentration and an increased content of abnormal spermatozoa in the semen. However, in 20.53% of the patients with Oligozoospermia we found relatively low quantity of metamorphic gametes and, at the same time, the number of morphologically normal and vital germ cells predominated.

Besides, the comparison of the number of spermatozoa and motility in the semen makes it possible to draw a conclusion on the degree of the functional disturbances in Varicocele, and the degree of man infertility (Table 4).

Table 4

Motility, number and velocity of spermatozoa in cases with Varicocele

Patients	Motility (%)			Velocity
Number gametes (%)	Progressive motile	Non-progressive	Immotility	(μ/s)
Varicocele ($n = 329$)				
Normospermia (51.98)	45.19 ± 3.43	23.52 ± 1.41	32.04 ± 3.60	9.40 ± 0.63
Oligozoospermia (48.02)	28.21 ± 4.19	21.46 ± 2.17	50.50 ± 5.18	6.44 ± 0.8
Control group ($n = 20$)	64.17 ± 5.07	15.90 ± 5.13	19.93 ± 4.04	16.52 ± 2.93

In most patients with Normospermia, the sperm motility is recognized as progressive (50%) and non-progressive (30%) with velocity values between 4 and 18 µm/s, and the remaining 20% of the cases show sperm immotility. In nine cases of Oligozoospermia, there are no spermatozoa with high motility, the percentage of poorly motile gametes is between 10% and 50% with velocity < 5 µm/s, and the akinetic germ cells are 50% and 90%. In all other cases, the sperm motility does not differ substantially from those in Normospermia.

Based on the above mentioned criteria for the degree of male infertility we can assess the fertilizing ability of patients with Varicocele. We estimated it preserved in 33.74% of the cases, relatively preserved – in 33.43%, poor – in 39.81% and missing – in 8.52% of the cases.

Discussion. The ratio between the number of abnormal germ cells and the total number of spermatozoa in the ejaculate is one of the main factors for determining the rate of male fertility. Generally, the reduced number of gametes in the semen and/or the increased presence of morphologically immature abnormal spermatozoa (on the account of the normal) are the consequences of the disturbances in the normal spermatopoetic process. Most of the reproductive system disorders can cause permanent damage to the spermatogenesis which results in infertility and, in some cases, sterility [10].

The morphometric studies on the two structural sperm head components – length and width, as well the ratio L/W (in normal ellipse-oval shape), the head area and perimeter are used for assessing the biological and functional sufficiency of the germ cells [11, 12]. These parameters are components of the morphological characteristics of the spermatozoa and their values reflect the morphometric variety. Each aberration in the normal structure of the gamete can give valuable indication of disturbances in the male reproductive system and fertilizing ability.

In the ejaculate samples from Varicocele patients we found increased number of morphologically damaged spermatozoa ($p < 0.001$). Most often, the disturbances affect the shape and the size of the head and rarely the flagellum.

Our results indicate a tendentious manifestation of germ cells with elongated head configuration together with microcephalic. They suggest that Varicocele affects the stages of spermiogenesis – early and/or late spermatid. We can assume that these changes depend on the severity and the duration of the disease and they are individual [7].

The sperm head impairment as well the injury of the flagellar segments reduce the germ cells motility and their fertilizing ability, respectively [13], and they are a common cause of male infertility.

There is an increased concentration of toxic metabolites or endocrine substances secreted by the adrenal glands and the kidney as a result of the venous reflux and the testicular edema, which are an important factor for suppression of spermatogenesis. The prolonged venous stasis and edema [14] result in elevated temperature [15] and testicular hypoxia [16].

On the other hand, the reduced oxygen saturation has an impact on the cell-tissue metabolism which is probably the main reason for the high percentage of germ cells with damaged morphology and the presence of spermatozoa with elongated and microcephalic heads in the ejaculate.

Therefore the results concerning the concentration of gametes with normal and/or impaired morphology show a tendency toward higher percentage of damaged cells mainly in Normo- and Oligozoospermia ejaculates from Varicocele patients. Nevertheless, some cases of Oligozoospermia with a low content of abnormal cells and prevalence of the normal gametes arouse interest. This is a fact of great importance for applying an adequate specific or individual therapy to the patient [3]. These examples suggest that patients with reduced expression of spermatozoa but high percentage of morphologically normal gametes with progressive motility can have a better fertilizing ability and more favourable prognostic assessment in comparison to Normospermia cases with predominant motile or immotile gametes [17,18].

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