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GLOBOZOOSPERMIA DHE RËNDËSIA KLINIKE CITOMORFOLOGJIKE

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Abstrakt

Analiza citomorfologjike e spermës është një pjesë thelbësore per diagnostikimin e infertilitetin mashkullor. Përveç vlerësimit të morfologjisë së spermës, analiza e spermes (spermiograma)duhet të përfshijë vlerësimin cilësor të elementeve qelizore të ndryshme nga spermatozoidet. Ngjyrosja e duhur e analizes rutinë të spermës na jep mundesine te të diferencojme "qelizat e rrumbullakëta" (të cilat duhet të diferencohen në lidhje me llojin e tyre dhe anomalitë citomorfologjike leukocitet, si neutrofilet dhe makrofagët. Për më tepër, mund të identifikohen eritrocitet, qelizat epiteliale si dhe aglutinimi i spermatozoideve.

GLOBOZOOSPERMIA AND CLINICAL RELEVANCE OF CYTOMORPHOLOGICAL

Abstract

Cytomorphological semen analysis is an essential part of the diagnostic work-up for male infertility. Apart from the assessment of sperm morphology, it should include the qualitative evaluation of cellular elements other than spermatozoa. Appropriate staining of routine semen smears allows one to differentiate "round cells" (which need to be differentiated with regard to their type and cytomorphological abnormalities) from the completely distinct population of leukocytes, such as neutrophils and macrophages. Moreover, erythrocytes, epithelial cells as well as agglutination of spermatozoa can be identified.

Introduction

Cytomorphological analysis of sperm pathology represents an important step beyond mere counting of spermatozoa with a normal shape. Systematic assessment of morphological sperm defects and characterization of their severity give some insight into testicular pathologies, i.e. those affecting spermato- and/or spermiogenesis, as well as disturbances of epididymal function.

The first to mention 'Rundkopfspermatozoen' (round-headed spermatozoa in German) after light microscopic analysis (Figura 1) was Meyhöfer (1965) discovered that their round shape was caused by a round nucleus lacking an acrosome. Intensive research during the eighteenth and nineteenth centuries established the testicular origin and fundamental role of spermatozoa in fertilization. The introduction of modern morphological, biochemical and molecular techniques during the twentieth century resulted in characterization of various distinct sperm abnormalities in infertile males. Standardized assessment of the conventional semen parameters, namely sperm concentration, motility and morphology, is mandatory for characterization of semen quality and an integral part of male infertility investigation.

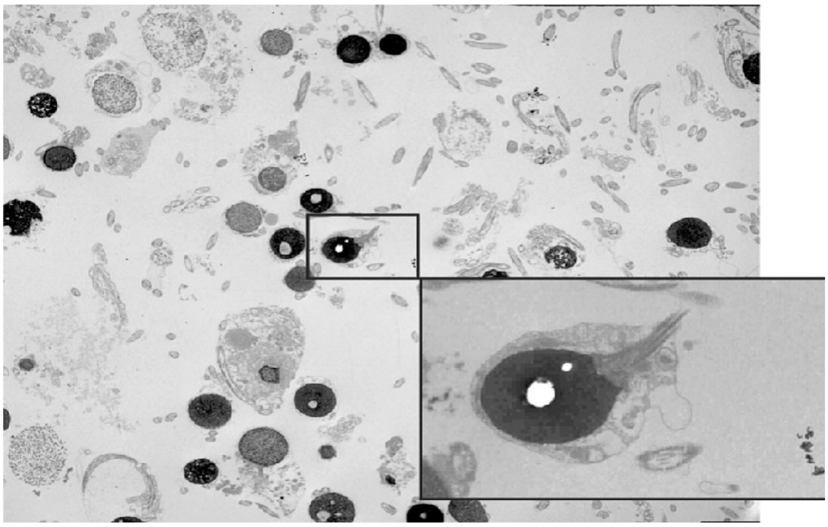

Figure 1 : Globozoospermia. Overview imaged by Transmission Electron microscopy

CASE REPORT

The first case of globozoospermia, was found after the examination of 3000 patients undergoing routine andrological screening, indicating an incidence of 0.03%.

Patient H.K a 37-year-old-man admitted to our clinic, had two marriages , seven years with the first and four years with his second wife. He affected consultations and semen analysis 22 times in 6 different laboratories. The semen analysis which he reported has a: 43,0 x 10^ 6 Concentration (million/ml) ; progressive sperm motility by 54%- 68% and normal morphology (Figure 2) .

Case 2; Patient B.I a 32-year-old-man, married by 6 years. He affected 14 consultation and semen analysis in 4 different laboratories. The semen analysis which he reported has a: 72 x 10^ 6 Concentration (million/ml) ; progressive sperm motility 64% and normal morphology .

Both patient, had no previous history of varicoceles or cryptorchidism and was not exposed or workplace gonadotoxines (radiation, chemotherapy agents, pesticides, excessive heat and drugs such as nicotine, alcohol, marijuana and steroids). Physical examination showed a normal body mass index, testicular volume of 4.5 cm (L),2.5 cm (W),2.5 cm (H), no gynecomastia. Serum LH, FSH, testosterone and prolactin levels were normal. This brings us that the diagnostic value of further cytomorphological semen analysis is often neglected

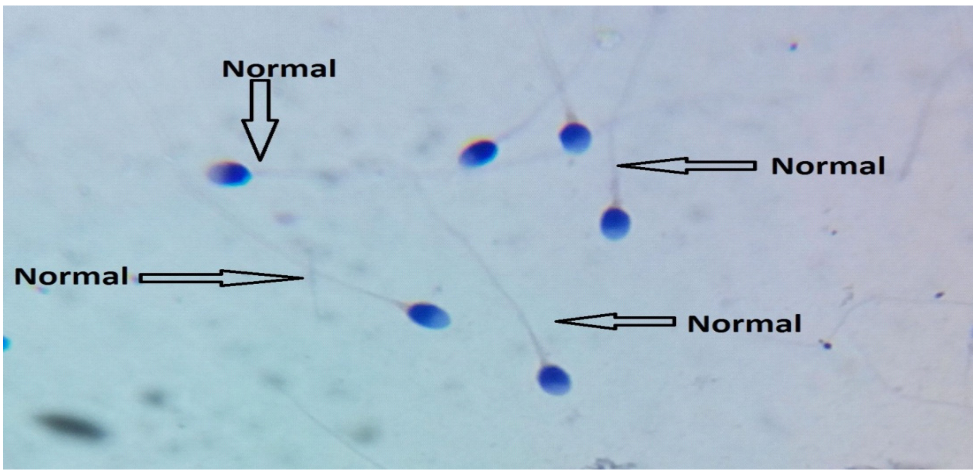

Figure 2 : Normal morphology.

(Medical center –Dani Andrology)

METHODOLOGICAL ASPECTS

The history of sperm morphology evaluation consists of two approaches or phases, which are also reflected in the evolution of sperm morphology evaluation criteria in the consecutive WHO manuals, from the first in 1980 to the soon-to-be-published fifth edition [1, 2–3]. The two approaches are the early, or liberal, approach [4] and the strict (Kruger) approach, as introduced by Menkveld in 1987 [5] and described in detail by Menkveld et al. in 1990 [6].

Strict Kruger criteria

One of the most important aspects of the strict evaluation criteria is that the range allowed for these minute normal biological variations has to be kept as small as possible. For this reason, the socalled 'borderline' or 'slightly abnormal' head forms are regarded as abnormal. In the fifth edition of the WHO manual [1], a new approach has been adopted with regard to the establishment of so-called 'normal,' or reference, values. By strict evaluation, the description of a morphologically normal spermatozoon is furthermore supported by the morphological appearance of spermatozoa found tightly bound to the human zona pellucida [7, 8]. In the fifth edition of the WHO manual [1], a new approach has been adopted with regard to the establishment of so-called 'normal,' or reference, values. The proposed value in the fifth edition is very low, 4% morphologically normal spermatozoa, based on the fifth centile of combined data from several reports in recent publications, in which semen analyses were performed according to known and standardized methodologies [9]. For optimal differential staining of spermatozoa, Papanicolaou or Shorr stain are recommended, alternative procedures include commercially available kits for rapid staining such as "Diff-Quik". Two hundred spermatozoa should be evaluated under oil-immersion at a magnification of 1000–1250×. Notably, spermatozoa with acrosome disorders have been shown to exhibit disturbances of chromatin condensation (Hofmann and Hilscher 1991; Chemes and Rawe 2003) (Figura 3).

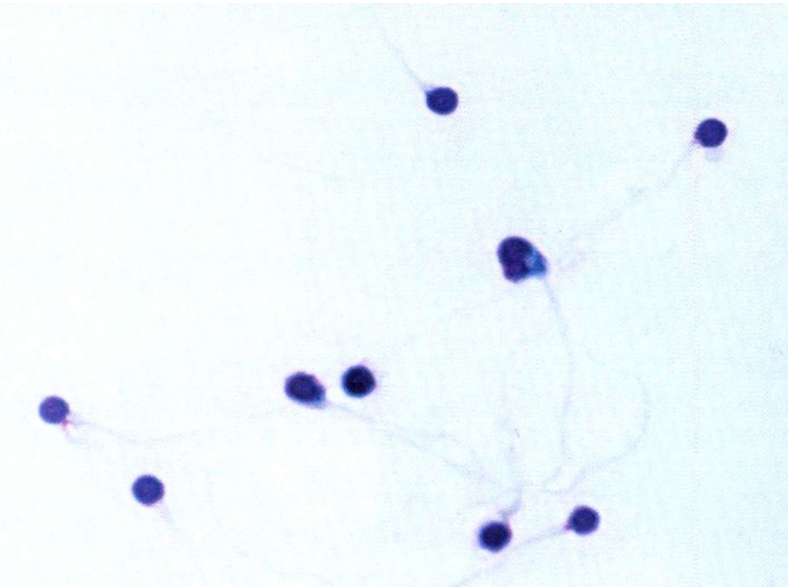

Figura 3 : Globozoospermia. Overview imaged by phase contrast microscopy under oil-immersion (Klinika Dani)

Sperm morphology evaluation

Systematic assessment of morphological sperm defects and characterization of their severity give some insight into testicular pathologies, i.e. those affecting spermato- and/or spermiogenesis, as well as disturbances of epididymal function, but the high-degree acrosome disorders and related combined defects are considered to be mostly of genetic origin and not reversible. Appropriate staining of routine semen smears allows the assessment of different abnormalities in individual patients is heterogeneous (Chemes and Rawe 2003). On the other hand, some patients reveal "systematic" defects such as globozoospermia affecting the majority of spermatozoa in a semen sample and resembling similar defects in other individuals. The first group will have a poor prognosis because of mostly genetically determined sperm pattern defects, such as globozoospermia; short tail syndrome; spermatozoa with small heads, in most cases combined with very small acrosomes; cases of large-headed spermatozoa, mostly combined with the presence of large acrosomes; and the presence of large or small acrosomes alone. All of these types of abnormal spermatozoa have been associated with abnormal sperm functions and, thus, with poor in vivo and IVF rates.

Genetically determined sperm defects

Globozoospermia

Globozoospermia is classified as total owing to the absence of any sperm with acrosome (type 1) and partial in that the ratio of roundheaded spermatozoa is less than 100% (i.e., 20-90%) and acrosomal vesicles are present in the rest of the spermatozoa (type 2) (10). The most important morphological characteristics of these spermatozoa are including round headed, acrosomeless, round nucleus, no post-acrosomal sheath and coiled tail. Normally, the highly condensed chromatin protects the sperm DNA from radical oxygen species; poorly condensed DNA is more prone to damage (Sakkas et al, 1999). It is shown that globozoospermia as well as other abnormal morphologic forms of human spermatozoa is associated with DNA strand breaks and abnormal chromatin structures. Although, some studies have shown no significant increase in DNA denaturation or sperm DNA fragmentation in globozoospermic cases compared fertile men (11-12,13), Sutovsky et al in 2001 with the introduction of SUTI (sperm-ubiquitin tag immunoassay) technique showed that globozoospermic are highly ubiquitinated that indicated their damaged DNA (14). In fact, these results suggest that abnormal globozoospermic spermatozoa can be carrier of abnormal chromatin as a possible source of DNA fragmentation. A genetic basis for globozoospermia was suspected and is supported by limited number of reports of families with two or more affected siblings. Although, some authors suggested that the inheritance of familial globozoospermia may be autosomal recessive (15), autosomal dominant (16), or polygenic , the exact mode of inheritance is not clear. In a recent study (17), a consanguineous family with three affected brothers, in whom the authors identified a homozygous mutation in the spermatogenesis-specific gene SPATA16 has been presented. In our patient, we also failed to present any microdeletion in chromosome Y.

Morphological characteristics of globozoospermia

100% round headed (20)	Disturbed midpiece (4)
Acrosomeless (8)	Abnormally arranged mitochondria (4)
<100% round headed (20)	Acrosome separated from nucleus (2)
Round nucleus (14)	Post-nuclear ring absent (2)
No postacrosomal sheath (12)	Chromatin treads (2)
Coiled tails (partially) (11)	Atypical implantation of the sperm tail (1)
Abnormally condensed nucleus (9)	Double heads (1)
Vacuoles in nucleus (6)	Tail abnormalities (1)
Separated nuclear envelope (6)	Invagination (1)
Cytoplasmic droplet (6)	

DISCUSSION

There is increasing evidence that sperm morphology plays a significant role in ICSI outcome (Chemes and

Rawe 2003). Although fertilization and pregnancy rates after ICSI were reported tobe independent fromthe average percentage of normal forms in a semen sample, recent data suggest that pathomorphology of the individual sperm. However, well defined morphological abnormalities such as complete globozoospermia and pin-head have been strongly associated with sterility even in assisted reproductive cycles (18, 10). Of interest, the outcome of intra-cytoplasmic sperm injection (ICSI) cycles found to be lower when compared with patients with male factor rather than specific morphological abnormalities (19 -22)). Therefore, examination of sperm morphology should not be underestimated to define specific sperm abnormalities in male patients with infertility. Genetic causes may account for 10-15% of severe male infertility including chromosomal aberrations and single gene mutations. Therefore, someone may hypothesize that in patients with globozoospermia, a higher risk of creating aneuploid embryos after ICSI treatment should be warranted.

In conclusion, the familial appearance of globozoospermia is extremely rare and the underlying inheritance is not clear. To be concluded although this study did not find any Y-chromosome microdeletions in patients with globozoospermia, the exact genetic locus resulting in this pathology requires further study.

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