Importance of Papanicolaou Staining for Sperm Morphologic Analysis

Comparison With an Automated Sperm Quality Analyzer

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Key Words: Leukocytospermia; Papanicolaou; Sperm analyzer; Sperm morphologic features

DOI: 10.1309/AJCpLCSPP24QPHR

Abstract

Without experience or proper training, the evaluation of the morphologic features of sperm can become the most confusing and time-consuming area of semen analysis. This study defined the role of Papanicolaou staining compared with an automated sperm analyzer in the management of infertility. We compared the morphologic features of sperm using Papanicolaou staining and an automated Sperm Quality Analyzer IIC-P (SQA-IIC-P; Medical Electronic Systems, Caesarea, Israel) and evaluated the role of factors affecting these features in semen samples from 201 infertile men. The sensitivity of the SQA-IIC-P was 85.5%, and the specificity was 87.3% compared with Papanicolaou staining. Although the positive predictive value of this instrument was 93.7%, the negative predictive value was only 73.3%. Moreover, sperm indices that are important for in vitro fertilization could be derived only by using the manual method. A strong correlation was seen with smoking and leukocyte sperm (P < .001).

The SQA-IIC-P can be used as a screening tool to evaluate the morphologic features of sperm. However, Papanicolaou staining to study the cytomorphologic features of sperm and the calculation of sperm indices should be done for quantification of defects.

Materials and Methods

The study included semen samples from 201 infertile men who came to our institution for semen analysis. A detailed
medical history (ie, smoking, alcohol use, occupational hazards, sexually transmitted diseases, trauma, surgery for varicocele or inguinal hernia, cryptorchidism, and drugs) was obtained in each case. Semen samples were prepared by masturbation after 3 days of abstinence and collected in sterile containers. Samples were analyzed with the SQA-IIC-P using a special capillary. From the remaining samples, smears were made, fixed, and stained by a modified Pap staining method for manual morphologic analysis. A total of 200 spermatozoa were scored per slide with an oil immersion objective.

The WHO criteria were applied, and a 30% cutoff was used for normal morphologic findings. All borderline forms were considered abnormal. Indices such as the TZI and the SDI were derived in each case, using 1.6 as the cutoff for both indices. TZI is the number of defects per total defective sperm, and SDI is the number of defects per total number of sperm. A microscopic evaluation for leukocytospermia was also performed to evaluate for the possible presence of infection or inflammation. Leukocytospermia was defined as more than 1 × 10⁶ leukocytes per milliliter of the semen sample.

**Statistical Analysis**

Statistical analysis was done to calculate the sensitivity, specificity, positive predictive value, and negative predictive value for the SQA-IIC-P in comparison with the manual method. The morphologic features of the sperm by the manual method and the analyzer were compared by using linear regression analysis. The study of variables affecting the morphologic features of sperm was done by using the χ² and Mann-Whitney tests.

**Table 1**

Cytomorphologic Sperm Defects on Papanicolaou Staining

<table>
<thead>
<tr>
<th>Classification</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head defects (mean ± SD, 141.65 ± 21.18; range, 54-189)</td>
<td>Large, small</td>
<td>Tapered</td>
</tr>
<tr>
<td></td>
<td>Pyriform</td>
<td>Round</td>
</tr>
<tr>
<td></td>
<td>Amorphous</td>
<td>Vacuolated</td>
</tr>
<tr>
<td></td>
<td>Small acrosomal area (40% of head area)</td>
<td>Double heads</td>
</tr>
<tr>
<td></td>
<td>Any combination of these</td>
<td></td>
</tr>
<tr>
<td>Midpiece defects (mean ± SD, 43.48 ± 21.167; range, 9-125)</td>
<td>Bent neck</td>
<td>Asymmetrical insertion into the head</td>
</tr>
<tr>
<td></td>
<td>Thick or irregular</td>
<td>Any combination of these</td>
</tr>
<tr>
<td>Tail defects (mean ± SD, 22.07 ± 15.038; range, 4-76)</td>
<td>Short</td>
<td>Multiple</td>
</tr>
<tr>
<td></td>
<td>Multiple</td>
<td>Hairpin</td>
</tr>
<tr>
<td></td>
<td>Broken</td>
<td>Bent</td>
</tr>
<tr>
<td></td>
<td>Bent</td>
<td>Irregular width</td>
</tr>
<tr>
<td></td>
<td>Coiled</td>
<td>Any combination of these</td>
</tr>
</tbody>
</table>

Results

The manual results were qualified as normal or abnormal based on WHO criteria. The morphologic defects observed with Pap staining were divided into head, midpiece, and tail defects. They were seen singly or in combination. Some important morphologic defects are shown in Image 1. An abnormal TZI was seen in 27 (13.4%) of 201 cases, and an abnormal SDI was seen in 3 (1.5%) of 201 cases.

In comparison with the Pap staining method, the results of the SQA-IIC-P for the morphologic features of sperm showed a sensitivity of 85.5% and a specificity of 87.3%. The positive predictive value was 93.7%, but the negative predictive value was lower (73.3%) owing to 20 abnormal cases that were not detected by the SQA-IIC-P. Linear regression analysis for the morphologic features of sperm showed good correlation between the manual method and the SQA-IIC-P (P ≤ .001; r = 0.7706).

While assessing variables affecting the morphologic features of sperm, we observed that the majority of the cases with abnormal morphologic features were in men with a history of smoking (n = 67), followed by alcohol use (n = 41). Other important variables in the histories were tuberculosis, lower abdominal surgery, sexually transmitted diseases, occupation, drug intake, and trauma. Only smoking showed a significant correlation with abnormal morphologic features and a high TZI (P < .001). Leukocytospermia was seen in 89 (64.5%) of 138 cases with abnormal morphologic features in comparison with 11 (17%) of 63 cases with normal morphologic features. This difference was statistically significant (P < .001). However, none of these variables were associated with specific morphologic defects.

Discussion

The morphologic features of sperm are the end result of a highly complex process of cellular modifications occurring during spermatogenesis. To standardize semen analysis, 5 major published classification systems have endured in clinical practice. Of these, the third and fourth editions of the WHO classifications are the modern classifications that are most recommended by fertility physicians. The WHO classification has set an empiric reference value of 30% normal forms or more as a normal result. The strict criteria define normal spermatozoa as having oval-shaped heads with a regular outline and an acrosomal cap covering more than one third of the head surface area. All borderline forms are considered abnormal. A TZI of 1.6 or more is associated with a lower pregnancy rate, and an SDI value of 1.6 or more is the cutoff for failure of in vitro fertilization. These indices can be derived only by using the manual method. The best stain for semen smears and observing the morphologic
features of sperm is the modified Pap stain, as done in the present study.

During the past 20 years, to avoid subjectivity, numerous studies that incorporate image analysis techniques such as computer-assisted semen analysis in the assessment of the morphologic features of sperm have appeared. The SQA-IIC-P, an upgraded version, is an inexpensive device that provides a quantitative estimation of sperm motility (the sperm motility index [SMI]). It is an automated system; the basic technology is different from computer-assisted semen analysis technology (signal processing vs image processing), and it requires no subjective calibration. The inability of the SQA-IIC-P and other automated analyzers to assess the midpiece and tail region is a major drawback, and these analyzers provide the percentage of normal morphologic results without quantifying specific abnormalities. Moreover, because the morphologic findings for the sperm are derived from SMI, sperm with low motility may falsely appear abnormal to the analyzer despite having normal morphologic

<table>
<thead>
<tr>
<th>Papanicolaou Staining</th>
<th>Abnormal</th>
<th>Normal</th>
</tr>
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<tbody>
<tr>
<td>Abnormal</td>
<td>118 (true-positive)</td>
<td>8 (false-positive)</td>
</tr>
<tr>
<td>Normal</td>
<td>20 (false-negative)</td>
<td>55 (true-negative)</td>
</tr>
</tbody>
</table>

SQA-IIC-P, Sperm Quality Analyzer IIC-P.
* Sensitivity, 85.5%; specificity, 87.3%; positive predictive value, 93.7%; negative predictive value, 73.3%. 

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features in smears. In the present study, although there was a good correlation between the methods, cases in which there was no agreement of results had more midpiece and tail defects (false-negative) or a lower value by the SQA-IIC-P system owing to low SMI values (false-positive).

Few studies have compared the Pap staining method and automated methods for identifying defects in the morphologic features of sperm. Shibahara et al7 and Agarwal and Sharma8 observed a good correlation in the morphologic features of sperm between the SQA and manual methods. In contrast, Johnston et al9 found different semen characteristics by these 2 methods and concluded that the morphologic features of sperm as determined by a semen analyzer are influenced by sperm motility, leading to results different from those of the manual method.

The morphologic features of sperm are influenced by various lifestyle and occupational factors and by infections. Auger et al10 found an increase in the abnormal morphologic features of sperm in cases with a history of medical treatment of the mother during pregnancy, higher birth weight, and previous treatment for cryptorchidism. Ozgur et al11 found increased tail abnormalities of the sperm in smokers. Venkatesh et al12 explained the role of reactive oxygen species in the pathogenesis of abnormal spermatozoa in smokers; they cause oxidative damage to mitochondrial DNA leading to mutations and impaired germ cell functions. Various studies have shown a relationship between leukocytospermia and structural damage of the sperm.13,14 The sperm damage may commence during spermatogenesis, and cytokines released by leukocytes have been found to interfere with Sertoli cell function, leading to abnormal spermatogenesis. Aziz et al14 reported a significant positive correlation between leukocytospermia and sperm tail defects, acrosomal damage, and a high SDI score. A similar study by Thomas et al13 demonstrated leukocytospermia leading to midpiece abnormalities by unknown mechanisms. In the present study, a statistically significant correlation of smoking and leukocytospermia was observed with abnormal morphologic features and higher teratozoospermia scores. However, none of these variables were associated with any specific morphologic defects.

The SQA-IIC-P might be used as an initial screening test for the evaluation of the morphologic features of sperm. However, cytomorphologic analysis by Pap staining, along with sperm indices, should be performed for quantification of defects and to detect cases missed by the analyzer owing to more midpiece and tail defects. It helps clinicians make decisions for in vitro fertilization. Certain lifestyle modifications such as quitting smoking and controlling infections can improve the morphologic features of sperm in patients with fertility problems.

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