ABSTRACT

Background: Assessment of sperm morphology is an important criterion in determining the semen quality and it plays a major role in male infertility.

Aim: To assess the morphological defects of the spermatozoa in patients with normal sperm counts.

Materials and Methods: This was a cross-sectional study carried out in a tertiary care centre for a period of one year. A total of sixty semen samples with normal sperm counts were included. Morphological assessments of spermatozoa were performed on smears stained with Hematoxylin and Eosin and Papanicolaou-stains. The defects were categorized based on the abnormalities in different parts such as head, middle piece and tail.

Results: All cases 60 samples despite normal sperm count showed morphological defects. The percentage of defects ranged from 1% to 20% but none of the sample showed abnormality more than 20%. The most common defect observed was in the region of head (tapering head - 86.67%) followed by thick middle piece defect (71.67%). Ninety five percentages of cases showed multiple defects.

Conclusions: Qualitative defects are observed in all patients with normal sperm counts

Key Words: Sperm count, Sperm morphology, Hematoxylin stain.

INTRODUCTION

Across the globe about 15% of married couples undergo infertility problem. About 20-30% of these cases are due to defects in semen and 30%-40% have combined defects in both men and women. So, male-factor associated infertility is observed in one third of the infertility couples. Semen analysis is the principal baseline investigation carried out for the detection of male infertility.[1-3]

Semen analysis performed by using the WHO criteria encompass quantitative as well as qualitative parameters.[4] Quantitative examination includes measurement of semen volume and sperm counts. Qualitative analysis includes assessment of viscosity, morphology and motility.

Assessment of sperm morphology is the major benchmark in determining the quality of a semen sample and it plays a key role in detecting male infertility. Qualitative analysis can be carried out by assessing the semen sample for identification of defects in different parts such as head, middle piece, and tail.[5] Accuracy of semen morphology assessment mainly depends on vigilant preparation, fixation and staining of the smear, since any of these procedures can significantly influence the sperm dimensions.
Available literatures were mainly focused on the factors influencing male fertility. Hence, the current study was undertaken to find the occurrence of diverse qualitative defects in patients having normal sperm counts.

**MATERIALS AND METHODS**

The current study was a prospective cross sectional analysis carried out in a tertiary care hospital during a period of one year from July 2016 to June 2017. A convenient sampling of 60 samples of semen with normal sperm count of 15 million/ml or more was included. Sample collection was performed following the standard WHO protocols. All Samples were collected in a sterile-capped container. After collection the samples were kept for proper liquefaction, and then each sample was taken for quantitative and qualitative analysis including volume, appearance, viscosity, sperm count, progressive/total motility, morphology and presence/absence of red blood cells/white blood cells.

Sperm count was carried out on properly liquefied sample by using Improved Neubauer chamber and motility study was carried out immediately on fresh sample; however sperm morphology was studied by making smears fixed in alcohol and stained by using Papanicolaou and Hematoxylin and Eosin staining. Morphological abnormalities were classified according to the defects detected in the various parts including head, middle piece, and tail. The commonly noted abnormalities were small/large head, round/tapering head and bifid head, bent neck, no tail, broad middle piece, and coiled tail for tail. The frequencies of these morphological abnormalities were studied.

**RESULTS**

In the study all the 60 samples of semen with normal sperm counts were examined for various morphological abnormalities. Various morphological abnormalities detected in all samples with normal sperm counts were categorized based on the type of defect and shown in Table 1. The most common defect was observed in the region of head followed by the defects in middle piece. The percentage of defects ranged from 1% to 20% but none of the sample showed abnormality more than 20%. Most common defect observed was tapering head. The defect which was least commonly presented was coiled tail. Fifty seven cases (95%) out of 60 cases showed multiple defects, the combination of defects was shown in Table 2. The most frequent combinations noticed were abnormalities in the head and middle piece, followed by other common combinations were combined defects in the head, neck and middle piece. A few samples showed multiple defects in the head region alone.

<table>
<thead>
<tr>
<th>Part of sperm</th>
<th>Abnormalities detected</th>
<th>Number of cases with abnormalities n=60 (%)</th>
<th>Percentage of sperms showing morphological abnormalities &lt;5%</th>
<th>5%-20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>Tapering</td>
<td>52 (86.67)</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Round</td>
<td>32 (53.33)</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>14 (23.33)</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>3 (05)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Middle Piece</td>
<td>Bent neck</td>
<td>21 (35)</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Thick Middle Piece</td>
<td>43 (71.67)</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Excess residual Cytoplasm</td>
<td>6 (10)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Tail</td>
<td>Coiled Tail</td>
<td>13 (21.67)</td>
<td>11</td>
<td>2</td>
</tr>
</tbody>
</table>
**DISCUSSION**

Sperm morphology is a major criterion in the analysis of semen. The morphological assessment is carried out by the microscopic examination of sperm using smears stained by either H&E or Pap. Among all the parameters in semen analysis, sperm morphology is one of the most dominant indicators of male fertility. Although the significance of sperm morphology has been recognised in the current scenario, the limitations like poor standardization in preparation, staining techniques and evaluation, the accurate potential of this parameter has not been exploited.

Morphologically diverse types of defects have been observed in various parts of the sperm. Studies have shown that these defects have a prognostic relevance since few such defects are irreparable, and those defects which occurred due to environmental factors can be reversed.

Few authors described about the association abnormal morphology with low sperm count, they also observed that the morphological defects of the sperm increases with decrease in the sperm count. There were not enough literature on the morphological defects associated with normal sperm counts.

Similar study had shown that the most common defects observed were in the head region. The observations in the current study also showed that the most common abnormality was tapering head. Few authors have described that defects such as megalohedra and tapering of the heads are reversible. This is mainly because of any current medications or stress.

A severe form of morphological abnormality associate with genetic aberration is amorphous head, and these sperms are incapable of fertilization. Globozoospermia is a severe and a rare form of male infertility in which spermatozoa will be small with rounded head and without acrosome; it is also genetically determined and the sperms are unable to bind zona pellucida due to the lack of acrosin. Small headed spermatozoa will bear tiny and abnormally formed acrosome. The abnormalities in the spermatozoa neck such as defects in the head neck attachment or misalignment and bent neck were genetically determined and carry poor prognosis. Immature spermatozoa usually exhibit excess residual cytoplasm and it is associated with creation of reactive oxygen species due to ongoing stress.

The other commonest abnormality observed in the present study was broad middle piece. Tail defects are least commonly recorded in the present study. Tail malformation such as short tail syndrome is hereditary determined associated with poor chances of future
Qualitative defects in normal sperm counts patients

Coiled tail defect cause infertility because of defective propulsion. Studies had shown that smokers had a higher proportion of spermatozoa with tail abnormalities.

Literature had shown that the morphological defects majorly occur in combinations. The findings have been correlated with the association of multiple defects and increased occurrence of spontaneous abortions. Many authors had found that there was association of sperm morphological defects with seasonal variations. Our study also showed multiple abnormalities.

There are few limitations in the current study such as it was a single hospital based study, small sample size and lack of follow-up. It is suggested that multicentre study with a larger sample size and adequate case follow up to support the study findings.

CONCLUSION

Sperm morphology assessment remains the baseline necessity for the diagnosis and management of male factor associated infertility when advanced techniques are unavailable, inaccessible or unaffordable. Qualitative defects are often seen even in patients with normal sperm counts and should be noted.

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Conflicts of interest: There are no conflicts of interest

REFERENCES