ORIGINAL ARTICLE

The impact of chronic testicular inflammatory infiltration on spermatogenesis in azoospermic men, evidence-based pilot study

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KEYWORDS
Spermatogenesis; Lymphocytic inflammatory infiltration; Testis; Azoospermia

Abstract Introduction: The testes are immune privileged organs but they are subjected to acute and chronic inflammations that can impair spermatogenesis. Spermatogonial stem cells are less sensitive to injury, capable of self-renewal, and act as a reservoir that can restart impaired spermatogenesis. Objective: To study the association of chronic testicular inflammatory infiltration with an area of complete spermatogenesis in testicular biopsies of men with non-obstructive azoospermia (NOA). Design: Retrospective study. Patients and methods: Review of 109 archived Bouin fixed, paraffin embedded and hematoxylin and eosin stained open testicular biopsies of azoospermic men. Main outcome measure: The absence/presence (focal/widespread) of chronic interstitial inflammatory infiltration and absence/presence of late spermatids. Results: Obstructive picture was found in 25 (22.9%) whereas non-obstructive patterns were found in 84 (77.1%) of the reviewed biopsies. Focal interstitial lymphocytic infiltration (ILI) was found in 9 (36%) obstructive biopsies whereas in non-obstructive patterns 32 (38.1%) had focal and 27 (32.1%) had widespread ILI. In NOA late spermatids were present in 32 (38.1%) biopsies. Finding late spermatids was significantly highest when the infiltrate was focal and significantly lowest when the infiltrate was widespread. Conclusion: In NOA, the possibility of finding an area of complete focal spermatogenesis in the open testicular biopsy is significantly highest when there is a focal type of ILI, but the possibility
1. Introduction

Spermatogenesis starts at puberty when the immune-tolerance is completed. The newly formed spermatogenic cells develop new antigens considered foreign for the own immune system. In the testes, modulation of the immune response protects the developing spermatogenic cells from self-destruction [1,2]. The modulation of the testicular immune response involves the formation of blood-testis barrier and presence of immunosuppressive factors secreted by macrophages, Sertoli, Leydig and peritubular cells [1–3]. In mice, disruption of the blood-testis barrier led to increased apoptosis, arrest of spermatogenesis, Sertoli cell only picture, aggregation of Sertoli cells in the apical compartment and infertility [4].

Modulation of the testicular immune response does not interfere with the development of an inflammatory reaction when an infectious agent attacks the testis or when pathology is induced, as experimental autoimmune orchitis [5]. The inflammatory response is marked by infiltration of the interstitial spaces with immune cells (such as macrophages, dendritic cells and T cells) and secretion of pro-inflammatory cytokines such as IL-6, TNF-α, IL-17 and IL-23 [1–3,6–8]. Previous published researches confirmed the infiltration of the testicular interstitial tissue with CD4 and CD8 lymphocytes during the acute and chronic inflammations [9]. Also it was confirmed that Th17 (CD4) cells are markedly involved in the chronic inflammation in testicular biopsies of men diagnosed as NOA [10].

Noguchi et al. [11] reported early regression of spermatogenesis and testicular atrophy in boars following epididymoorchitis especially in genetically predisposed animals. Inflammation of testes has deleterious effects on spermatogenesis however the spermatogenical stem cells, less sensitive to injury, are capable of self-renewal and act as a reservoir for spermatogenesis [12–15].

Prediction of the presence of testicular sperms in NOA men is an important issue before TESE/ICSI procedure. FSH estimation is considered the best, non-invasive, predictor for sperm retrieval before TESE procedures [16] however FSH had a moderate predictive value [17]. Furthermore, with the situation of failed TESE, the clinician is in need for predictive factors before making a decision to repeat the TESE procedure. Using micro-TESE procedure Berookhim et al. [18], reported a sperm retrieval of 44.5% in patients with a previous histological diagnosis of Sertoli cell-only.

Hypothesis: Inflammatory conditions of testes have deleterious effects on spermatogenesis that can lead to non-obstructive azoospermia. However regeneration of foci of spermatogenesis is a possibility. This research aimed to study the association of chronic ILI with finding an area of complete spermatogenesis in testicular biopsies of azoospermic men.

2. Patients and methods

A waiver of getting consent was taken for this retrospective study, from the Ethics Committee, Andrology Department, Faculty of Medicine, Cairo University. During the period of February 13, 2015 till March 19, 2015 we reviewed 109 randomly selected archived Bouin fixed, paraffin embedded and hematoxylin and eosin stained open testicular biopsies of azoospermic men who attended the Andrology clinic of the university hospital. Each testicular biopsy was reviewed for the histopathological diagnosis [19], absence/presence (focal/widespread) of interstitial inflammatory infiltrate with meticulous search for the presence of late spermatids in biopsies diagnosed as NOA. Widespread infiltration meant pervasive infiltration of the tissue or most of it. Scattered foci of infiltration were considered focal infiltration. Multi-focal infiltrations affected more than 30–40% of the tissue was considered as widespread. Confounders were avoided by studying all the variables on the same testicular tissue.

2.1. Statistical analysis

Data were entered using the statistical package SPSS version 20 (SPSS Inc., Chicago, IL, USA). Data were summarized using numbers and percentages for the qualitative data. Comparisons between groups were done using Chi square test for the qualitative data. P value < 0.05 was set as statistically significant.

3. Results

In the studied biopsies, a histopathological diagnosis of obstructive azoospermia was found in 25 (22.9%) whereas 84 (77.1%) had histopathological diagnosis of NOA. Interstitial lymphocytic infiltrate (ILI) was found in 68 (62.4%) biopsies, focal in 41 (37.6%) and widespread in 27 (24.8%), whereas an inflammatory infiltrate was absent in 41 (37.6%) biopsies.

In obstructive azoospermia a focal type of ILI was found in 9 (36%) biopsies whereas 16 (64%) did not have infiltration. In NOA ILI was found in 59 (70.2%) biopsies whereas 25 (29.8%) did not have infiltration. In NOA, the numbers and percentages of different histopathological patterns, ILI (absent/focal/widespread) and late spermatids (absent/present) in each pattern are represented in Tables 1 and 2. Comparative study of the histopathological patterns demonstrated significant differences regarding both the detection of an area of complete spermatogenesis and the absence/presence (focal vs widespread) of ILI.

In cases of NOA, the total numbers and percentages of biopsies in which late spermatids (sperms) were absent/present and ILI absent/focal/widespread when spermatids were absent/present are represented in Table 3. Comparative study...
Table 1  Presents data of biopsies regarding absent vs focal vs widespread interstitial lymphocytic infiltrate in different histopathological patterns of non-obstructive azoospermia.

<table>
<thead>
<tr>
<th>Infiltrate</th>
<th>ESA</th>
<th>TF</th>
<th>HSG</th>
<th>KF</th>
<th>MA</th>
<th>PSA</th>
<th>SCO</th>
<th>Count</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>12</td>
<td>25</td>
<td>57.1%</td>
</tr>
<tr>
<td>Focal</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>18</td>
<td>6</td>
<td>3</td>
<td>32</td>
<td>42.9%</td>
</tr>
<tr>
<td>WS</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>15</td>
<td>27</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>23</td>
<td>15</td>
<td>30</td>
<td>84</td>
<td>100%</td>
</tr>
</tbody>
</table>

\[ a \] Widespread.
\[ b \] Early spermatid arrest.
\[ c \] Tubular fibrosis.
\[ d \] Hypospermatogenesis.
\[ e \] Klinefelter like pattern.
\[ f \] Mixed atrophy.
\[ g \] Primary spermatocyte arrest.
\[ h \] Sertoli cell only.

Table 2  Presents data of biopsies regarding the absence/presence of sperms in different histopathological patterns of non-obstructive azoospermia.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>ESA</th>
<th>TF</th>
<th>HSG</th>
<th>KF</th>
<th>MA</th>
<th>PSA</th>
<th>SCO</th>
<th>Count</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>11</td>
<td>30</td>
<td>52</td>
<td>14.3%</td>
</tr>
<tr>
<td>Present</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>18</td>
<td>4</td>
<td>0</td>
<td>32</td>
<td>85.7%</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>23</td>
<td>15</td>
<td>30</td>
<td>84</td>
<td>100%</td>
</tr>
</tbody>
</table>

\[ b \] Early spermatid arrest.
\[ c \] Tubular fibrosis.
\[ d \] Hypospermatogenesis.
\[ e \] Klinefelter like pattern.
\[ f \] Mixed atrophy.
\[ g \] Primary spermatocyte arrest.
\[ h \] Sertoli cell only.

Table 3  Elaborates the absence/presence of late spermatids (sperms) and type of interstitial lymphocytic infiltration in testicular biopsies of men with non-obstructive azoospermia.

<table>
<thead>
<tr>
<th>Infiltrate</th>
<th>ESA</th>
<th>TF</th>
<th>HSG</th>
<th>KF</th>
<th>MA</th>
<th>PSA</th>
<th>SCO</th>
<th>Count</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>20</td>
<td>7</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>52</td>
<td>80.0%</td>
</tr>
<tr>
<td>Focal</td>
<td>8.0%</td>
<td>21.9%</td>
<td>92.6%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>61.9%</td>
<td></td>
</tr>
<tr>
<td>Widespread</td>
<td>5</td>
<td>25</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32</td>
<td>20.0%</td>
</tr>
<tr>
<td>Present</td>
<td>20.0%</td>
<td>78.1%</td>
<td>7.4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>38.1%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>32</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>84</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

\[ P \] < 0.001

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demonstrated significant differences with late spermatids (sperms) highest detected in biopsies when the ILI was focal and lowest detected when the ILI was wide spread (Table 3).

4. Discussion

Inflammation of testes may take a patchy distribution and regional differences of spermatogenesis were detected in 32% of testicular biopsies of men diagnosed as NOA [19].

In the current study, a histopathological diagnosis of obstructive azoospermia was found in 25 (22.9%) biopsies whereas 84 (77.1%) had a diagnosis of NOA. Interstitial lymphocytic infiltration (ILI) was commonly encountered in the studied biopsies a finding confirmed by Schuppe et al. [20]. ILI of testes was less commonly encountered in biopsies of obstructive azoospermia and it was only of focal type. This gave a clue that the presence of a focal ILI did not have a pervasive deleterious effect on the testicular tissue. Supporting this finding, Terayama et al. [8] reported that the spermatogenic regression in mice was more frequent in the tubules surrounded by the ILI in cases of experimentally induced autoimmune orchitis.

In cases of NOA this study considered the mere detection of late spermatids (sperms) in the biopsy as an evidence for the presence of an area of complete spermatogenesis. Johanson’s score modified by De Krest and Holstein was not suitable for the current study as it can give a mean score, for example 3 or 4, that is not indicative of the presence or absence of late spermatids in cases of NOA [19].

The frequency of detection of late spermatids (sperms) and ILI was significantly different among the histopathological patterns of NOA. The association of an area of complete spermatogenesis with focal ILI was most frequently encountered in biopsies of mixed atrophy, early spermatid arrest and hypospermatogenesis. The wide spread ILI (Figs. 1 and 2) was most frequently found in biopsies in which late spermatid was absent, namely, in tubular fibrosis, Klinefelter like pattern and Sertoli cell only (SCO).

In mixed atrophy, ILI was encountered in 23 (100%), the focal type (Fig. 3) in 18 (78.3%), biopsies indicating the definitive role of the inflammatory process in inducing this pathology. ILI was least encountered in biopsies of spermatogenic arrest and hypospermatogenesis. In these pathologies, late spermatids were found, with absence of ILI, in 5 biopsies in which the impairment of spermatogenesis can be explained by a non-inflammatory etiology of the testis. Supporting this, Pilatz et al. [21] reported incremental deterioration of the function of testes in mice following bacterial epididymitis without any inflammatory affection of the testis.

Both late spermatids (sperms) and inflammatory infiltration were absent in 20 biopsies (12 of them were SCO) and impaired spermatogenesis can be explained by a non-inflammatory etiology as congenital SCO or spermatogenic arrest.

In NOA, comparative study of the total numbers, associating the late spermatids (absent/present) with the ILI (absent/focal/widespread) demonstrated significant differences with late spermatids (sperms) being highest detected in biopsies when the infiltrate was focal and lowest detected when the infiltrate was widespread indicating that the focal type of ILI is
associated with the presence of an area of complete spermatogenesis. This may guide the clinical TESE procedure.

5. Conclusion

In NOA men, the possibility of finding an area of complete focal spermatogenesis in the testicular biopsy is significantly highest when there is a focal type of ILI, but the possibility is lowest when the infiltrate is widespread. This may guide the clinical TESE procedure.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

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References