PhD Thesis

STUDY OF MORPHOLOGICAL CHANGES IN THE SEMINIFEROUS TUBULE WALL WITH AGE

- ABSTRACT -

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Key words: testis, ageing, seminiferous tubule wall, immunohistochemistry, morphometry.
Introduction

Ageing remains one of the biggest mysteries of medical science. Worldwide, the population age is increasing which entails finding swift answers to two fundamental questions: how and why do we age. We know too well that age makes us weaker, more vulnerable to illness, eventually leading to death. What actually determines aging and what we can do to slow the occurance?

It seems that, ultimately, humans age because during their biological evolution, nature has relied more on reproduction than on maintaining the body integrity for a long time which would have allowed us to live forever. Understanding this truth opens new paths for investigating how genes determine our life span.

The relatively narrow approach to morphological changes induced by aging in the testicular parenchyma in the literature on the one hand, and on the other hand the constant concern of the medical school of Craiova for the study of the stromal structures and their echo on their function both in terms of normal and pathological conditions, were the two main reasons that led to the shaping of the present study.

Based on these considerations, this paper aims to study the morphological changes occurring with age of the supporting component of the noble seminal epithelium, namely the seminiferous tube wall, but restricted only to cases of patients with malignant epithelial proliferation of the prostate which led to orchiectomy as a treatment.

CURRENT DATA

Chapter I - Anatomy of the testis

Chapter I – „Anatomy of the Testis” describes the classical anatomical elements of the adult testis. Aspects regarding the location, shape, orientation, size, color and consistency, external configuration and anatomical relations of the testis are described. The testicular sheaths, its supporting structures as well as the arterial vasculature, venous and lymphatic drainage and innervation are also described.

Chapter II - Histology of the testis

Chapter II - "Histology of the Testis" describes the tissue, cellular and stromal components of the testis.

In the first part the emphasis is on the testicular interstitial components (Leydig cells, macrophages, lymphocytes and nerve fibers, intratesticular vascular architecture, innervation), and then on the detailed decription of the testicular tubular compartment, which is also the main topic addressed in the research project. The peritubular tissue, Sertoli cells and germ cells together with the kinetic process of spermatogenesis are described in detail.

Chapter III - Senescent testis

The aim of this study is the morphological changes with age of the seminiferous tubule wall. Accordingly, Chapter III of the thesis deals with the senescence from the morphological, physiological, psychological and social point of view. Senescence of the human body and especially its implications and its hallmarks regarding the male gonad and male reproductive function are discussed (physiology of aging, theories of senescence, sexuality and senescence, endocrine aspects in senescence, testicular morphology in the elderly, fertility risks related to advanced paternal age).
PERSONAL STUDY

Chapter IV – Material and Methods

The topic discussed in this doctoral thesis is based on research conducted in a research contract in the PNCDI II, funded by the Ministry of Education, Program Partnerships in priority areas PC, no. 41-015/2007 entitled "Cellular and molecular fundamentals of the influence of the changes in the seminiferous tubule on aging of the testis – experimental and human study". The project was carried out between 2007 and 2011 and was coordinated by professor Iancu Emil Pleșea having as partners the following institutions: the University of Medicine and Pharmacy of Craiova, of which I was part of the research team, County Emergency Hospital of Craiova, the "Victor Babes" National Research Institute in Bucharest, the University of Medicine and Pharmacy of Tg. Mureș, Fundeni Institute in Bucharest, the Academy of Economic Studies in Bucharest, and a private company involved in research, SC Pneumatic & Vacuum Technology SRL in Bucharest.

The basis of this study was represented by a group of 74 patients admitted in the urology clinics of the partner medical institutions of the project who diagnosis of prostate adenocarcinoma (ADKP) was established in the Pathology Departments of the same institutions and who underwent bilateral orchietomy for therapeutic purposes.

Table 1: Case distribution depending on their origin

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fundeni Clinical Institute</td>
<td>30</td>
</tr>
<tr>
<td>County Emergency Hospital of Tg. Mureș</td>
<td>14</td>
</tr>
<tr>
<td>County Emergency Hospital of Craiova</td>
<td>30</td>
</tr>
</tbody>
</table>

The parameters analyzed were: clinical parameters (period, age) and histopathological parameters (wall thickness of seminiferous tubules, thickness of the basement membrane, lamina propria thickness, thickness of the external layer of the lamina propria, thickness of the inner layer of the lamina propria, fibro-hyaline collar thickness; layout of the fibro-hyaline collar; maturation stage of the seminal epithelium, presence of complete tubular sclerosis). Each of these parameters was analyzed individually and correlations were made between them, as well as with the seminal epithelium and age.

For histological staining several techniques, namely the classical staining techniques (hematoxylin-eosin and Masson trichrome for connective tissue components) and immunohistochemical labeling to highlight specific components of the seminiferous tube wall.

Table 2: Antibodies used for the immunohistochemical labeling

<table>
<thead>
<tr>
<th>Antibody</th>
<th>M/P</th>
<th>Clone</th>
<th>Source</th>
<th>Specificity</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo a-Hu SMA</td>
<td>M</td>
<td>1A4</td>
<td>DAKO</td>
<td>Smooth muscle fibers from the interstitium and arteriolar wall</td>
<td>1:50</td>
</tr>
<tr>
<td>Mo a-Hu Collagen IV</td>
<td>M</td>
<td>CIV 22</td>
<td>DAKO</td>
<td>Collagen fibers from the basement membrane</td>
<td>1:50</td>
</tr>
<tr>
<td>Mo a-Hu CD34</td>
<td>M</td>
<td>QBEnd 10</td>
<td>DAKO</td>
<td>Endothelial cells</td>
<td>1:50</td>
</tr>
<tr>
<td>Mo a-Hu Vimentin</td>
<td>M</td>
<td>V9</td>
<td>DAKO</td>
<td>Cells with mesenchymal origin</td>
<td>1:50</td>
</tr>
</tbody>
</table>
**Acquisition of microscopic images.** Histopathological aspects were selected with an Olympus CX31 microscope, using the ×4 eyepiece. For image acquisition we used optical planapocromate corrected objectives with ×20 and ×40 magnification. The most significant images were taken with an Olympus ColorViewII digital camera directly into the computer using the AnalySIS Pro software. The images were processed using the same software.

**Morphometric measurements.** For the morphometric measurements the functions of the AnalySIS Pro software Measure module were used. Previously, it was calibrated for all objective (×4, ×10, ×20 and ×40) using a micrometer calibration scale produced by IOR.

**Statistical indicators.** For numeric parameters the following statistical indicators were calculated: minimum value, maximum value, mean, standard deviation (STDEV), coefficient of variation (CV).

To assess the evolution trend with age of different morphological features evaluated we used two statistical tools namely the Pearson correlation test and the χ² test. The evolution trend correlated with the age was considered significant if the p-value was < 0.05. The upward or downward trend of the variables was also established materialized by the regression line its R² coefficient. Graphs were made with the help of XLSTAT 2009 2, trial version.

**Chapter V – Assessment of the seminiferous tubule wall components**

**Age of patients.** In the study group, the mean patient age was 71.9 years, with a minimum of 56 and a maximum 85 years.

**Assessment of tubular wall components.**

**Average thickness of the tubular wall.** Most values of the the mean wall thickness of the seminiferous tubule wall were located in the first part of the variation interval for this parameter, the regression showing a strong downward trend.

**Average thickness of the basement membrane.** Both the mean thickness per tube and per case were located around the mean value, in the range 0.4-0.8 µm.

**Average thickness of the lamina propria.** Both the mean thickness per tube and per case were located in the first part of the variation range, namely between 5-11 µm, and 5-15 µm.

**Average thickness of the external layer of LP.** Both the mean thickness per tube and per case were located in the first part of the variation range, namely between 1.5-7.5 µm, and 1-7 µm.

**Average thickness of the inner layer the LP.** Both the mean thickness per tube and per case were located in the first third of the variation range, and the number of cases with high average values was increasingly lower.

**Average thickness of fibro-hyaline collar.** Both the mean thickness per tube and per case were located in the first third of the variation range, namely between 1-4 µm and 1-3 µm respectively.

**The degree of extension of the FHC.** Mean percentage of tubular circumference occupied by the FHC ranged between 12.46 and 100% for each case and between 7.98 and 100% for each tube, with averages of 65.70% and 66.71%.

The case distribution according to the presence of tubular sclerosis revealed that half of the cases showed tubular sclerosis phenomena. In most cases affected by it the percentage of was between 10-49% (average damage), the rest being divided between the two extremes, minimum and maximum damage, with a greater number of cases in the first category.

**Chapter VI – Correlations between the components of the seminiferous tubule wall**

The correlative study of the seminiferous tubule wall components suggests that its changes in thickness are mainly due to changes in the LP.
Within the inner layer of the LP, which is in direct contact with the BM, there is a degenerative process of reticular fibrillar structures, consisting in a collagen fiber increase with rearrangement in bundles, focal condensation and even transformation into an amorphous hyaline material.

All these alterations named fibro-hyaline collar, are not diffusely spread which is demonstrated by the variability of morphometric measurements, which range from almost complete absence of alterations in some tubules, to their presence throughout the entire circumference of the tubule.

Test values of the correlation tests between the LP components confirm the location of these alterations within its internal layer.

Another conclusion that can be drawn from these tests is that this fibro-hyaline collar goes shows a simultaneous evolution both in terms of its thickness and its circumferential extension.

The most significant correlation of the seminal epithelium was with this fibro-hyaline collar, both in terms of its thickness and circumferential extension. Hence the strong correlation with the inner layer of the lamina propria and the lamina propria per se, and the tubular wall thickness. Note that usually circumferential type processes were associated with more severe degrees of involution of the seminal epithelium.

These data and previously published data confirm and complete the data from the literature that show that the disruption of testicular function and reduction or absence of spermatogenic activity are associated with the thickening of collagen fiber layer and the material between peritubular cells. In this case the tubule wall becomes fibrotic or, based on the histological appearance, hyalinized [Pleşea et al. 2009a; Pleşa et al. 2009b; Pop et al. 2009, Weinbauer et al. 2010; Pleșea et al. 2010a; Pleșea et al. 2010b; Cotoi et al. 2010, Pop et al. 2011a; Pop et al. 2011b].

Chapter VII – Correlations of the components of the seminiferous tubule wall with ageing

Summarizing the correlation with age of the studied peritubular tissue changes we can say that the only component that changes significantly with aging is the inner layer of lamina propria and especially the fibro-hyaline collar that develops within, thus confirming the studies in the literature [Honoré 1978; Trainer 1997]. Overall, the general trend of evolution of the morphometric parameters evaluated is increasing with age, increasing the besides the inner layer and the FHC is relatively mild.

Thus, this study contradicts some data from the literature concerning the basement membrane thickness and confirms other studies on this subject [Xi et al. 1982; Sasano and Ichijo 1969; Johnson et al. 1984, Neaves et al. 1984; Obafunwa et al. 1993, Meacham and Murray 1994, Richardson et al. 1995; Plas et al. 2000; Dakouane et al. 2005]. The data presented about the thickness of the lamina propria, partially confirm the literature in that, indeed, we observed an increasing trend in terms of its thickness, parallel to the tubular involution, but without the existence of significant correlations [Johnson 1986; Paniagua et al. 1987]. As noted, neither the involution of seminal epithelium nor tubular sclerosis itself correlate with advancing age. This was a somewhat heterogenous process in terms of correlation with age; there are studies that confirm our data and describe changes ranging from complete testicular atrophy to the presence of both normal and atrophic seminiferous tubules in the same testis [Rolf et al. 2010].

As a consequence of aging the fibro-hyaline collar may explain both the involution of the seminal epithelium and the focal nature of this process and the lack of correlation with the age of the patient. The presence of this fibro-hyaline collar was observed both in tubules with normal spermatogenesis or hypospermatogenesis as well as in tubules with spermatogenic arrest in different stages.

It was either discrete and discontinuous, either quite obvious, with irregular thickenings,
occupying, not infrequently, the entire circumference of the tubule.

In this first phase the sclerosis is not diffusely spread in all seminiferous tubules, and progressively generalized, which is proven by the polymorphous aspects captured on the same section and microscopic observation of situations in which sperm cells were seen in the lumen of a tubule with atrophied epithelium and intense sclerosis. The presence of this FHC, especially when circumferential, is associated more frequently with advanced stages of epithelial atrophy. The interposition of this layer between the BM and intramural capillaries may be an important mechanism to explain alterations of the senescent germinal epithelium.

**Conclusions**

The fundamental morphological change observed in the wall of seminiferous tubules in the elderly is the condensation of collagen fibers within the inner layer of the lamina propria in apposition with the basement membrane of the seminal epithelium. This fibrillary densification also described by other authors, may undergo a subsequent degeneration process, with the advent of hialinizare areas.

This phenomenon which we generically called "fibro-hyaline collar" showed a focal and uneven development pattern regarding its thickness and its extension within the tubular circumference in both seminiferous tubules and the entire testicular parenchyma.

The focal pattern of the FHC presence within individual tubules correlates with age, but its presence and evolution is not generalized to the entire testicular parenchyma with age.

Correlation analysis of all components of the seminiferous tubule wall (basement membrane, inner layer and outer layer of lamina propria) with the morphological changes of the seminal epithelium observed in the elderly showed that the only component that affects epithelial involution is clearly the fibro-hyaline collar.

Our observations allow us to advance the hypothesis that the fibro-hyaline collar intervenes directly in the epithelial atrophy by interposing itself between the basement membrane of the epithelium and the intramural segments of the peritubular capillary network.

Thus, the presence of the fibro-hyaline collar is a key element of the onset of the tubular sclerosis process which can be systematized as follows:

- appearance of the FHC within the inner layer of the LP
- onset of epithelial atrophy
- consecutive shrinkage of the seminiferous tubules, which causes its thickening and the reduction of the lumena up to its disappearance
- tubular sclerosis

The focal and uneven pattern of degeneration of the seminiferous tubule wall within the inner layer of the lamina propria, leading to tubular sclerosis, may be considered one of the fundamental factors that contribute to the preservation of the reproductive function in males until extreme age.

**REFERENCES**

2. Andrew W. (1971). The anatomy of aging in man and animals, Grunne and Stratton,


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- Published papers:
  - 1 book (coauthor);
  - 11 articles published in ISI journals;
  - 18 abstracts published in ISI journals;
  - 7 articles published in journals indexed in international databases;
  - 1 abstract published in journals indexed in international databases;
  - 6 articles in journals indexed in national databases;
  - 25 papers presented in international conferences.
- Second Prize for the paper „Morphological changes in lamina propria of the seminiferous tubule in aging testis” presented at the 18th European Students' Conference, 2007, Berlin, October, 7th-11th;
- Editorial Secretary al Romanian Journal of Morphology and Embriyology.

PAPERS PUBLISHED OR PRESENTED RELATED TO THE TOPIC OF THE PhD THESIS (REFERENCES IN THE TEXT)


4. Plessea IE, Pop OT, Enache SD, Mandache E, Gherghiceanu M, Stanoiu B, Butaru A.
Comparative study of peritubular tissue in human and rat ageing testis. 20th Ljudevit Jurak International Symposium on Comparative Pathology", Zagreb, Croatia, June, 5th-6th.


