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Early histopathological aspects of benign prostatic hyperplasia: myxoid-inflammatory nodules

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ABSTRACT

Objectives: Infiltration of benign prostatic hyperplasia (BPH), nodular prostatic hyperplasia (NPH) or prostatic adenoma by mononuclear cells, predominantly lymphocytes, is a common finding in surgical specimens. The biological significance of this infiltration, very similar to chronic inflammation, is unknown, but it is suspected as being related to the pathogenesis and progression of the BPH. The identity and number of the infiltrating cells is not well known. The objectives of the present study were: 1) to describe in more detail the histologic pattern of early lesions BPH, that is “myxoid” nodules. 2) to count and locate these myxoid nodules and to analyse the possible influence of age 3) and describe the patterns of mononuclear cell infiltration in BPH.

Materials and methods: One hundred and seventy-three specimens of BPH tissue were reviewed. These samples were processed routinely for histological examination and immunohistochemical examination was performed in selected cases in order to further define the cellular composition of the described lesions. The immunohistochemical stains were performed automatically. Vimentin, specific smooth muscle actin and desmin were used to show the stromal cells and CD3, CD20 and CD68 monoclonal antibodies were used to quantify the populations of T-lymphocytes, B-lymphocytes and macrophages, respectively.

The number of myxoid nodules identified in each patient was counted and the maximum and minimum diameter measured. These data have been compared in two groups of patients, those younger than 57 years and those over 80. Statistical analysis has been performed with SPSS 13.0. Student's t test was used for bivariate analysis and Pearson's r for correlation.

Results: Interstitial infiltration involving T and B lymphocytes with less macrophages was a constant finding of the early nodules of BPH. The bivariate analysis with Student's t test has shown a statistically significant difference between the mean number of myxoid nodules ($p=0.02$), significantly lower in the younger patients. Although we have found differences between the mean diameters of the smallest and the largest nodules in both groups, these differences did not reach statistical significance. We have not shown any significant correlation between the number of nodules and the patient age ($p=0.11$) in the Pearson's r correlation.

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Conclusion: Inflammatory cells and myxoid nodules are a constant finding in BPH surgical samples. The mean number of myxoid nodules is significantly lower in younger patients. Interstitial distribution is the most common pattern of mononuclear cell infiltration. B and T-cell lymphocytes are the most frequently found inflammatory cells in early myxoid nodules.

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Aspectos histopatológicos iniciales de la hiperplasia benigna de próstata: nódulos mixo-inflamatorios

R E S U M E N

Palabras clave:

Hiperplasia benigna de próstata

Nódulos mixoideos

Diferencias de edad

Células inflamatorias

Objetivos: La infiltración de la hiperplasia benigna de próstata (HBP; hiperplasia nodular de próstata, adenoma prostático) por células mononucleares, con predominio de linfocitos, es un hallazgo frecuente en piezas quirúrgicas. Se desconoce la importancia biológica de esta infiltración, pero se sospecha que guarda relación con la patogenia y la progresión de la HBP. No se conocen demasiado bien la identidad ni el número de células infiltrantes. Los objetivos del presente estudio fueron: 1) describir con más detalle el patrón histológico de las lesiones iniciales de HBP, es decir, los nódulos «mixoideos»; 2) contar y localizar estos nódulos mixoideos y analizar la posible influencia de la edad, y 3) describir los patrones de infiltración por células mononucleares en la HBP.

Materiales y métodos: Se examinaron 173 muestras de tejido de HBP. Estas muestras se procesaron sistemáticamente para su examen histológico y se realizó un examen inmunohistoquímico en casos seleccionados para definir mejor la composición celular de las lesiones descritas. Se realizaron tinciones inmunohistoquímicas de forma automatizada. Se utilizaron anticuerpos contra vimentina, actina específica del músculo liso y desmina para poner de manifiesto las células del estroma y los anticuerpos monoclonales CD3, CD20 y CD68 para evaluar las poblaciones de linfocitos T, linfocitos B y macrófagos, respectivamente.

Se contó el número de nódulos mixoideos identificados en cada paciente y se midió el diámetro máximo y mínimo. Estos datos se compararon en dos grupos de pacientes, menores de 57 y mayores de 80 años. El análisis estadístico se realizó con el programa informático SPSS 13.0. Se empleó la prueba de la t de Student en el análisis bifactorial y la prueba de la r de Pearson en el análisis de correlación.

Resultados: La infiltración intersticial por parte de linfocitos T y B, con acompañamiento minoritario de macrófagos, fue un hallazgo constante en los nódulos iniciales de HBP. El análisis bifactorial con la prueba de la t de Student reveló una diferencia estadísticamente significativa entre el número medio de nódulos mixoideos ($p = 0,02$), significativamente más bajo en los pacientes más jóvenes. Aunque se constataron diferencias entre los diámetros medios de los nódulos de menor y mayor tamaño en ambos grupos, estas diferencias no alcanzaron significación estadística. No se identificaron correlaciones significativas entre el número de nódulos y la edad del paciente ($p = 0,11$) con la prueba de la r de Pearson.

Conclusión: Los nódulos mixoideos con células inflamatorias crónicas de distribución intersticial son un hallazgo constante en las piezas quirúrgicas de HBP. El número medio de nódulos mixoideos es significativamente menor en los pacientes más jóvenes. Los linfocitos B y T son las células inflamatorias más abundantes en los nódulos mixoideos iniciales.

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Introduction

Although known for a long time, lymphohistiocytic infiltration in prostate hyperplasia¹ (Moore 1937, 1943) has only recently gained interest since it became suspected that such infiltration could be related to the pathogenesis of such hyperplasia or prostate cancer. This hypothesis gave rise to improved definition of the lymphohistiocytic infiltration patterns in prostate hyperplasia, and to more precise and detailed identification of the components of

such infiltration. In earlier studies, our group has defined the inflammatory infiltration pattern and the different T lymphocyte populations that participate in this reaction².

The objectives of the present histological study are the following: 1) to describe in more detail the histological pattern of early benign prostatic hyperplasia (BPH) lesions, i.e., “myxoid” nodules; 2) to count and locate these myxoid nodules and analyze the possible influence of age; and 3) to describe the patterns of mononuclear cell infiltration in BPH.

Materials and methods

We analyzed 173 transurethral resections of the prostate (TURP) specimens corresponding to operations performed during the year 2008, and diagnosed with BPH. Following conventional diagnosis, a sample was selected, composed of patients under 57 years of age (9 subjects) or aged 80 years or older (20 subjects) at the time of the diagnosis. A pathologist (FM) examined the hematoxylin-eosin stained sections to count the number of myxoid nodules present in each patient, along with the maximum and minimum diameter of the nodules, using the measurement tool incorporated to the DMD108 microscope (Leica®).

In two cases immunohistochemical techniques were applied on an automated basis using a TECH-MATE 500® processor (Dako). Heat was used as the antigen recovery method. The primary antibodies used were polyclonal CD3 (Dako) in 1:100 dilution, monoclonal CD20 (Dako) in 1:1.000 dilution, and mononuclear CD68 (Dako) in 1:10.000 dilution for the lymphoid populations, and vimentin, desmin and smooth muscle actin (all from Dako) for the stromal populations. EnVision® (Dako) was used to detect immune reaction *in situ*.

The statistical analysis was performed using the SPSS version 13.0 statistical package for Microsoft Windows. A first descriptive study was made, with qualitative variables given as percentages, and quantitative variables as means and standard deviations. In the two-factors analysis we used the Student t-test for the comparison of means, while the Pearson *r* test was used for the correlation between quantitative variables, after confirming a normal (Gaussian) distribution of all the analyzed values based on the Kolmogorov-Smirnov test. Statistical significance was considered for $p < 0.05$ in application to all the variables.

Results

Histopathology and immunohistochemistry

The lymphohistiocytic infiltrate in the whole BPH pieces was very heterogeneous in terms of grade and distribution, with the definition of two patterns: diffuse interstitial (with isolated cells among the fibroblasts, myofibroblasts and smooth muscle cells) and nodular (in which the cells formed dense aggregates with a tendency to form nodules and follicles without germinal centers).

Myxoid nodules were identified (figs. 1 and 2) in the connective tissue of the chorion immediately subjacent to the urethral mucosa, in the depth of the periurethral sphincter, and within the internodular fibromuscular tissue. These myxoid lesions are micronodules, sometimes presenting well defined margins, but in other cases with less clearly defined margins. They are composed of a myxoid matrix in which fusiform stromal cells can be seen, expressing vimentin but not smooth muscle actin (fig. 3) or desmin. In all the nodules we identified a diffuse interstitial, mononuclear infiltrate exhibiting a uniform distribution (fig. 4). These cells were

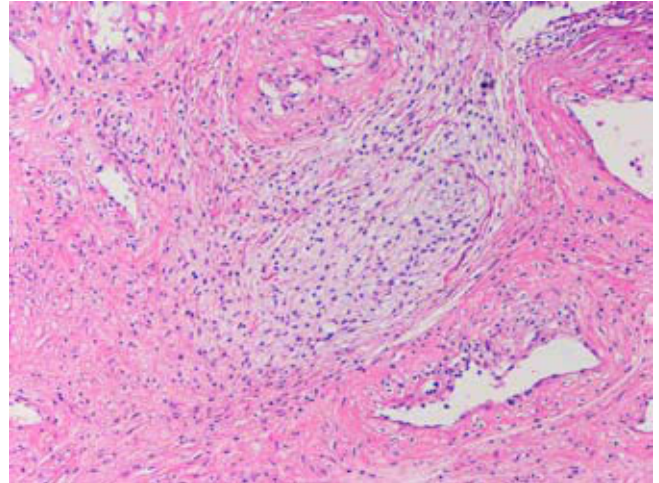


Figure 1 – Incipient stromal nodule with abundant myxoid ground substance in the central part of the image (hematoxylin-eosin, ×200).

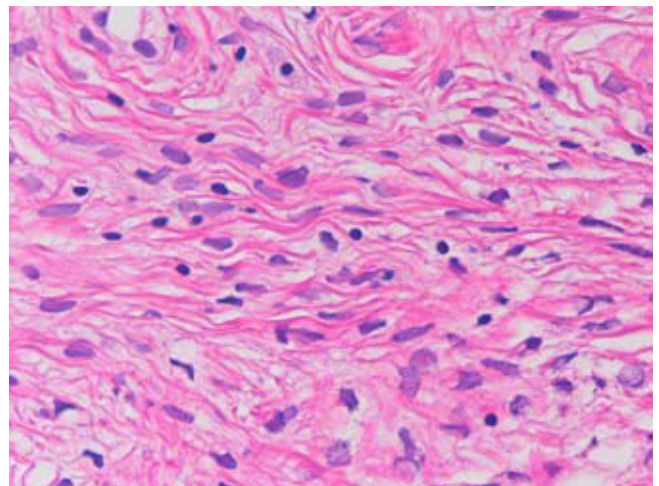


Figure 2 – Detail of the previous figure showing fusiform stromal cells and isolated lymphoid cells with a round hyperchromatic nucleus within the ground substance, and collagen fibers (hematoxylin-eosin, ×600).

immunohistochemically positive for T (CD3) (fig. 5) or B lymphocyte markers (CD20), in similar proportion. We also observed a less abundant population of histiocytes (CD68-positive), and very occasional mast cells.

Statistical analysis: The mean patient age of the global sample was 73.83 years (standard deviation 13.77), versus 53.89 (2.14) and 82.8 years (2.14) for the young and elderly subjects, respectively. The mean number of paraffin blocks examined per patient was 9.28 (2.15), 9 (2.3) and 9.4 (2.11), in the global sample and in the young and elderly subjects, respectively.

The mean number of nodules identified per patient was 0.48 (2.4), 0.33 (0.7) and 2 (2.7), in the global sample and in the young and elderly subjects, respectively. The mean diameters of the nodules of smaller and greater size were 1.27 (1.06) and

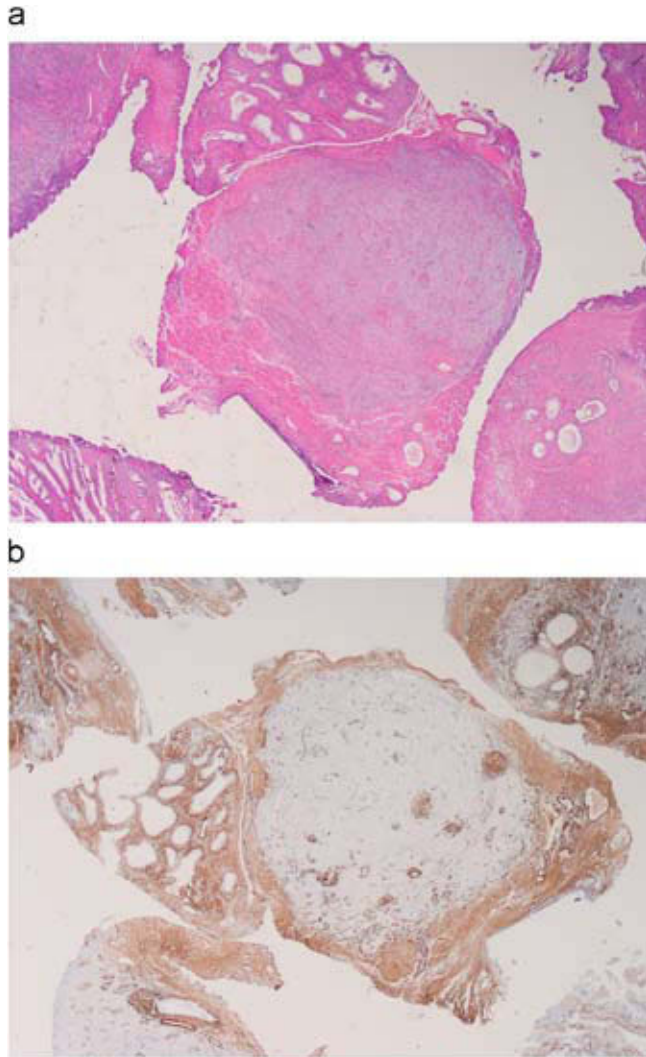


Figure 3 – a) Low magnification view of a myxoid nodule among more developed nodules corresponding to benign prostatic hyperplasia (hematoxylin-eosin, $\times 40$). b) The same nodule is immunohistochemically negative for specific smooth muscle actin (actin immunohistochemistry, $\times 40$).

3.17 (2.05) in the global series. These values in turn were 1.06 (0.98) and 3.04 (2.1), and 2.5 (0.7) and 4 (1.41) in the young and elderly subjects, respectively.

The two-factors analysis with the Student t-test showed the mean number of nodules to be significantly lower among the young patients ($p=0.02$). Although important differences were noted in the mean diameters of the smaller and larger nodules between the two age groups, statistical significance was not reached. However, with $p=0.07$, a tendency towards significance can be assumed in the case of the diameter of the smaller nodules. No significant correlations were found between the number of nodules and the age of the patient ($p=0.11$), based on the Pearson r test.

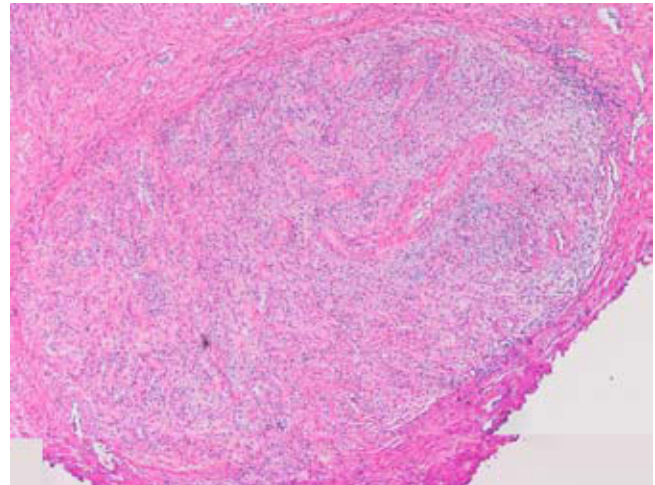


Figure 4 – A larger myxoid nodule showing diffuse mononuclear cell infiltration of the interstitial stroma (hematoxylin-eosin, $\times 100$).

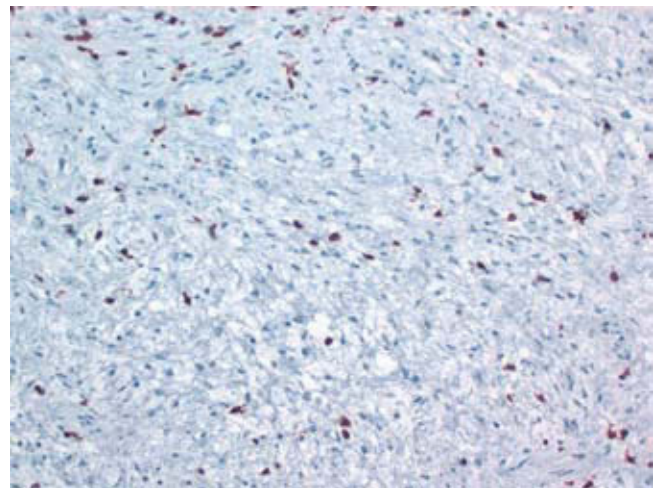


Figure 5 – Detail of immunohistochemical labeling for CD3, showing T lymphocyte reactivity (immunohistochemistry for CD3, $\times 400$).

Discussion

In routine practice, and particularly in the case of retropubic tumor resection samples, the usual histological study often reveals the presence of a lymphohistiocytic infiltrate, with an incidence of 40-87%, depending on the literature source²⁻⁵. In our experience, such an infiltration is always present when specifically searched for — though it shows considerable variability in terms of intensity, and presents two differential and often coexisting patterns: a first pattern is characterized by a predominant diffuse mononuclear cell infiltrate, while the other shows nodular groups of lymphohistiocytic cells

in addition to the mentioned infiltrate. Gerstenbluth et al. found the degree of inflammatory infiltration to increase with patient age and the volume of the adenoma — being insignificant in individuals under 55 years of age or in glands weighing less than 35 grams⁵.

This cellular infiltration of the stromal nodules must be distinguished from the secondary inflammatory changes that often accompany benign prostatic hyperplasia (BPH). Some cases show acute inflammatory foci (neutrophils) and chronic granulomatous inflammatory zones (histiocyte accumulations) or foci in the form of plasma cell infiltrations with no direct correlation to the characteristic lymphohistiocytic infiltrate of BPH.

In our study we analyzed the initial histopathological lesions of BPH. To this effect we selected two populations of males with BPH — young and elderly (under age 57 and over age 80 years) — with the purpose of comparing the histopathological alterations identified in the TURP pieces, and of defining the morphological evolution of the lesions. We identified no morphological features indicative of diffuse stromal hyperplasia (increase in size) in the transition region. Without ignoring this possibility, we observed micronodular lesions located mainly in the connective tissue of the chorion immediately subjacent to the urethral mucosa, in the depth of the periurethral sphincter.

Some of these nodules exhibit clearly defined margins, though others are poorly defined, and all are composed of a myxoid matrix containing fusiform stromal cells and disperse mononuclear inflammatory cells such as lymphocytes, histiocytes and very sporadic mast cells.

We found the number of nodules to be significantly lower in the young patients, showing an increase with advancing age (from 0.33 to 2). We consider that these may be the earliest histologically identifiable lesions of BPH. The stromal cells were seen to be positive for vimentin but negative for smooth muscle actin and desmin. This immunophenotype indicates a fibroblastic origin of the cells. In addition, this cell population showed metabolic activity, with the secretion of mucopolysaccharides and proteoglycans that conform the characteristic myxoid or mucoid matrix of these nodular lesions. The mast cells can also contribute to this mucoid matrix.

The mentioned matrix is important from the biological perspective, since it allows the diffusion of certain soluble extracellular factors such as growth factors and cytokines, and moreover also increases the migration of the different types of cells found in the nodules. In this environment, the stromal cells interact with the inflammatory cells — fundamentally B and T lymphocytes and histiocytes. It seems clear that this interaction must be important, since a constant inflammatory reaction has been identified, of uniform distribution. The mentioned reaction is also present in the most evolved nodules, where some phenotypic characteristics have been seen in the fusiform cells of the stromal component indicating leiomyofibroblastic (expression of actin) and even smooth muscle cell differentiation (with the additional expression of desmin). Such “maturation” of the nodules is also characterized by an increase in vascularization.

In our samples we have seen all the characteristic phases of the morphogenesis of prostatic hyperplasia. The earliest lesion is the myxoid nodule with an abundant cellular matrix rich in mucopolysaccharides, and with a low cellular density, corresponding to fibroblastic elements that are not modified either functionally or phenotypically. It has been seen that this phase is subsequently and concomitantly followed by an increase in capillary vascularization and morphological and functional maturation of the fibroblasts, myofibroblasts and myocytes⁶. According to the data published by Bierhoff⁶ and our own experience⁷, the developed stromal nodules show a lymphohistiocytic infiltrate from the start, and are also accompanied by mast cells in the very early stages. This underscores the importance of the lymphohistiocytic infiltrate in the etiopathogenesis of BPH, whether of a primary or secondary nature. These activated cells may release inflammatory mediators and growth factors⁷, which in turn progressively contribute to increased tissue damage and activation of hyperplastic tissue proliferation — presumably through the inhibition of apoptosis regulating factors such as BCL-2^{5,8}. The absence of granulocytes rules out the hypothesis of bacterial infection, while the abundance of T lymphocytes reinforces the immune hypothesis.

To summarize, BPH begins with microscopic myxoid lesions composed of immature fibroblastic and hematolymphoid cells over a myxoid background, which undergo morphological and functional maturation to become stromal nodules. The chronic inflammatory reaction is fundamentally linked to the pathogenesis of this disorder. We have observed an increase in the number of stromal myxoinflammatory nodules with advancing patient age. This alone seems to indicate the etiopathogenic role of these lesions in BPH, and the progressive nature of the disorder.

Conflicts of interest

The authors declare no conflicts of interest.

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