AgNOR histochemical expression in benign prostatic hyperplasia and prostatic adenocarcinoma

R Rita*, Delyuzar and L I Laksmi

Anatomical Pathology Department, Universitas Sumatera Utara, Medan, Indonesia
*Corresponding author: tabsroza82@gmail.com

Abstract. Benign prostatic hyperplasia and prostatic adenocarcinoma were common diseases and usually occurred after the 5th decade of life. The problem in diagnosing using Hematoxylin and Eosin staining was how to differentiate whether it is benign or malignant zone. Therefore, proliferating markers, such as AgNOR, could be helping to over this difficulty. A descriptive study using consecutive sampling as the method of sample recruiting was conducted to describe AgNOR histochemical expression in benign prostatic hyperplasia and prostatic adenocarcinoma. AgNOR staining was done in 13 benign prostatic hyperplasia samples and 7 prostatic adenocarcinoma samples, which have been confirmed using p63 immunohistochemical staining before. Benign prostatic hyperplasia usually showed lower AgNOR proliferating activity while all of theprostatic adenocarcinoma (100%) had high AgNOR proliferating activity.

1. Introduction

The prostate is an organ involved in themale reproduction system. There is only three pathologic processes that affect the prostate gland with sufficient frequency to merit discussion namely inflammation, benign prostatic hyperplasia (BPH) and tumor. The incidence rates of prostatic diseases, such as BPH and prostatic adenocarcinoma, increase with age. The 2010 American Urological Association (AUA) Guideline reported that there was an increasing incidence rate of BPH around the world and predicted that there are more than 50% 0f 60-year-old men microscopically showed BPH appearance.[1-3]

There are various difficulties in differentiating benign and malignant zones of theprostatic lesion in hematoxylin and eosin (H&E) staining. The key of diagnostic criteria in differentiating benign and malignant prostatic lesion is the loss of abasal cell adenocarcinoma. Several IHC markers have been used to stain prostatic basal cell, such as high molecular weight cytokeratin (HMWCK), p62, etc. p63 is a core protein that is coded by the gene of the 3q27-29 chromosome, that is homologue with p53 (a tumor suppressor gene), which regulates the growth and development of epithelium of skin, cervix, breast, prostate, prostatic stem cell and urogenital tract.[4-5]

Proliferating markers, such as argyrophilic nucleolar organizing region (AgNOR) and proliferating cell nuclear antigen (PCNA), are very helpful in differentiating whether it is abenign, premalignant, or malignant lesion. Nucleolar organizing regions (NORs) are genetic loci on chromosomes that are composed of ribosomal DNA (rDNA) and proteins, some of which are argyrophilic characteristic. They are located in the nucleoli of cells and on the short arms of chromosomes 13, 14, 15, 21 and 22 in association with proteins. Since RNA is the main location for protein synthesis, the amount of

NORsmay reflect the activity of the nucleus and the cell itself. Therefore, the AgNOR value can be considered as a measure of the rate of cell proliferation.[2,4,6-11]

Khanna et al. (2014) reported that there was a statistically significant difference between AgNOR value per nucleus of benign lesion compared to amalignant lesion. The mean number of silver-stained nucleolar regions, such as AgNOR count per nucleus, was observed to be 2.1 in BPH, of which lowest value was 1.2,and thehighest value was 3.0. In another hand, the average AgNOR count per nucleus in malignant specimens was 5.15, ranging in 3.8 as the lowest value and 7.1 as the highest value. The mean of AgNOR count per nucleus was shown to be highest in poorly differentiated prostatic adenocarcinoma.[12]

2. Methods

This descriptive study used cross-sectional design aiming to observe AgNOR histochemical expression in benign prostatic hyperplasia and prostatic adenocarcinoma. This study was conducted at the Universitas Sumatera Utara, Anatomical Pathology Department and Haji Adam Malik Public Hospital, Pathology Laboratory. The population in this study were all paraffin-embedded tissue blocks diagnosed as BPH and prostatic adenocarcinoma (malignant) lesions. These tissues were taken from transurethral resection of theprostate (TURP) and prostatectomy from the Universitas Sumatera Utara, Anatomical Pathology Department and Haji Adam Malik Public Hospital, Pathology Laboratory.

The procedure was a follow. First, we sought for BPH and prostatic adenocarcinoma patients medical records at places stated above. Based on the data, the histopathology slides were then examined by two pathologists and the author. Samples were dichotomised into BPH and prostatic adenocarcinoma. Later, we sought for the paraffin blocks and undergone the slicing process. The first slice was stained with p63 immunohistochemical staining and the second slice was stained with silver nitrate in order to observe the Nucleolar Organizer Regions (NORs). p63 immunohistochemical staining was performed using the REAL EnVision method. Rabbit p63 monoclonal antibody was used as aprimary antibody with 1:50 – 1:100 dilutions and the immunogen used was human p63 recombinant protein fragments. The study instrument was thep63 expression on the prostatic microscopic samples.

3. Results

This study was done in Universitas Sumatera Utara, Anatomical Pathology Department. According to HE study, the population was 47 slides diagnosed with either BPH or prostatic adenocarcinoma. However, after sample size calculation was done, there only 20 samples needed, and they were randomly recruited in the study to undergone diagnostic confirmation using p63 study. Among those samples, there were 13 (63%) patients diagnosed with BPH and 7 (35%) patients diagnosed with prostatic adenocarcinoma. Moreover, adenocarcinoma patients were then categorized using Gleason grade group.

3.1. Descriptions of BPH and prostatic adenocarcinoma patients based on p63 immunohistochemical study

Of 20 BPH and prostatic adenocarcinoma patients that were confirmed using p63 immunochemistry, 13 patient was diagnosed BPH. Most of them (69.2%) showed moderate positive staining while 23.1% showed weak positive staining, and only 1 patient (7.7%) showed strong positive staining. Meanwhile, all prostatic adenocarcinoma samples showed negative staining.

3.2. Distributions of prostatic adenocarcinoma patients based on Gleason grade group

We categorized the samples using Gleason grade group. Out of 7 prostatic adenocarcinoma patients, 5 (71.4%) of, 1 (14.3%) patient showed Gleason grade group 4, and 1 (14.3%) patient showed Gleason grade group 3.

3.3. Distributions of BPH and prostatic adenocarcinoma patients by age
The mean age of BPH patients was 65.3 years old, ranging from 40-78 years old, whereas the mean age of adenocarcinoma patients was 62.86 years old with the range of 50-80 years old.

3.4. Distributions of samples by the AgNor proliferating activity
Among 20 samples, 60% showed high AgNOR proliferating activity, while 40% had low AgNOR proliferating activity.

3.5. Cross distributions of samples by the AgNor proliferating activity
Among 13 BPH samples, 12 samples (92.3%) showed low AgNOR proliferating activity. All of those seven prostatic adenocarcinomas (100%) had high AgNOR proliferating activity.

3.6. Cross distributions of grading prostatic adenocarcinoma diagnosis based on AgNOR proliferation index
Among 5 prostatic adenocarcinoma patients with Gleason grade group 5, there were two patients who had AgNOR proliferating index of 9-100%, two patients with an index of 61-70% index, and one patient had an index of 20-30%. One prostatic adenocarcinoma patient who was Gleason grade group 4 had AgNOR proliferating index of 61-70%. One of prostatic adenocarcinoma patient with Gleason grade group 3 had a proliferating index of 81-100%.

4. Discussion
This study showed that mean age o BPH patients was 65.23 years old. The youngest age was 40 years old while the oldest was 78 years old. On the contrary, mean age-old adenocarcinoma was 62.86 years old, ranging from 50-80 years old. The incidences of BPH and prostatic adenocarcinoma were increasing along with age. The result of this study was in accordance with the AUA guideline. The AUA 2010 guideline stated that more than 50% male who aged more than 60 years old microscopically has BPH appearance. In other literature, they also stated that BPH was observed in 20% of 40-year-old men, increasing and reached 70% at the age of 60, and 90% at the age of 80. Meanwhile, prostatic cancer was mostly detected in >60-year-old men. Three-fourths of all cases around the world occur in 65-year-old men or older. Only 1% was detected clinically in <50-year-old men.[13]

From the samples used in this stud, 65% samples were diagnosed with BPH. This result is in accordance with Subathra and Sangeetha study (2014), in which they reported that BPH was more frequent than malignant lesion.[2] Therefore, in this study, we also found that the number of BPH patients was more than prostatic adenocarcinoma patients.

This study found that from 20 samples, 65% showed AgNOR proliferating activity. Of 13 BPH patients, 12 samples (92.3%) showed low AgNOR proliferating activity. Meanwhile, all prostatic adenocarcinoma samples showed high AgNOR proliferating activity. The pAgNOR study has not been done using prostate as sample however it has been done for other diseases. The result of this study was parallel with several studies stated in following. Ibnerase et al. (2005) found that most malignant effusion cases have AgNOR proliferating index > 90%.[14] Khan et al. (2008) reported that proliferating index was significantly higher ($p<0.05$) in grade III transitional cell carcinoma of bladder than in grade II tumor.[15] Hossain et al. (2012) assess the role of AgNOR multiparameter analysis for thyroid FNA smear in differentiating benign and malignant lesions. The mean of pAgNOR in benign lesions was 0.63 (SD ± 1.33) with the range of 0-74, while mean pAgNOR in malignant lesion was 14.02 (SD± 3.01) with the range of 11.9-19. From 166 case, 148 has 8% pAgNOR (considered as a benign lesion), and 9 of them showed 11% pAgNOR (considered as a malignant lesion). That study also found the sensitivity, specificity, PPV, NPV, and accuracy of AgNOR in differentiating thyroid benign and malignant lesion in FNA were 83.33%, 100%, 100%, 98,21% and 98.36%, respectively.[16]Syed et al. (2014) studies about AgNOR in primary oral squamous cell carcinoma. They found that pAgNOR showed a significant difference in in poorly differentiated carcinoma

(p=0.001), which is higher in poorly differentiated carcinoma. Many studies have shown that the proportion of proliferating cell was significant, which is higher in poorly differentiated carcinoma. Many studies have shown that the proportion of proliferating cell was significantly increased in prostatic adenocarcinoma than in BPH. As was indicated by AgNOR count per nucleus, the AgNOR count per nucleus was twice higher in prostatic adenocarcinoma than in BPH. As was indicated by AgNOR count per nucleus, the AgNOR count per nucleus was twice higher in prostatic adenocarcinoma that in BPH with a p-value of 0.001. This means that the proliferating activity was increased in adenocarcinoma. Many studies have shown asignificant increase in theproportion of proliferating activity in carcinoma compared with thebenign lesion.

5. Conclusion

After the study was done to 20 BPH and prostatic adenocarcinoma samples, it could be concluded that mostly BPH showed<8% AgNOR expression, which meant a low proliferating activity. However, prostatic adenocarcinoma mostly showed a very high AgNOR expression, which is >8%.

References

[1] Epstein J I 2010 The lower urinary tract and male genital system *Robbins and cotran Pathologic basis of disease*ed ed V Kumar, A K Abbas, *et al.* (China: Saunders Elsevier) pp 993-1001
[2] Subathra K and Sangeetha N 2014 Histopathological study of prostatic lesions and assessment with agnor index *Int. J. Pharm. Bio. Sci.* **5(2)** 253-60
[3] Parijatham B O, Ranjitham P, Poorana P P and Subhashree A R Role of immuno-hisochemical stains in prostatic neoplasms: a hospital based study research *J. Pharmaceu. Biol. Chem. Sci.* 0975-8585
[4] Hendrianto, Lubis H M N D and Lukito J S 2012 Perbedaan tampilan imunohistokimia p63 antara neoplasia intraepitel prostat dengan adenokarsinoma prostat *Majalah Patologi* **21(1)** 8-13
[5] Pathology Outline 2010 Stains p63 *Diagnostic pathology* [Accessed: June 20, 2016] **5** 8 Available from: http://pathologyoutlines.com/topic/stainsp63.html
[6] Elemam I B Y, Elsheikh M A, Elnour A M A, Elhaleem H M M A and Alobaid A E A 2015 Assessment of proliferation activity by using nucleolar organiser regions count among sudanese patients with prostate cancer and benign prostate hyperplasia *J. Pharm. Biomed. Sci.* **5(11)** 863-6
[7] Rajeshwari K, Damle R, Dravid N and Karibasappa G N 2015 Arygyrophilic nucleolar organizer regions (AgNORs) as proliferative marker in various prostatic lesions *Indian J. Pathol. Oncol.* **2(3)** 126-30
[8] Dhillon P , Barry M, Stampfer M J, Perner S, Fiorentino M, Fornari A, Ma J, *et al.* 2009 Aberrant cytoplasmic expression of p63 and prostate cancer mortality *Cancer Epidemiol. Biomarkers Prev.* **18(2)** 595-600
[9] Eroz R 2015 Argyrophilic nucleolar organizing regions associated proteins in oncocytology *Oncocytology* **5** 21-4
[10] Torabi- Nezhad S and Kheradmand P 1999 Differentiation between prostatic carcinoma and benign prostatic hyperplasia by AgNOR staining *MJIRI* **12(4)** 333-8
[11] Khanna A, Patil R and Deshmukh A 2014 Assessment of the potential of pathological stains in human prostate cancer *J. Clin. Diag. Res.* **8(1)** 124-8
[12] Rosai J 2011 Male reproductive system: prostate and seminal vesicles, testis, testicular Adnexa, penis and dcrotum *Rosai and ackerman's surgical pathology* vol 10
[13] Caliskan S, Koca O, Akyuz M, Ozturk M and Karaman M 2015 Clinical significance of single microscopic focus of adenocarcinoma at prostate biopsy *Prostate Int*. **3** 132-4
[14] Ibnerasa S N, Chaudhry N A and Khan S A 2005 AgNOR proliferative index in malignant pleural and peritoneal effusions *Inter. J. Pathol.* **3(2)** 86- 90

[15] Khan A H, Khan S A, Chaudrhy N A and Tayyab M 2008 Argyrophilic nuclear organiser regions (AgNORs) pattern in different grades of transitional cell carcinoma of urinary bladder *Annals* **14(3)** 86-9
[16] Hassain M I, Hassan M Q, Bhattacharjee P, Ahamad M S U and Rahman Z 2012 Hindawi publishing corporation, pathology research international Article ID 908106 doi: 10.1155/2012/908106