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Assessment of Proliferation Activity by Using Nucleolar Organiser Regions Count among Sudanese Patients with Prostate Cancer and Benign Prostate Hyperplasia

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ABSTRACT

Background Prostatic adenocarcinoma is the most prevalent cancer and the second cause of cancer related death among men; the tumour proliferative activity is difficult to measure histologically. Increasing evidence suggests that the factors controlling cell cycle progression also modulate the rate of ribosome biogenesis; and can assess the proliferative activity. The present study aimed to assess the proliferation activity in prostate cancer.

Materials and Methods A total of 40 various prostatic lesions were studied, 20 cases of prostatic adenocarcinomas (study group) and 20 cases of benign prostatic hyperplasia (BPH) as (control group). Sections of 3-µ thickness was obtained from each formalin-fixed paraffin-embedded tissue block using rotary microtome and it was stained using haematoxylin and eosin (Mayer's technique) and AgNOR stains.

Results The majority of patients with BPH and prostate adenocarcinoma were in their sixth to eighth decade of life. The BPH samples displayed fewer AgNORs (mean 2.0 dots/cell) compare to adenocarcinomas (mean 4.1 dots/cell), p value was (0.001). Therefore this data indicate that analysis of silver staining-positive material in intact interphase cells may help distinguish between benign and malignant prostatic tumours.

Conclusions AgNOR have a value in distinguishing between BPH and adenocarcinoma of the prostate.

KEYWORDS prostate, carcinoma, prostatic hyperplasia, AgNORs

INTRODUCTION

Prostate is the gland in male reproductive system.[1] Most prostate cancers are slow growing; however, some grow relatively fast.[2,3] the cancer cells may spread to the bones, lymph nodes and other parts of the body[4]. Initially it may cause no symptoms.[3] In later stages it can cause difficulty in urination, haematuria, or pelvic pain.[5] The benign prostatic hyperplasia (BPH) may produce similar symptoms. Later symptoms may include fatigue, due to low levels of red blood cells.[3] The risk factors of prostate cancer include older age, family history, and ethnicity. About 99% of cases occur in those over1 the age of 50 years. First degree relative affected increases the risk 2- to 3-fold. In the United States it is more common in the African American population than the Caucasian population. Other risk factors are processed red meat diets, high consumption of milk products and low intake of certain vegetables.[2] The prostate cancer diagnosed by biopsy and medical imaging may then be done to determine if the cancer has spread to other parts of the body.[5] Regarding prostate cancer screening, it is controversial.[2,3] Prostate-specific antigen testing increases cancer detection but does not decrease mortality.[6] The United States Preventive Services Task Force (USPSTF) recommends against screening using the PSA testing, due to the risk of over-diagnosis and over-treatment as most cancer diagnosed would remain asymptomatic.

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The USPSTF concludes that the potential benefits of testing do not outweigh the expected harms.[7] 5α-reductase inhibitors therapy appears to decrease low grade cancer, but it does not affect high grade one and thus are not recommended for prevention.[2] Supplementation with vitamins or minerals does not appear to be of preventive benefit.[2,8] Many cases can be safely followed up with active surveillance or watchful waiting. Treatments may include a combination of surgery, radiation, hormone or chemotherapy.[5] The prostate cancer can be curable, if confined to the gland.[3] Metastasis to the bones needs pain medications, bisphosphonates and targeted therapy. Outcomes depend on a person's age, aggressiveness, extension of the cancer other health problems. Most people with prostate cancer do not end up dying from the disease.[5] In the United States, the five year survival rate is 99%.[9] It is the second most common type of cancer globally and the fifth leading cause of cancer-related death in men.[10] In the year 2012 it occurred in 1.1 million men and caused 307,000 deaths worldwide.[10] It was the most common cancer in males in 84 countries,[2] occurring more commonly in the developed countries. Rates have been increasing in the developing countries.[11] Detection increased significantly in the 1980s and 1990s in many areas due to increased adoption of PSA testing.[2] Studies of autopsy from males aged over 60 years, who died from unrelated causes, had found prostate cancer in 30% to 70% of samples.[3]

The nucleolar organiser regions (NORs) were first described by Heitz (1931) and McClintock (1934) as weakly stained chromatin regions around which, at the end of telophase, nucleoli are reformed after their disappearance during the mitotic phase of cell cycle. NORs located on short arm of acrocentric chromosomes (chromosome number 13, 14, 15, 21 and 22) contain ribosomal genes which encode for ribosomal RNA and play an important role in protein synthesis[12,13]. NORs are considered to be a marker of both DNA transcriptional activity and DNA transcriptional potential[14]. Associated with these regions are certain acidic and argyrophilic, non-histonic proteins called NOR-associated proteins (NORAPs)[15]. These structures contain all necessary components for r RNA synthesis and are the sites where the transcription of ribosomal genes occurs[16]. The silver-stained NORs are called argyrophilic nucleolar organiser region (AgNORs)-associated proteins. AgNORs in normal cells are usually tightly aggregated within one or two nuclei, thus making individual Ag-NORs indiscernible. An increase in the mean AgNOR count of a cell population could be the result of a defect in nucleolar aggregation, association, ploidy; or increased transcriptional activity[17,18]. Theoretically, a neoplastic cell population could show any or all of the above defects and therefore demonstrate an increased AgNOR count. Several studies have shown that AgNOR frequency within nuclei is significantly higher in malignant cells than in normal, reactive; or benign neoplastic cells. AgNOR expression is directly related to the ribosome biosynthesis rate, which, in proliferating cells, is directly related to the length of the cell cycle. The shorter the cell cycle, the greater the synthesis of rRNA for each time unit and, therefore, the greater the quantity of AgNOR present in the nucleolus. Thus, the AgNOR value can be considered as a measure of the rate of cell proliferation[19].

MATERIALS AND METHODS

This study was a prospective case-control study conducted in Khartoum state, during the period from March to June 2015. Samples involved in this study were collected from Sudanese patients with prostatic cancer and prostate hyperplasia. Paraffin-embedded formalin-fixed tissues were used to perform AgNOR technique. Twenty samples from case group (patients with prostate cancer) versus 20 samples from control group (patients with prostate hyperplasia). One of the two sections was stained by hematoxylin and Eosin staining method to confirm diagnosis of each block. The second section was stained separately by using argyrophilic silver technique.

METHODS

Hematoxylin and eosin staining method

Sections of 3-μ thickness were obtained from each formalin-fixed paraffin-embedded tissue block by using rotary microtome. Sections were stained in haematoxylin and eosin (Mayer's technique) to confirm the histopathological diagnosis. Sections were dewaxed in two changes of xylene for 2 min, hydrated through ethanol 100%, 90%, 70%, 50% and water for 2 min for each, then stained in Mayer's haematoxylin for 7 min, then washed in running tap water for 10 min, then stained in eosin for 3 min, then washed in distilled water and dehydrated through ascending ethanol concentrations, then cleared in xylene and finally mounted in distyrene plasticizer xylene (DPX).

AgNOR staining

Solution A (50% silver nitrate solution) and Solution B (gelatine solution) were prepared separately. Two parts of solution were mixed with one part of solution B to form the working solution. Sections were de-waxed in xylene and rehydrated with alcohol and water consecutively. After washing with distilled water, sections were incubated in freshly prepared working solution for 45 min in darkness and at room temperature. Then sections were washed with distilled water for 1 min and

dehydrated, cleared and mounted in DPX. The AgNOR positive sites were seen as intra-nucleolar black dots and the background were observed to be pale yellow.

Counting of AgNOR positive sites

Each slide was considered as a single unit in which 20 nuclei were counted for the presence of black appearing AgNOR dots in the nucleolar region by using oil immersion lens. AgNOR dots from each nucleus (total 20 nuclei) were analysed and mean AgNOR count for each slide were calculated.

All results were analysed by Statistical Package for the Social Sciences. The means were obtained (t test) were used to analyse the numerical count of AgNOR in test and control groups. p value was obtained to assess the significance of the results; the data was presented in the form of tables.

RESULTS

A total of 40 cases were used in the present study, which included 20 cases. The patient's age ranged from 52 to 95 years which categorised into four groups as follows: 51-60, 61–70, 71–80 and more than 80 years. And 50% of the prostate adenocarcinoma cases (10 cases), fall in the age of 61–70 years; 55% of the benign prostate hyperplasia was in the age group of 61–70 years, as given in Table 1. The peak incidence of prostate adenocarcinoma and prostate hyperplasia was in age group 61–70 years.

The average of AgNOR was 4.1 in prostatic adenocarcinoma and 2.0 in (BPH), the average of AgNOR dots per nucleus between cases of BPH (control) and cases of carcinoma (study group) was statistically significant (p value 0.001) as given in Table 2.

The average of AgNOR/nucleus, was calculated against the age group in the prostate adenocarcinoma patients, the maximum averages was reported in the sixth and seventh decant as given in Table 3.

DISCUSSION

Tumour differentiation and proliferative activity are important predictors of biological behaviour. Routine histopathological evaluation is fairly adequate for assessing differentiation. Tumour proliferative activity is difficult to measure, however. Silver staining for the NOR is useful for assessing tumour proliferation. The present study aimed to analyse the AgNOR count expression in benign prostate hyperplasia and prostate adenocarcinoma lesions and to assess whether these can be useful in differentiating them.

In prostate lesions, many studies have shown a significantly increased proportion of proliferating cells in adenocarcinoma compared with benign prostate hyperplasia. As indicated by AgNOR count per nucleus, the AgNOR count per nucleus in prostate adenocarcinoma was 2-fold increases compared to the benign hyperplasia, the p value was 0.001; similar observations were reported in other previous studies[20,21]. That means the proliferation activity was increased in the adenocarcinoma; many studies have shown a significantly increased proportion of proliferating activity in carcinoma compared with benign lesions[22]. Some studies have demonstrated AgNOR number to have a role in differentiating between benign and cancerous lesions[23,24]. However AgNOR counts are of no use for diagnosis of any single case, because of overlapping counts as reported by Hansen and Ostergard[20] concluded that, despite statistically significant differences, AgNOR counts are of no use for diagnosis of any single case. AgNOR typing, however, may contribute to a differential diagnosis between benign and malignant lesions.

Our findings were in agreement with the study conducted by Ahsan et al.[25] they observed a significantly different count in nodular hyperplasia and carcinoma. They concluded that the AgNOR technique is rapid, simple; and reproducible, although somewhat tedious and laborious.

Also the study clarified that the prostate adenocarcinoma and benign prostate hyperplasia were more common in elderly men, as a high frequency in the sixth

Table 1 The frequency of prostate adenocarcinoma and prostate hyperplasia according to the age.

Age group (years)	Prostate adenocarcinoma	Prostate hyperplasia	Total
51–60	2	4	8
61–70	10	11	24
71–80	5	4	11
More than 80	3	1	4
Total	20	20	47

Table 2 Comparison of the average of AgNOR between adenocarcinoma and benign hyperplasia of the prostate.

Study group	Average of AgNOR	p value
Prostate adenocarcinoma	4.1	
Prostate hyperplasia	2.0	0.001

Table 3 Average of AgNOR in the prostate adenocarcinoma.

Age group (years)	Mean of AgNOR dots/nucleus
51–60	3.8
61–70	4.3
71–80	4.2
More than 81	3.4

and seventh decants, and the rate of proliferation indicated by the numerical AgNOR/nucleus, was correlated with the age of patients. Our findings were agreed with Carter and colleagues[26], showed that 50% of men between 70 and 80 years of age showed histological evidence of malignancy.

CONCLUSION

AgNOR count may be helpful in distinguishing between adenocarcinoma and benign hyperplasia of the prostate.

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