

Assessment of Expression of Ki-67 in Benign and Malignant Prostatic Lesions among Sudanese Patients

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Abstract

Citation: Mohamed AA, Abbas MY, Alharbi H, Babiker AY. Assessment of Expression of Ki-67 in Benign and Malignant Prostatic Lesions among Sudanese Patients. Open Access Maced J Med Sci. <https://doi.org/10.3889/oamjms.2018.309>

Keywords: Prostatic cancer; Ki-67; Sudanese patients; Prostatic lesions

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Received: 17-Mar-2018; **Revised:** 17-Sep-2018; **Accepted:** 18-Sep-2018; **Online first:** 20-Oct-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

BACKGROUND: Prostatic cancer is one of the most common cancers of males in a Sudanese population. The early detection is very important, as it is only curable at an early stage.

AIM: The objective of this study was to investigate the expression pattern of Ki-67 in benign and malignant prostatic lesions to improve the diagnosis that may help in better management and prevention of disease.

MATERIAL AND METHODS: Fifty-eight formalin fixed paraffin blocks from diagnosed cases of prostatic tumours with different grade, and stages were included in this study. Ki-67 expression was examined immunohistochemically by using monoclonal mouse anti-human Ki67 IS626. The results were correlated with Gleason score and tumour differentiation and stage.

RESULTS: The frequency of histological types was as follow: 11 cases of benign prostatic hyperplasia (19%) and 47 cases of prostatic cancer (81%). Our results stated that prostatic adenocarcinoma among Sudanese patients was of low grade which means tumours are less aggressive. Furthermore, the findings demonstrate that Ki-67 expression in prostatic carcinoma smears was correlated significantly with the degree of Gleason score ($P < 0.05$).

CONCLUSIONS: We found that the prostatic adenocarcinoma among Sudanese patients was less aggressive. Furthermore, Ki-67 expression was proportional to the grade of a tumour and it was a useful prognostic and diagnostic biomarker.

Introduction

Prostatic cancer is the second most common cancer of males in Western societies and those emulating Western lifestyles and diets. It was the fourth of the top 10 cancers found in Khartoum state – Sudan between 2009 -2010 (rate = 7.3 per 100,000) [1]. In the United States, the new cases were estimated to be 11,500; approximately one in seven American men diagnosed with prostate cancer during their lifetime [2]. Detection of this disease earlier, as a consequence of the introduction of the prostate-specific antigen (PSA) blood test, has been acknowledged by the National Cancer Institute (NCI) as one factor contributing to lowering the mortality rate over the past few years [3]. The factors that determine the risk of developing clinical prostate Cancer are not

well known; however, a few have been identified; an important risk factor seems to be heredity (4). The most important risk factor such as food consumption, the pattern of sexual behaviour, alcohol consumption, exposure to ultraviolet radiation and occupational exposure have all been discussed as being of etiological importance [5]. Other factors increasing risk include low intakes of vitamin E, selenium, lignans and isoflavonoids [6]. The reviewed international trends in prostate cancer mortality and reported significant reductions in prostate-cancer mortality in the UK, USA, Austria, Canada, Italy, France, Germany, Australia and Spain with downward trends in the Netherlands, Ireland and Sweden [7]. Detection of this disease earlier, as a consequence of the introduction of the prostate-specific antigen (PSA) blood test, has been acknowledged by the National Cancer Institute (NCI) as one factor contributing to

lowering the mortality rate over the past few years. The use of PSA testing has been estimated to provide a diagnostic lead-time of up to 10 years [3] [8]. Ki-67 is a novel proliferative marker that can be readily detected by immunohistochemistry. The expression of Ki-67 is shown in all stages of the cell cycle, except G0, whereas resting cells entering from G0 lack Ki-67 in the early part of G1 [9]. The fact makes it an excellent marker for determining the so-called growth fraction of a given cell population. The usefulness of the Ki-67 labelling index has been well established for various types of malignant neoplasms [10]. Ki scoring is essential for diagnosis for tumour grade, based on proportional of tumour-positive cells has usually used as an indication for evaluation, and many reports have shown clinical significance in a variety of cancers regardless of whether the origins epithelial or non-epithelial [11]. The concordance rate of the Ki was determined by classifying the Ki 67 into less than 5% (< 5%) per 100/high power field (HPF), scored as zero (low), 5%-10% per 100/HPF, scored as 1 (intermediate), and higher than 10% (10% <) per 100/HPF, scored as 2 and 3 (higher) [12]. Tissue microarrays (TMAs) are now widely accepted as a fast and cost-effective tool for use in almost any application requiring in situ tissue analysis [13]. According to its applications, TMAs was classified into predictive TMAs used to establish markers that predict response to therapy [14] and TMAs for validation of markers discovered by extracted protein, DNA- or RNA-based studies [15]. TMAs used to correlate staining results with clinical endpoints [16].

To the best of our knowledge, this is the first study to score ki-67 expression in prostatic cancer among Sudanese patients. The overall objective of this study was to improve the diagnostic and prognostic by investigating the expression of ki-67 this will provide data which may help in better management and control of this disease.

Material and Methods

This is a descriptive retrospective study, was conducted at the University of Science and Technology to Laboratories Administration and different laboratories in Khartoum State, Sudan.

A total of 58 patient, 11 cases of benign prostatic hyperplasia and 47 cases of prostate cancers were collected, and immunohistochemical staining was performed to evaluate the expression of Ki-67. The study was approved by the local Ethics Committee of the University of Science and Technology. Poorly fixed, overheated and too tiny specimen where excluded.

Formalin-fixed paraffin-embedded tissue blocks were sectioned by using Rotary microtome and

low profile disposable knives by using 4 microns as the thickness of choice. Sections were then floated on a floating water bath adjusted to 45°C. Finally, clear-coated glass slides were used to pick up the floated sections and slides were left in a 60°C overnight in a hot air oven. Next day after oven drying sections were subjected to xylene and then to decrease graded of alcohols (100%, 90%, 70%, and 50%) for dehydration. The section was boiled in preheated retrieval buffer, performed in citrate buffer with pH 6.0 for 40 min at 95°C in a water bath followed by cooling at a refrigerator for 10 min. After cooling the slides, Phosphate Buffer Saline (PBS) was added to the slides for 5 min. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide (Envision FLEX Peroxidase-Blocking reagent K8000/K8010) for 7 min and then washed with PBS for 7 min. The slides were then incubated with primary antibody (monoclonal mouse anti-human Ki67 IS626) for 30 min followed by phosphate buffer saline for 7 min. Then the slides were incubated with secondary antibody (EnVision FLEX/HRP K8000/K8010) for 30 min, and then washed with PBS for 7min, and also sections were incubated with streptavidin peroxidase. Substrate-DAB Chromogen (K8000/K8010 Substrate Working Mix) was added for 10 min. The DAB Chromogen was prepared as described by the manufacturer (Dako) by adding one drop from the chromogen to 1ml of the substrate buffer. Slides were then washed in distilled water for 20 min and counterstained with Mayer's hematoxylin. The slides were evaluated individually in a standard light microscope for immunohistochemical staining. Positive controls were included from the beginning.

A hundred fields were examined for Ki-67 expression. Expression of Ki-67 less than 5 per cent per 100 high power fields (HPF) was scored as zero (low). Expression from 5 to 10 per cent of Ki-67 per 100/(HPF) was scored as 1 (intermediate) and expression more than 30 per cent of ki-67 per 100/(HPF) was scored as 2 and 3 (high) [10].

Data were analysed by using the Statistical Package for the Social Sciences 20.0, SPSS, Inc., Chicago, IL) (SPSS).

Results

Fifty-eight cases of a prostate tumour were included in this study, patients' age ranged between 50-82 years with (mean 66 years). The frequency of histological types was as follow; 11 cases of benign prostatic hyperplasia (19%) and 47 cases of prostatic cancer (81%). 27 cases of low grade (57.4%), 20 cases of high grade (42.6%). Table 1, shows there

was a significant correlation between age and prostatic cancer ($P < 0.05$).

Table 1: Association between age and diagnosis cross tabulation

Cross-tabulation	Diagnosis		Total	
	B.PH	CA		
Age group (Years)	(50-60)	11	1	12
	(61-70)	0	20	20
	(71-82)	0	26	26
Total		11	47	58

Chi square p value = 0.000**

Chi-square p-value less than 0.05 that's considered as statistically significant. ** Significant.

According to the Ki-67 expression among different tumour grades in the current study, the Gleason scoring was: twenty-seven cases of low-grade prostatic cancer were included, 74% cases were scored as score zero, while the remaining cases (16%) were scored as score 1 shown in Figure 1. On the other hand, Twenty cases of high-grade prostatic cancer were involved in this study; fourteen cases were scored as score 1, only two cases were scored as score 2 whereas the remaining three cases scored as score 3 as shown in Table 2.

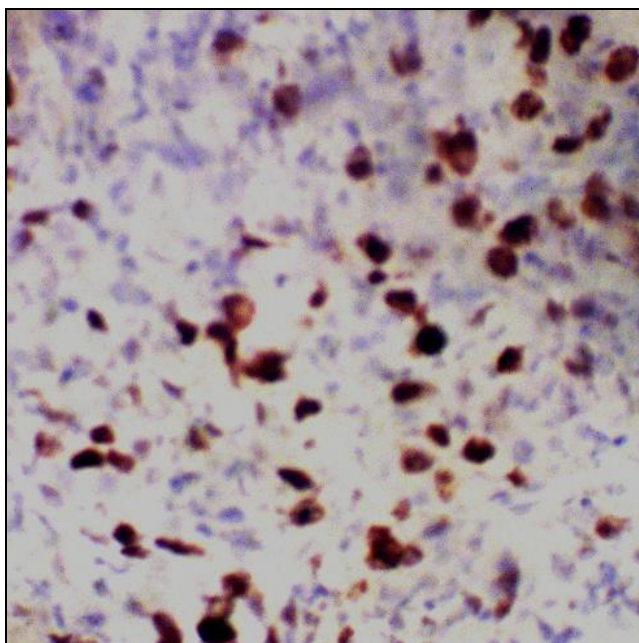


Figure 1: Expression of Ki-67 in malignant prostatic cancer

The result showed that there was a significant correlation between Ki-67 score and tumour grades ($P < 0.05$). This means that a high score is associated with increased Gleason grade as shown in Figure 2.

Table 2: Association between scoring (Gleason) Ki-67 and Grading cross tabulation

Cross-tabulation		Grading		Total	Percentage Of low grade	Percentage High grade
		Low grade	High grade			
Scoring (Gleason) Ki67	Low (<5% = Score 0)	20	1	21	95.5%	4.7%
	Intermediate (5-10% = Score1)	7	14	21	33.3%	66.6%
	High (score 2 =10-30% and score 3 = >30%)	0	5	5	00.0%	100%
Total		27	20	47		

Chi square p value = 0.000**

Chi-square p-value less than 0.05 that's considered as statistically significant. ** Significant.

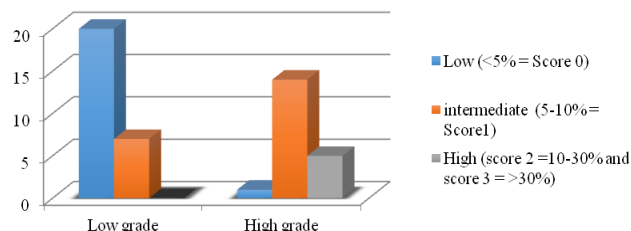


Figure 2: Distribution of scoring (Gleason) Ki-67 in low and high grade

Discussion

Prostatic cancer is one of the serious types of carcinomas that affect men all over the world. Although no strong data is available, a significant increase in the disease is noticed in Sudan. In this study, the expression of Ki-67 in 47 cases of prostatic adenocarcinoma was evaluated and scored according to KI Score system. In this study found that Ki-67 expression was significantly is low in benign prostatic hyperplasia (19%) as compared with prostatic carcinoma (81%), ($P < 0.05$). This finding is in agreement with Nornazirah et al., [17] and Renuka et al., [18] who also found that the ki67 marker is highly expressed in prostate carcinoma as compared with benign prostatic hyperplasia, ($P = 0.01$) and ($P = 0.05$) respectively. The results found in this study concerning the patient age is acceptable and in comparison to global age. Older ages who found the most vulnerable group in this study is agreement with Fantony et al., [19] and Mohamed S A. Aziz et al., [20] who stated that with increasing age, men are significantly more likely to have high-risk prostate cancer. It is also in agreement with the American Cancer Society report (2016) that Prostate cancer is very rare in men younger than 40. About 60% of all prostate cancer cases are diagnosed in men 65 years of age and older, and 97% occur in men 50 and older [21].

In this study, there were significant differences between different prostatic Gleason scores. Since Ki-67 is a proliferative biomarker indicating proliferation of tumour cells expressing it is expected to be associated with the aggressiveness of the tumour proliferation index [22]. This explains the results that no Ki-67 expression was detected among benign prostatic tumours. In contrast, positive staining of Ki-67 was detected in all cases of prostatic malignancy regardless of tumour grade.

In the current studied group when the Gleason's score correlated with expression of Ki-67, there was an increased expression of Ki-67 with the increase in the grade of a tumour. This is strongly

agreed with Sulik and Guzinska who there was a relationship between Gleason score and high expression of Ki-67 in prostate cancers [23] it is also agreed with Bantis et al. their findings demonstrate that Ki-67 expression in prostatic carcinoma smears, correlated significantly with the degree of Gleason score [24]. Despite this agreement, Ojea *et al.* found that Ki-67 is less effective than classic factors such as PSA and Gleason score [25].

In conclusion, we found that the prostatic adenocarcinoma among Sudanese patients was of low grade (Gleason's score less than 4) which means tumours are less aggressive. Furthermore, Ki-67 expression was proportional to the grade of a tumour and it was a useful prognostic biomarker. We recommend to investigate for Ki-67 expression routinely for the diagnoses of prostatic cancer and to extend the investigation to involve more tumour biomarkers.

Acknowledgement

The authors sincerely thank all who participated in this study.

References

- Saeed IE, Weng HY, Mohamed KH, Mohammed SI. Cancer incidence in Khartoum, Sudan: first results from the Cancer Registry, 2009–2010. *Cancer Med.* 2014; 3:1075–84. <https://doi.org/10.1002/cam4.254> PMID:24821265 PMCID:PMC4303176
- Baade PD, Coory MD, Aitken JF. International trends in prostate-cancer mortality: the decrease is continuing and spreading. *Cancer Causes Control.* 2004; 15:237-41. <https://doi.org/10.1023/B:CACO.0000024212.66334.26> PMID:15090718
- Bartsch G, Horninger W, Klocker H, Reissigl A, Oberaigner W, Schonitzer D, et al. Decrease in prostate cancer mortality following introduction of prostate specific antigen screening in the federal state of Tyrol, Austria. *J Urol.* 2000; 163:88.
- Grozescu T and Popa F. Prostate cancer between prognosis and adequate/proper therapy. *J Med Life.* 2017; 10(1):5–12. PMID:28255369 PMCID:PMC5304372
- Ali Yousif Babiker, Arshad H Rahmani, Mohamed S Abdalaziz, Aqel Albutti, Salah M Aly, Hussain Gadelkareem Ahmed. Expressional analysis of p16 and cytokeratin19 protein in the genesis of oral squamous cell carcinoma patients. *Int J Clin Exp Med.* 2014; 7(6):1524-1530. PMID:25035775 PMCID:PMC4100961
- Gann PH, Hennekens CH, Sampfer MJ. A prospective evaluation of plasma prostate specific antigen for detection of prostatic cancer. *JAMA.* 1995; 273:289-94. <https://doi.org/10.1001/jama.1995.03520280035036> PMID:7529341
- Partin AW, Stutzman RE. Elevated prostate-specific antigen, abnormal prostate evaluation on digital rectal examination, and transrectal ultrasound and prostate biopsy. *Urol Clin North Am.* 1998; 25:581-9. [https://doi.org/10.1016/S0094-0143\(05\)70049-5](https://doi.org/10.1016/S0094-0143(05)70049-5)
- Gerdas J, Lemke H, Baisch H, et al. Cell cycle analysis of a cell proliferation associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol.* 1984; 133:1710-5. PMID:6206131
- Scholzen T, Gerdas J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol.* 2000; 182:311–322. [https://doi.org/10.1002/\(SICI\)1097-4652\(200003\)182:3<311::AID-JCP1>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9)
- Arshad H. Rahmani, Ali Yousif Babiker, Mohammed A. Alsahli, Saleh A. Almatroodi, Nazik Elmalaika O. S. Husain. Prognostic Significance of Vascular Endothelial Growth Factor (VEGF) and Her-2 Protein in the Genesis of Cervical Carcinoma. *Open Access Maced J Med Sci.* 2018; 6(2):263-268. <https://doi.org/10.3889/oamjms.2018.089> PMID:29531585 PMCID:PMC5839429
- Hasegawa T, Yamamoto S, Yokoyama R, Umeda T, Matsuno Y, Hirohashi S. Prognostic significance of grading and staging systems using MIB-1 score in adult patients with soft tissue sarcoma of the extremities and trunk. *Cancer.* 2002; 95:843–51. <https://doi.org/10.1002/cncr.10728> PMID:12209729
- Kononen J, Bubendorf L, Kallioniemi A, Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med.* 1998; 4:844–7. <https://doi.org/10.1038/nm0798-844>
- Skacel M, Siva A, Xu B, Tubbs RR. From array to array: confirmation of genomic gains and losses discovered by array-based comparative genomic hybridization utilizing fluorescence in situ hybridization on tissue microarrays. *J Mol Histol.* 2007; 38:135–40. <https://doi.org/10.1007/s10735-006-9051-8> PMID:17043918
- Bubendorf L, Kolmer M, Kononen J, Koivisto P, Mousset S, Chen Y, et al. Hormone therapy failure in human prostate cancer: analysis by complementary DNA and tissue microarrays. *J Natl Cancer Inst.* 1999; 91:1758–64. <https://doi.org/10.1093/jnci/91.20.1758> PMID:10528027
- Kallioniemi OP, Wagner U, Kononen J, Sauter G. Tissue microarray technology for high-throughput molecular profiling of cancer. *Hum Mol Genet.* 2001; 10:657–6. <https://doi.org/10.1093/hmg/10.7.657> PMID:11257096
- Nornazirah Azizan, Firdaus Hayati, Nur Maya Sabrina Tizen, Wirda Indah Farouk, Noraidah Masir. Role of co-expression of estrogen receptor beta and Ki67 in prostate cancer. *Urol Ann.* 2015; 7(4):488–493.
- Renuka Verma, Veena Gupta, Jagjeet Singh, Monica Verma, Gopal Gupta, Sumiti Gupta, Rajeev Sen, and Megha Ralli. Significance of p53 and ki-67 expression in prostate adenocarcinoma. *Investig Clin Urol.* 2018; 59:232-237.
- Fantony JJ, Howard LE, Csizmadia I, Armstrong AJ, Lark AL, Galet C, Aronson WJ, Freedland SJ. Is Ki67 prognostic for aggressive prostate cancer? A multicenter real-world study. *Biomarkers in medicine.* 2018; 15(0). <https://doi.org/10.2217/bmm-2017-0322>
- Mohamed S A. Aziz, Hagir Elfadil Mohagiri, Ali Yousif Babiker, Mohamed A. Alsahli, Saleh A. Almatroodi, Arshad Rahmani. Immunohistochemical Detection of Alpha-Methyl-Co-Racemase (AMACR) in Adenocarcinoma of Prostate. *RJMSci.* 2016; 707-710.
- Bechis SK, Carroll PR, Cooperberg MR. Impact of age at diagnosis on prostate cancer treatment and survival. *J Clin Oncol.* 2011; 29:235–241. <https://doi.org/10.1200/JCO.2010.30.2075> PMID:21135285 PMCID:PMC3058279
- Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer. *Molecular medicine reports.* 2015; 11(3):1566-72. <https://doi.org/10.3892/mmr.2014.2914> PMID:25384676
- American cancer society. *Cancer Facts & Figures.* www.cancer.org/research/cancerfactsstatistics/2016-cancer-facts-and-figures.pdf; 2016.
- Sulik M, Guzinska-Ustymowicz K. Expression of Ki-67 and PCNA as proliferating markers in prostate cancer. *Rocz Akad Med Bialymst.* 2002; 47:262–9. PMID:12533969
- Bantis A, Giannopoulos A, Gonidi M, Liossi A, Aggelonidou E, Petrakakou E, et al. Expression of p120, Ki-67 and PCNA as proliferation biomarkers in imprint smears of prostate carcinoma and their prognostic value. *Cytopathology.* 2004; 15:25–31. <https://doi.org/10.1046/j.0956-5507.2003.00090.x> PMID:14748788
- Ojea Calvo A, Mosteiro Cervifio MJ, Dominguez Freire F, Alonso Rodriguez Iglesias B, Benavente Delgado J, Barros Rodriguez JM. Prognostic factors of prostate cancer: usefulness of ki-67 expression in preoperative biopsies. *Arch Esp Urol.* 2004; 57(8):805. PMID:15560269