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Stratification of Ki 67 proliferation marker labelling index – Use in Gleason Score Grading of Prostatic Carcinoma

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Abstract
Introduction: *Prostatic carcinoma is a common cause of morbidity and mortality in men worldwide. Gleason score is the most accepted method of grading Prostatic carcinoma. At times it is difficult to determine Gleason score which is partially subjective. Ki -67 expression in Prostatic carcinoma has shown correlation with Gleason score. This study is designed to evaluate whether the Ki-67 proliferation marker labelling index can be useful in determining the grade of Prostatic carcinoma using routine histology as gold standard.*
Methods: *54 cases of adenocarcinoma prostate having adequate tissue available for immunohistochemistry was included in the study. 4 micrometer thick sections were obtained for H&E and immunohistochemical staining with MIB 1 – following antigen retrieval.*
Results: *All the prostatic carcinoma cases studied are acinar adenocarcinomas. The most common age group is between 61-70 years. Majority of patients have a Gleason score of 7 (40.7%) and grade group 5 (37%). The mean Ki67 labelling index in low grade tumours is 17.3% and in high grade tumours is 55.1%. Ki67 LI in low grade tumours is between 0-30% and in high grade tumours is >30% and thus a value of 30% is used as a cut off to discriminate the two.*
Conclusion: *The findings of the study indicate that Ki67 labelling of needle biopsy of prostate can be used as an additional diagnostic parameter for differentiating between low and high grade prostatic carcinoma. This may be useful in the clinical management of these patients. It could help in determining the subset of patients having favourable prognosis and also identify those who might benefit from Active surveillance.*
Keywords: *Gleason score (GS); Ki67labelling index (Ki67LI); Prostatic carcinoma; grading.*

Introduction

Prostatic cancer develops from prostate gland secretory cells. It often progresses slowly: may remain localised, but can grow into a large aggressive tumour. It is a common cause of morbidity and mortality in men worldwide. In terms of prevalence of cancer in men, Prostatic cancer is the second worldwide, the first in developed countries and sixth in developing countries. It is the sixth cause of cancer death in men in developed and in developing countries. Its pathogenesis depends on dihydrotestosterone–

androgen receptor complex (DHT–AR) which regulates gene expression in cancer cells. The aim of the treatment is androgen blockade and the therapeutic options include surgery, hormone-therapy, chemotherapy and radiotherapy [(1)].

Gleason grading remains the standard approach to histologic grading of adenocarcinoma of the prostate. Since the 2004 WHO classification, there have been modifications to the Gleason grading system, and these were incorporated into the 2016 WHO section on grading of prostate cancer. A new set of Grade Groups was recently developed, resulting in 5 prognostically distinct grade groups. The new grading system more accurately reflects prostate cancer biology than the Gleason system [(6)]. While the data in present study has been displayed according to Grade Groups and Gleason Scores, the final analysis has been done on basis of Gleason Scores.

Ki-67 protein is a cell proliferation marker that belongs to the regulatory proteins. It assists during the cell mitosis and disappears when the cell passes to the resting phase or at the time of DNA repair. Antibodies against Ki-67 proteins are often used for evaluation of proliferating activity of cancer cells, including Prostatic cancer cells[(2)].

Ki -67 expression in Prostatic carcinoma has shown correlation with Gleason score.[(2)] Gleason score is the most accepted method of grading Prostatic carcinoma in histopathology. High Grade Prostatic Carcinomas are known to have worse prognosis and have to be managed differently from Low Grade Carcinomas. At times it is difficult to determine Gleason score which is partially subjective. This study is designed to evaluate whether the Ki 67 labelling index can be useful in determining the grade of Prostatic carcinoma using routine histology as gold standard. A definite difference in Ki67 labelling index in carcinomas with lower and higher Gleason scores, could be used in conjunction with histology to determine the grade of Prostatic carcinoma. This is a simple additional method which has implications in prognostication and management of prostatic carcinomas

Materials and Methods

The current study was across sectional study conducted in the Department of Pathology, Government Medical College Thrissur. Fifty four histopathologically diagnosed cases of Prostatic carcinoma diagnosed over a period of three years were included in study, blocks were retrieved and 4 micrometer thick sections were obtained for H&E and immunohistochemical staining. Patients were divided into two groups according to Gleason score: Group 1(low grade)- Gleason score 2-7 and Group 2 (high grade) - Gleason score 8-10. The Ki-67 stained slides were evaluated by light microscopy with high dry objective, counting a total of 1000 tumour cells within at least 5 different fields (having maximum proliferation of cells) and expressing the results as percentage of positive cells by the formula- Labelling Index = [no. of positive tumour cells/total no of cells counted]. Data thus obtained was entered in Microsoft office excel 2007 sheet. This was then analysed using software SPSS version 16.0. The statistical test used is the Chi square test. P value <0.001 was considered statistically significant in the tests for correlation.

Results

The age of the patients ranged from 54-88 years. The maximum number of patients were in the age group of 61-70 years as shown in Table 1.Majority of patients are in grade group 5 as shown in Table 2. Majority of patients had a Gleason score of 7 as shown in Table 3. Of the total 54 cases 26 low grade tumours had Ki 67 labelling index in the range of 0-30% and 26 high grade tumours had >30% Ki 67 labelling index. (Low grade – Gleason score</=7 and High grade – Gleason score >7) as shown in Table 4. There is a difference in Ki67 labelling index between low (Gleason score </=7) and high grade (Gleason score >7) prostatic carcinoma and this difference is statistically significant (p value <0.001). The mean Ki67 labelling index in low and high grade tumours were 17.3% and 55.1% respectively. In the present study Ki67 labelling index in low

grade tumours was between 0-30% and high grade tumours was >30%.

Table 1: Age distribution

Age group (Years)	Frequency	Percentage
51-60	5	9.2
61-70	26	48.1
71-80	18	33.3
81-90	5	9.2

Table 2: Grade group

Grade group	Frequency	Percentage
1	5	9.2
2	12	22.2
3	10	18.5
4	7	12.9
5	20	37

Table 3: Gleason score

Gleason score	Frequency	Percentage
4	1	1.8
5	0	0
6	4	7.4
7	22	40.7
8	7	12.9
9	19	35.2
10	1	1.8

Table 4: Ki 67 labelling index in low grade and high grade tumours

Grade	Ki 67 labelling index 0-30%	Ki 67 labelling index >30%
Low grade	26	1
High grade	1	26

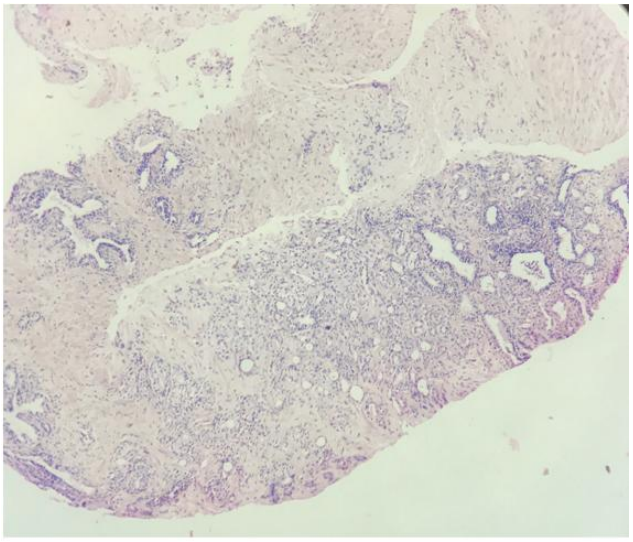

Image 1: Low grade prostatic carcinoma - Gleason score 6 (H&E)

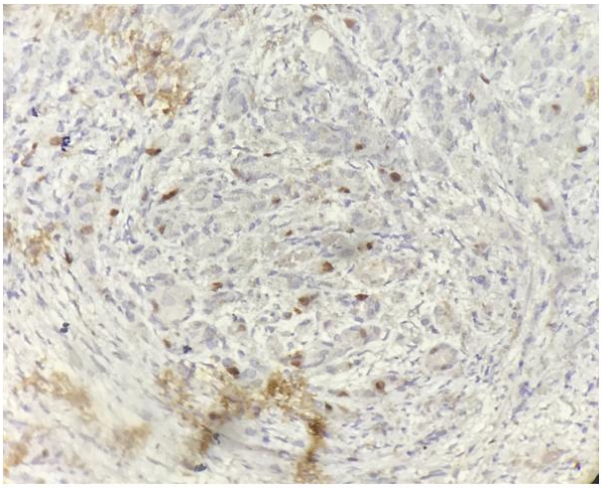

Image 2: Low grade prostatic carcinoma - Gleason score 6 (Ki67 LI)

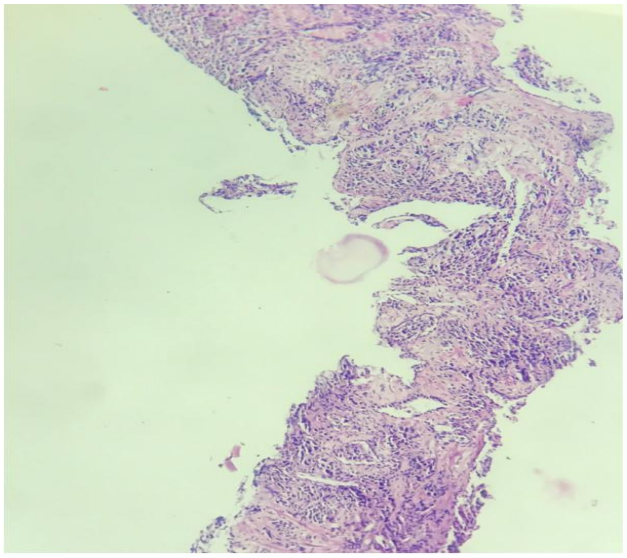

Image 3: High grade prostatic carcinoma - Gleason score 10 (H&E)

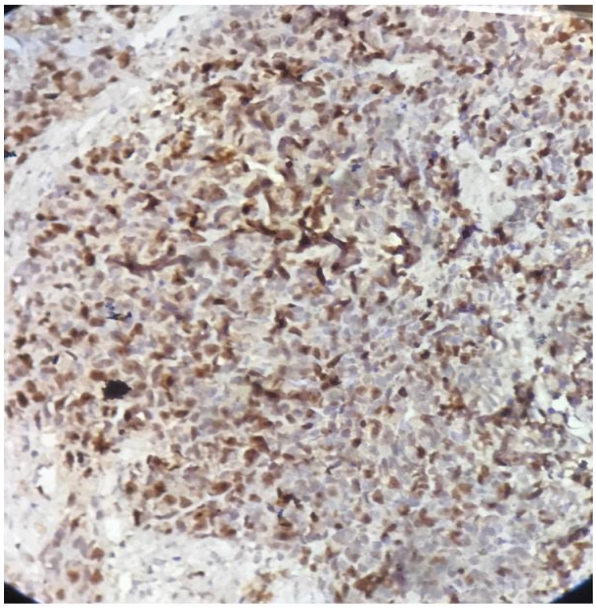

Image 4: High grade prostatic carcinoma - Gleason score 10 (Ki67 LI)

Discussion

Prostate cancer is a life-threatening illness in men worldwide. At the time of diagnosis there is a challenge of determining which case will remain indolent or be aggressive[1]. Proliferation markers may augment the role played by Gleason score in predicting Prostatic carcinoma prognosis[8].

Gleason score is a significant element of histopathological examination which has been the single most powerful predictor of prostate cancer prognosis[4]. At times it is difficult to determine Gleason score because it is partially subjective. Review of literature suggests that there is a statistically significant association between Ki67 indices and the Gleason scores grouped into low and high risk prognostic groups.

The aim of this study is to stratify Ki67 proliferation marker labelling index in those with low and high Gleason score and thus determine the cut off levels which may assist in grading Prostatic carcinoma. In the present study Gleason scoring was studied in 54 cases of Prostatic carcinoma which was then divided into low grade (Gleason score 2-7) and high grade (Gleason score 8-10). Ki67 labelling index was studied in each of the cases.

All prostatic carcinoma cases studied were acinar adenocarcinoma which is similar to the previous studies conducted by B Rugwizangoga et al[1] and Mwakyoma HA et al[9].

Age group: The most frequent age group in the present study is between 61-70 years. The mean age of patients is 69.16years. This is similar to that described in literature[1]. Out of the 27 low grade cases, 23 were in the age group of 60-80 years and 4 cases were more than 80 years. Of the 27 high grade cases, 1 case was <60years, 25 cases were between 60-80 years and 1 case was >80years.

Gleason score: In the present study majority of patients had a Gleason score of 7 (40.7%) which is similar to the study conducted by B Rugwizangoga et al[1]. In the original Gleason score system, Gleason score 6 was predominating (39.05%)[7]. The overtime increase in the proportion of Gleason score 7 and above has also been documented[10-12].

Grade group: A new set of grade groups has been recently developed, with a broad consensus for acceptance by expert urologic pathologists and clinicians at the 2014 ISUP consensus conference on Gleason grading of prostatic carcinoma. These grade groups are as follows: -Grade group 1: Gleason score - 6 - Grade group 2: Gleason score 3 + 4 = 7 - Grade group 3: Gleason score 4 + 3 = 7 - Grade group 4: Gleason score 4 + 4 = 8, 3 + 5 = 8, 5 + 3 = 8 - Grade group 5: Gleason scores 9–10. In the present study majority of patients are in grade group 5 (13%).

Ki-67 labelling index: In the present study patients were divided into low grade and high grade based on the Gleason score (low grade – GS</=7 and high grade – GS 8-10).

B Rugwizangoga et al[1] studied 214 cases and found that there is statistically significant association between Ki67 indices and the Gleason scores grouped into low and high risk prognostic groups. They have also established a statistically significant association between Gleason score prognostic groups and survival if Gleason score 7 is considered as being of low risk prognosis rather than intermediate or high risk prognosis. The studies conducted by Diaz JI et al[3] and Liang Cheng et al[5] have also found that Ki67 labelling index correlates well with Gleason score.

The main aim of our study is to evaluate whether Ki67 LI can be useful in differentiating between low and high grade prostatic carcinoma using routine histology as gold standard. In the present study the mean Ki67 labelling index in low grade tumours was 17.3%. The mean Ki67 labelling index in high grade tumours was 55.1%. Compared to low grade tumours the mean Ki67 labelling index in high grade tumours was significantly increased. There is a difference in Ki 67 labelling index between low and high gradeprostatic carcinoma and this difference is statistically significant (p value <0.001). In the study conducted by B Rugwizangoga et al[1] the

mean Ki67 labelling index for low grade tumours was 9.0949 and high grade tumours was 16.5887.

The immunohistochemical study of the prostatic carcinoma indicated significant differences of the Ki 67 labelling index in relation to the grade of the tumour and this demonstrates that this marker can be used as an additional indicator in differentiating low and high grade prostatic carcinomas. The findings of this study may have implications for the clinical management of patients with prostatic carcinoma.

The treatment options for prostatic carcinoma include Active surveillance for low grade tumours and Radical prostatectomy for extraprostatic extension and External beam radiation for locally advanced disease. In the present study Ki67 LI in low grade tumours was between 0-30% and high grade tumours was >30% and thus a value of 30% can be used as a cut off to discriminate the two. Thus, this could help in predicting the therapeutic outcome: that is, it may help to distinguish patients who need Radical prostatectomy/ External beam radiation from those who may benefit from Active surveillance. Physicians could also counsel the patients regarding their prognosis, taking into account the Labelling Index.

Conclusion

54 cases of prostatic carcinoma were studied: 27 cases were low grade tumours (GS </=7) and 27 cases were high grade tumours (GS 8-10). The ages ranged between 54 years and 88 years and the maximum number of patients was in the age group of 61-70 years. Majority of patients had a Gleason score of 7 and grade group 5. The Ki67 labelling index was found to increase with increase in Gleason score of the tumour. There is a difference in Ki67 labelling index between low and high grade prostatic carcinoma and this difference is statistically significant. The mean Ki67 labelling index in low and high grade tumours was 17.3% and 55.1% respectively. Ki67 labelling index in low grade tumours was between 0-30% and high grade tumours was >30% and therefore a value of 30% can be used as a cut off to discriminate the two. The findings of the study indicate that Ki67 labelling of needle biopsy of prostate can be used as an additional diagnostic parameter for differentiating between low and high grade prostatic carcinoma. This may be useful in the clinical management of these patients. It could help in determining the subset of patients having favourable prognosis. Those patients who might benefit from Active surveillance could also possibly be identified. This study is limited by the small sample size. Further clinical studies to establish the prognostic significance and management guidelines, differentiating low and high grade prostatic carcinoma by the method of Ki67 labelling is necessary.

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