**International Society of Urological Pathology (ISUP)-Grade Grouping in Prostatic Adenocarcinoma and its Prognostic Implications**

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**ABSTRACT**

In this study, we evaluated the association of ISUP/WHO-grade groups with various pathological prognostic parameters and cancer-specific survival in patients with prostatic adenocarcinoma. We found 27 (15.7%) cases of grade group 1, 22 (12.8%) grade group 2, 30 (17.4%) grade group 3, 40 (23.3%) grade group 4 and 53 (30.8%) grade group 5 prostatic adenocarcinoma. We found that high-grade tumors (grade 3–5) had a higher frequency of perineural invasion and higher tumor volumes (>50%). Moreover, a significant association of tumor grade was noted with cancer-specific survival of patients, signifying prognostic significance of grade grouping in prostatic adenocarcinoma.

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**Introduction**

Prostate cancer leads to an estimated 366,000 deaths annually worldwide (1). In this era of oncologic practice, there is a continued search for clinical and pathological prognostic parameters. Apart from clinical and radiologically determined staging, preoperative prostate-specific antigen (PSA) levels are a well-known prognostic parameter (2). Histological parameters that predict prognosis include gleason score, tumor volume (tumor quantification), extraprostatic soft tissue/fat involvement, seminal vesicle invasion, perineural and lymphovascular invasion (3). Besides these well-defined histological parameters, biomarkers such as, ERG, PTEN, FSN and androgen receptor (AR) also possess some prognostic value, but their routine use is not considered justified in all patients, up till now (4–7). Among the above-mentioned pathological prognostic parameters, gleason score is given much importance in pathological reporting. Gleason score is a sum of major and minor architectural patterns in prostatic biopsy specimen, and the total score ranges from two to ten. Due to this wide range, International Society of Urological Pathology (ISUP) and World Health Organization (WHO) classification of tumors of Male Genital Tract (MGT) introduced the concept of grading in prostatic biopsies. ISUP/WHO-grades range from one to five, and they have good reproducibility in pathologic reporting (8). The need for this grouping was to stratify patients into prognostic groups to evaluate the treatment response in different prognostic groups. Prognostic reproducibility of ISUP/WHO-grade grouping was not well studied in our population; therefore, in this study, we evaluated the association of ISUP/WHO-grade groups with various pathological prognostic parameters and cancer-specific survival.
Materials and methods

A retrospective cross-sectional study was conducted at the Department of Histopathology, Liaquat National Hospital (LNH) from February 2011 until January 2018, over a period of 7 years. Approval from the institutional research and ethical review committee was taken for this study. A total of 172 cases were included in the study. The specimens were received in histopathology laboratory, LNH and included prostatic chips/transurethral resection of the prostate (TURP) and radical prostatectomies. After the gross examination, representative sections were submitted according to standard guidelines. For TURP specimens weighing less than 12 g, whole specimens were submitted. For those weighing more than 12 g, one cassette per additional 5 g was submitted. For radical prostatectomies, after the submission of urethral and bladder neck margins, seminal vesicles and vas deferens, systematic sampling were employed. If the tumor was not identified on initial sections, complete/total sampling was done. Clinical parameters were recorded by evaluating records of histopathology archives. Immunohistochemical studies including PSA, CK7 and CK20 were done where there was a need to confirm primary prostatic origin or to rule out metastatic carcinoma. Immunohistochemical stains p63 and 34-betaE12 were performed in cases of low grade prostatic carcinoma to differentiate from benign mimickers of malignancy. Slides of all specimens were retrieved, and re-reviewed for ISUP/WHO-grade grouping, and additional sections were performed from tissue blocks, where necessary. ISUP/WHO-grade grouping was done by senior histopathologists, and compared with other prognostic pathologic parameters.

Gleason scoring was performed on the basis of low-power architectural pattern assessment of tumor as follows (9).

Gleason score 3: Single and separate glands with size and shape variability, and wide-spread stromal invasion between normal non-neoplastic glands.

Gleason score 4: Coalescent and fused glands (>1 lumen) with absent intervening stroma. Cribriform, hypernephroid and glomeruloid patterns were also included in gleason score 4.

Gleason score 5: Single cells, cords of cells without lumen and presence of comedo necrosis qualify for gleason score 5.

The total gleason score was calculated by summing-up major gleason core (most frequent architectural pattern) and minor gleason score (second most frequent or highest grade pattern).

ISUP/WHO-grade grouping was performed based on gleason scoring as follows.

Grade group 1: Gleason score = 6.
Grade group 2: Gleason score 3 + 4 = 7.
Grade group 3: Gleason score 4 + 4 = 8; 3 + 5 = 8; 5 + 3 = 8.
Grade group 5: Gleason score 9-10.

The follow-up records of 106 patients were available. The disease-free survival of patients was determined by evaluating urological and oncological records.

Statistical analysis

Data analysis was performed using Statistical Package for Social Sciences (Version 26.0, IBM Inc., Armonk, NY). The Shapiro-Wilk test was used to check the normality of quantitative variables. Non-parametric Kruskal-Wallis H test was applied for the comparison of quantitative variables. Chi-square test was used to check the association between qualitative variables. Survival analysis was done by Tarone-Ware log-rank test. For chi-square test, effect size index \( w \) was calculated by Power Analysis and Sample Size (PASS-v11). It represents the magnitude of an effect, and tells about meaningful relationship between variables. It was considered to be small if \( w = 0.10 \), medium if \( w = 0.30 \), and large if \( w = 0.50 \). \( p \)-Values < 0.05 were considered significant.

Results

In this study, we found 27 (15.7%) cases of grade group 1, 22 (12.8%) grade group 2, 30 (17.4%) grade group 3, 40 (23.3%) grade group 4 and 53 (30.8%) grade group 5 prostatic adenocarcinoma. We noted 62 (36%) patients with perineural invasion, 3 (1.7%) with lymphovascular invasion, 12 (7%) with extraprostatic extension and 8 (4.7%) with the seminal vesicle invasion. Total 97
(56.4%) cases had tumor volume (tumor quantification) more than 50% of the examined tissue. Median follow-up time was 52 months and median cancer-specific survival of the patients was 43.5 months, respectively (Table 1).

We found that high-grade tumors (grade 3–5) had a higher frequency of perineural invasion than low-grade tumors (grades 1–2) with significant association ($p = 0.005$). Similarly, high-grade tumors were also found to have higher tumor volumes (>50%) than low-grade tumors with a significant $p$-value. Similarly, a significant association of tumor grade was noted with cancer-specific survival of the patients. Conversely, extraprostatic extension was noted more frequently in low-grade tumors with a significant association ($p = 0.0001$). Alternatively, no significant association was found with age ($p = 0.18$), lymphovascular invasion ($p = 0.47$) and seminal vesicle invasion ($p = 0.62$). The effect size was large for tumor quantification ($w = 0.52$), signifying a strong association, while it was medium for survival status ($w = 0.48$) and extraprostatic extension ($w = 0.37$). There was a small effect size for lymphovascular invasion ($w = 0.14$) and seminal vesicle invasion ($p = 0.12$) (Table 2).

Figure 1 shows the survival analysis (cancer-specific survival) of the patients with respect to tumor grade. A significant association was found between tumor grades and cancer-specific survival.

**Table 1. Descriptive statistics of population characteristics under study ($n = 172$).**

| Clinicopathological characteristics | Frequency (%) |
|-------------------------------------|---------------|
| Age (years)* | 67.0 (16.00) |
| Cancer-Specific Survival (months)* | 43.5 (22.25) |
| Grade groups |               |
| Grade 1 | 27 (15.7%) |
| Grade 2 | 52 (30.2) |
| Grade 3 | 40 (23.3%) |
| Grade 4 | 97 (56.4%) |
| Total gleason score | |
| Score 6 | 27 (15.7) |
| Score 7 | 52 (30.2) |
| Score 8 | 40 (23.3) |
| Score 9 | 53 (30.8) |
| Perineural invasion | |
| Present | 62 (36) |
| Absent | 110 (64) |
| Lymphovascular invasion | |
| Present | 3 (1.7) |
| Absent | 169 (98.3) |
| Extra-prostatic extension | |
| Present | 12 (7) |
| Absent | 160 (93) |
| Seminal vesicle invasion | |
| Present | 8 (4.7) |
| Absent | 164 (95.3) |
| Tumor quantification | |
| <10% | 38 (22.1) |
| 10–50% | 37 (21.5) |
| >50% | 97 (56.4) |
| Survival status ($n = 106$) | |
| Alive | 69 (65.1) |
| Expired | 37 (34.9) |

*Median (Inter-quartile range).
Discussion

In this study, we evaluated grade groups in prostatic acinar adenocarcinoma and found a high frequency of grade group 5 (30.8%) in our population. Moreover, there was a significant association of the grade group with perineural invasion, tumor volume and cancer-specific survival, signifying the prognostic value of grade grouping in prostatic carcinoma.

WHO/ISUP-grade grouping of prostatic adenocarcinoma is based on Gleason score, which is assessed in prostatic cancers based on low-power architectural patterns. Various studies have confirmed the prognostic implication of grade grouping in prostatic cancers (10).

Grade group 1 is Gleason score less than or equal to six (3+3 or below). Gleason patterns 1 and 2 are rarely encountered. Gleason pattern 3 is defined as a discrete well-formed glandular proliferation (Figure 2).

A large prospective cohort study with long-term follow-ups revealed cancer-related deaths or metastasis in less than 1% of grade group 1 prostatic cancers (11). We had 15.7% cases of grade 1 prostatic carcinoma, only 7.4% of which showed perineural invasion, with a 90% survival rate.

WHO/ISUP segregates Gleason score 7 into grade groups 2 and 3 on the basis of predominant pattern. Gleason score 3+4 = 7 is known as grade group 2, whereas a score of 4+3 is categorized as grade group 3. Gleason pattern 4 includes raggedly infiltrating or cribriform glands (Figure 3).

Studies have shown that the cribriform pattern is associated with recurrence and poor prognostic features, like extraprostatic extension (12). For similar reasons, Gleason score 4+4 = 8 was identified as grade group 4. In our study 23.3% cases were grade group 4, 37.5% of which revealed perineural invasion that is a marker of poor prognosis in prostatic adenocarcinoma. Moreover, poor cancer-specific survival was noted in grade group 4 than lower grade groups (1–3) in our study.

Gleason score 5 is defined by sheets, cords or isolated tumor cells without any obvious gland formation (Figure 4).

The presence of comedo-type necrosis is also included in Gleason pattern 5. Studies have shown that Gleason pattern 5 is frequently underreported and actual frequency is high in resection specimens (13). We also noted a high percentage of grade group 5 in our study, concordant with these findings.

A large-scale multi-institutional European study validated the prognostic value of ISUP/WHO-grade groups in prostatic carcinoma. They analyzed data involving 27,122 patients treated with radical prostatectomy at seven centers. The 4-year recurrence-free survival in grade groups 1–5 were 91.3, 81.6, 69.8, 60.3, and 44.4%, respectively (14). Concordant with the results of...
this study, we also found decreasing cancer-specific survival from grade groups 1–5 (53 months in grade group 1–27 months in grade group 5).

Apart from glandular neoplasms (adenocarcinoma); squamous cell carcinoma, urothelial carcinoma, basal cell carcinoma and neuroendocrine carcinoma can arise in the prostate as primary prostatic cancers. The most common histological sub-type of prostatic adenocarcinoma is acinar carcinoma. Besides acinar carcinoma,
intra-ductal and ductal adenocarcinoma also occurs in prostate; however, in our study we only included acinar carcinomas, as other variants are rare.

The identification of low grade (grade group 1) prostatic adenocarcinoma is a diagnostic challenge as small atrophic glands can resemble low-grade tumors. In these circumstances, the utility of basal cell immunohistochemical markers is pivotal. The loss of immunohistochemical stains, such as p63 and 34-betaE12 is considered diagnostic for prostatic carcinoma, in addition to the presence of histological features, such as prominent nucleoli and small infiltrating glands.

Screening for prostate cancer using the PSA test is still controversial and thus is not recommended unless risks and benefits are completely explained to the patients. Recently, a meta-analysis combining results of five randomized control trials, including 721,718 men, evaluated all aspects of prostate cancer screening. They showed that screening has no effect on all-cause mortality and may not even have any significant effect on the prostate cancer-specific mortality. However, sensitivity analysis of studies that were at low risk of bias demonstrated that screening seems to have a small effect on prostate-specific mortality. Therefore, the study concluded that at maximum, prostate cancer screening using PSA has a small effect on prostate cancer-specific mortality; however, there is no significant effect on overall mortality (15). Despite limitations of prostate cancer screening, many men in western countries undergo prostate cancer screening that leads to an early diagnosis of cancer in these patients. Alternatively, in Pakistan, apart from the lack of prostate cancer screening, most patients present late in the disease course leading to high cancer morbidity and mortality. The same trends were demonstrated in our study, revealing a higher frequency of grade-4 and grade-5 tumors, along with high tumor volume. These poor-prognostic features may be attributed to the late disease presentation and lack of screening programs in our population.

With the advent of time, new cancer biomarkers are emerging with promising results with respect to predicting prostate cancer biological behavior. Among these new biomarkers prostate cancer antigen 3 (PCA3), prostate health index (phi) and sarcosine showed significant results. All of these three markers were associated with high tumor volume, pathological T3 stage and high gleason score (>7) (16). Similarly, other studies reported that the prognostic significance of preoperative prostate-specific antigen isoform
p2PSA and its derivatives, %p2PSA and prostate health index as predictors of high tumor volume and advanced gleason score (17). As these new biomarkers are not available in under-developed countries, like Pakistan, therefore, evaluation of histological prognostic parameters, such as grade groups are still of utmost clinical importance.

We acknowledge a few limitations of our study, as the study design was retrospective. Second, sample size was small, especially cases with radical prostatectomies. Although we noted that low-grade tumors had a higher frequency of extraprostatic extension, the number of cases with extraprostatic extension was small; therefore definite conclusions regarding the association of tumor grades with the extraprostatic extension require large-scale studies.

Conclusion

In this study, we found a high frequency of grade groups 4 and 5 in prostatic adenocarcinoma in our population. We also validated the prognostic significance of grade grouping in prostatic adenocarcinoma as higher-grade groups (3–5) were associated with higher frequency of perineural invasion, high tumor volume (tumor quantification), and poor cancer-specific survival. Therefore, we recommend the evaluation of ISUP/WHO-grade groups in all cases of prostatic adenocarcinoma, in addition to gleason scoring for prognostic stratification of the patients in our population. Furthermore, the assessment of novel prognostic markers of prostatic adenocarcinoma is required in our population to improve survival and better patient management.

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