# Correlation of Gleason Grading System and nuclear parameters by nuclear morphometry in patients of Prostatic Adenocarcinoma: A study Protocol

**Abstract:**

***Background:*** Adenocarcinoma prostate is one of the major causes of death in men. The Gleason Grading System is the most commonly used mortality to assess the degree, yet, identical results among blinded pathologists are difficult to obtain and hence come down to an unobjectionable result.

***Aim and Objectives:*** To evaluate the relationship between histopathologically obtained Gleason's Grading Score and various nuclear morphometric parameters by the use of computer aided system in Prostatic Adenocarcinomas.

***Methods:*** A series of 31 new and histopathologically diagnosed cases of adenocarcinoma prostate will be taken over a period of one year and the following nuclear morphometric parameters will be studied: mean nuclear area, mean nuclear length, mean nuclear perimeter, mean nuclear roundness factor, mean nuclear area factor and mean nuclear form ellipse. These individual parameters will be correlated with the Gleason Score of the respective cases.

***Expected Outcome:*** The present study expects the nuclear atypia to be more in cases having a higher Gleason Score and to avoid the inter-observer contradictions in diagnosis. It surely can be used as a tool to quantify the aggressive of the malignancy, and thus, to assess disease progression and prescribe a justifiable management protocol. Nuclear morphometrical analysis can be found to be more accurate, objective and effective method in the diagnostic and prognostic significance of prostate adenocarcinoma.

***Keywords:*** Prostate, adenocarcinoma, nuclear morphometry, Gleasons grading



## Introduction:

Having diagnosed with Carcinoma of Prostate is never easy, but if caught in time, it can be easily treated. Carcinoma Prostate is mainly a condition occurring in the elderly, in more than 75 percent of men, mostly occurring in individuals above the age of 65. However, worldwide this entity has turned out to be a significant health concern in the recent decades. According to current literature, it has been found that Carcinoma Prostate occurs second most often in adult men globally, and fifth most common cancer amongst all individuals. Among the fatalities due to cancers in men, this is the sixth leading cause.[1]

Clinical and paraclinical studies suggest that i. hormonal effect of androgen, ii. hereditary and iii. the environmental factors, and iv. acquired somatic mutations play a role in the etiopathogenesis and growth of cancer. Androgen is of the prime importance amongst these. Prostatic Carcinoma rarely occurs in males who were castrated before puberty or in patients of liver cirrhosis having hyperestrogenism, putting forth that androgens somehow nurture the development of prostatic carcinoma. Hereditary factors are important as well, as there is an increased risk among first degree relatives of patients. Carcinoma Prostate is not so common in Asians as its incidence is highest among African-Americans. About 5 percent to 10 percent of prostatic carcinomas are inherited

genetically. About 75 percent of the cases are seen in men above the age of 65. The younger patients, too, can land up with these lesions. They are not known to be associated with any occupational carcinogens, smoking, sexually transmitted diseases, nodular hyperplasia or any dietary changes.[2]

Seventy percent of all the prostate carcinomas arise most commonly in the outer, peripheral zone (i.e the lateral and posterior part) of the prostate gland. It is easily palpable by the rectal examination. [3]

Grossly, these lesions are firm, grey-yellow, poorly demarcated and usually shows extension into adjacent structures. Microscopically, predominantly numerous small to medium sized crowded glands, are seen with various architectural patterns like solid, cribriform or papillary. There is nuclear enlargement, hyperchromasia and prominent nucleoli often measuring >3 microns in diameter. The loss of basal cells confirms the malignant transformation.

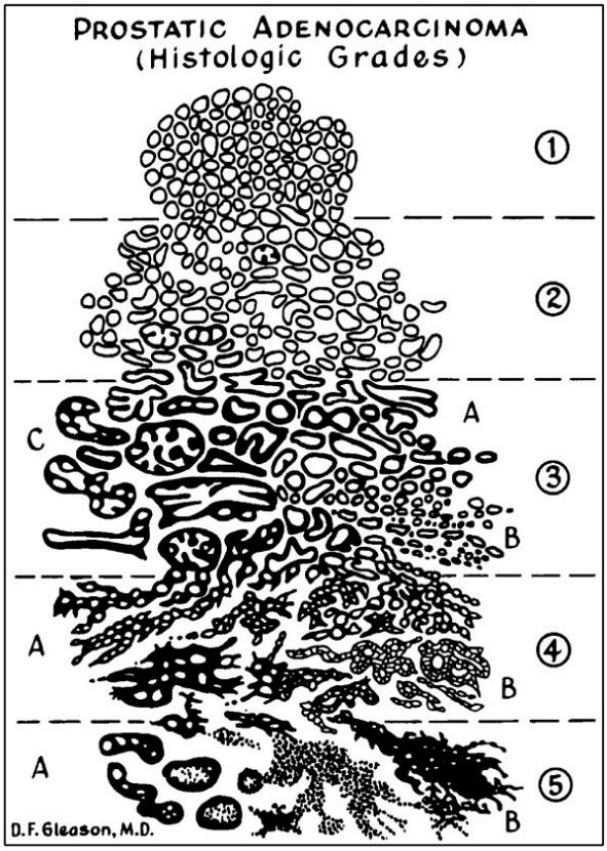
The Gleason grading system is an important tool for assessing the aggressiveness of Carcinoma Prostate. It is the principal method, worldwide in research and in daily practice. Dr. Donald F.Gleason, a pathologist in Minnesota, and some members of the Veterans Administration Cooperative Urological Research Group (VACURG) developed this technique in 1967 and updated in 2014. The histologic pattern of arrangement of carcinoma cells in H and E stained prostatic tissue sections, in low or medium magnifications is the basis for this. There are five grades based on glandular patterns of differentiation of prostatic cancer. [4]

The characteristic of cancer cells is morphologic changes in nuclei. The alterations in nuclear shape and size, pleomorphic nuclei, hyperchromasia, prominent large nucleoli and marked increased nuclear to cytoplasmic ratio (N:C) are some basic features of malignancy. Various researchers have used nuclear morphometric characteristics like nuclear length (shortest and longest dimensions), perimeter, area, volume, ellipticity, and circularity, to develop an objective method for predicting and grading the prognosis of prostatic malignancy.[5,6]

Quantitative nuclear morphometry is the process by which the alterations of the nuclei are converted into specific quantifiable parameters by using a digital image analysis technique. The image analysis permits pathologists to quantitatively measure the cytological smears and histopathological sections, as the visual impressions are supremely augmented by modality of quantitative morphometry. By this procedure, we can also get the precise dimensions and texture of the individual cells, which is not possible by routine diagnostic tools. [7,8]

To increase accuracy in the prognosis of Carcinoma Prostate patients, the present study is undertaken to assess the nuclear parameters of the prostatic adenocarcinoma cells using a quantitative nuclear morphometric system correlate the results with Gleason's Score evaluated by histopathological examination.

1.




[Fig 1: Nine growth patterns were consolidated into five grades, and these were illustrated in a drawing by Dr. Gleason.]

**Methodology:**

The present study is an observational, cross-sectional, and retrospective study conducted for one year in a rural tertiary care hospital in central India. Ethical clearance was obtained for the same, and informed consent will be taken from the participants in the study.

A sample size of 30 specimens was calculated with Krejcie and Morgan Formula. All cases diagnosed as adenocarcinoma prostate on prostatectomy specimen or prostate biopsy and primary cases of adenocarcinoma prostate without any history of previous treatment will be included in the study. All cases of recurrence or history of neoadjuvant therapy will not be taken into the study. Prior informed consent, clinical history and physical examination in new cases, and clinical details in previously diagnosed cases will be taken from those participating, considering the inclusion and exclusion part. Biopsy from clinically suspected cases will be taken and sent for histopathological examination. All histopathological samples will be fixed in formalin, then embedded in paraffin , then cut into 5-µm sections, and then stained with H&E. The cases are confirmed as "Adenocarcinoma of Prostate" on the histopathological examination will be respectively graded using the Gleason Grading System.

i. **Gleason's Grading**

The Gleason grading system is mainly based on the histological pattern of arrangement of malignant epithelial cells in Haematoxylin & Eosin stained prostatic tissue sections. In this method, histological patterns of Adenocarcinoma Prostate are categorized at a lesser magnification (100x and 400x) by differentiation of glandular and the stromal growth patterns in the sections.[9]

The basic histopathological patterns for corresponding grades are used to give a histologic score. It ranges from 2 to 10 by adding both the primary and secondary grades. (Fig 1) By simple visual inspection, the primary pattern is predominant pattern in the section and given the first number. The second most common pattern is the secondary pattern and given the second number. The sum of these two numbers given the final Gleason's score which can be categorized according to the grade groups like well-differentiated, moderately differentiated and poorly differentiated. If there is only one pattern in the tissue sample, the respective grade must be multiplied by two to get the score.

Pattern 1 is rarely diagnosed on biopsies as it is difficult to differentiate from the normal tissue. Pattern 2 needs larger areas of parenchyma to be diagnosed. Pattern 3 is best identified and Score 3+3 = 6 is usually denoted as Well Differentiated Adenocarcinomas of Prostate, which will show well circumscribed acini, arranged back to back and well-formed glands with no fusion. At times, amorphous crystalloid material called corpora amylacea can be identified in lumen on histopathology. Pattern 4 is made out of poorly formed or fused glands. Cribriform structures are typical of this patter. Luminal surfaces are appreciated but glands are not encircled. Pattern 5 is predominantly made up of solid nests, no luminal or glandular structures, cells may also be arranged in sheets or cords with occasional areas of comedo necrosis.[4]

**ii. Nuclear Morphometric Parameters**

On H and E stained histological section, the morphometric analysis will be performed. The microscope used will be Leica, DMLB100S which will be connected to a computer and video camera (Leica, DFC280), and the morphometric parameters will automatically measured for the microscopic images obtained, which will shifted to the computer and measured by an image analysis program (Leica, QWINPlus v.3.1.0). With sharply demarcated contours, about 150 nuclei will be included in the morphometric analysis of each case. Nuclei that will be markedly distorted during preparation and significantly overlapped will be excluded from the analysis. The following parameters are included:

- mean nuclear length (MNL) in µm, most extended orthogonal projection
- mean nuclear perimeter (MNP) in µm, the circumference
- mean nuclear area (MNA) in cubic µm, area enclosed inside the contour
- mean nuclear roundness factor (MNRF), - given by the equation - **$\text{perimeter}^2/(4\pi*\text{area})$**
- mean nuclear area factor(MNAF) – given by the equation - **$4\pi(\text{area}/\text{perimeter}^2)$**
- mean nuclear form ellipse (MNFe) as the measure for cell apoptosis, which will be given by the equation **longest diameter/shortest diameter**.[10]

All measurements will be made with the 400x objective and will be expressed in micrometers. The 30 prostatic adenocarcinoma cases evaluated the nuclear morphometric parameters will be compared with Gleason Score.

**Statistical Analysis:**

The calculation will be of Mean and Standard Deviation. In addition, a student t-test, Chi-square test and linear regression analysis will be used to compare means of nuclear morphometric parameters with Gleason's Score. A p-value that is less than or equal to 0.05 will be considered significant.

**Expected Results:**

The nuclear length, nuclear perimeter, nuclear area, nuclear roundness factor, and nuclear form ellipse are expected to positively co-relate with the Gleason's Score while a decrease in the value of nuclear area factor should show tumour progression, or a negative correlation with Gleason's Score. In prostatic adenocarcinoma, nuclear morphometrical analysis can be used as a reliable tool for diagnosis to supplement the Gleason Score. After the measurement of size and shape of the tumour

cells, the nuclear morphometric analysis, the observation is expected to help us to improve our understanding of the diagnostic and prognostic features of the prostatic adenocarcinoma.

**Discussion:**

Prostatic Carcinoma is a significant causes of death in men worldwide, and Gleason Grading System is a universally accepted method to classify the grade of carcinogenesis. Quantitative computerized nuclear morphometry is an objective way to supplement the Gleason Grading System and add prognostic and diagnostic values in the patients diagnosed with Adenocarcinoma Prostate.

**BEKTAŞ S et al.,** in their study observed the nuclei for their shape, size and characteristics of the prostatic adenocarcinoma cells with the help of a computerized analysis system and compared the results of various parameters with the Gleason score in 130 subjects diagnosed with prostatic adenocarcinoma cases having 77% of needle biopsies and 23% of radical prostatectomy specimens. All the nuclear morphometric parameters, like length form ellipse, roundness factor, and, perimeter were tested based on tissue sections using a computer-aided image analysis system. The results of nuclear shape factors and nuclear area were significantly concordant with the Gleason score. They concluded that the nuclear size and shape assessment might help in the evaluation of the histopathological status of the adenocarcinoma prostate.[10] **DIACONESCU S. et al.,** in the study appellation as "Nucleolar Morphometry in Carcinoma Prostate," analyzed morphometric nucleolar parameters and compared the results to the Gleason grading system in 35 cases of prostatic Carcinoma from the past of the Department of Pathology, District Hospital of Brasov. The average number of nuclei increased significantly in parallels with the Gleason grade. They concluded that the nucleolar number, perimeter, area, and diameter should be added to the list of histology features that helped diagnose Carcinoma Prostate on transurethral resection.[11]

**RW Veltri et al.** in a study entitled "Nuclear morphometry, nucleonics, and Carcinoma Prostate progression," in which 557 consecutive men were biopsied and it was studied. Together with quantitative nuclear grade and Gleason score to predict non-organ-confined Prostate Carcinoma. Therefore, it was confirmed that when quantitative nuclear grade is combined with the Gleason score, it was possible to improve the pathological stage prediction. **Vesalainen S. et al.,** conducted a study with 325 subjects diagnosed with adenocarcinoma prostate and took a long term follow up. These cases were subjected to histomorphological analysis for the Gleason score and many nuclear morphometric factors as well. The results showed that the nuclear morphometric measurements were marginally significant.[12-15]

**Interpretation:**

Adenocarcinoma of the prostate is one of the significant causes of fatality in men. Gleason Grading System being the most commonly used modality to grade the tumour, and to predict the prognosis, it is yet not easy to achieve identical results among pathologists. The advantage of nuclear morphometry is its accuracy, efficiency, and objectiveness, and it will supplement the diagnostic significance of the Gleason Score after correlating with the individual cases. After comparing those parameters with the Gleason Score, the conclusion will be drawn from the morphometric evaluation of nuclear parameters in prostatic adenocarcinoma cell nuclei.

**Limitations:** Interobserver and intra-observer variability and technical errors while processing can influence the interpretation of histopathological reporting of tumour sections.

**COMPETING INTERESTS DISCLAIMER:**

**Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.**

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