"AN IMMUNOHISTOCHEMICAL EVALUATION OF TUMOR ASSOCIATED MACROPHAGES (M1 & M2) IN CARCINOMA PROSTATE- AN INSTITUTIONAL STUDY.

BY

Dr. SOUMYA.M. HADIMANI, MBBS

DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

TAMAKA, KOLAR, KARNATAKA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

PATHOLOGY

UNDER THE GUIDANCE OF

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SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

APRIL/MAY 2022

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The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the Synopsis entitled **"An Immunohistochemical Evaluation of Tumor Associated Macrophages (M1 & M2) in Carcinoma Prostate- An Institutional Study"** being investigated by Dr.SOUMYA M HADIMANI, Dr. Subhashish. Das & Dr. Harish Kumar[1] in the Departments of Pathology & Urology[1] at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.**

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Thank you everyone and…..God bless.

Date: *Signature of the Candidate*

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LIST OF ABBREVATIONS

Pca- Prostate Carcinoma

TAM- Tumor Associated Macrophages

M1- CD68

M2-CD 163

PBCRs-population-based cancer registries.

IL- Interleukins

TGF- Tumor Growth Factor

TNF- Tumor Necrosis Factor

VEGF- Vascular Endothelial Growth Factor

BPH- Benign Prostatic Hyperplasia.

UGS- Urogenital Sinus.

PSA- Prostate specific antigen

PSAP- Prostate-Specific Acid Phosphatase (PSAP)

DRE- Digital rectal exam

AUA- American Urologic Association Recommendations

AJCC- American Joint Committee on Cancer Prognostic Stage Grouping of prostatic carcinoma

TURP-Transurethral resected prostatic(TURP)

ABSTRACT

BACKGROUND:

Prostate cancer is primarily a disease of the older men with more than three quarter of the cases occurring above 65 years of age. Studies showed that prostate carcinoma is the second most commonly diagnosed carcinoma in men worldwide and the fifth most common cancer overall. Tumor associated macrophages(TAM) are main component of inflammation along with leukocytes, vascular endothelial cells and fibroblasts together form a tumor microenvironment, with immune cells representing its vital component. Many studies suggested that TAMs cumulation in tumors correlates with a poor prognosis. In prostate cancer, TAMs can enhance cancer cell invasion by stimulating tumor angiogenesis, degrading the extracellular matrix, and also suppresses the anti-tumor functions of cytotoxic T cells resulting in poor prognosis. Therefore, TAMs are an alluring target for therapeutic intervention by targeting their various function. Hence the study is undertaken. On H and E section, it is difficult to differentiate M1 and M2 phenotypes. Hence Immunostaining is used to identify M1 and M2 sub population of macrophages. CD68 is been taken as a marker for M1 macrophage and CD163 is been taken as a marker for M2 macrophage. Only few studies determining expression of CD68 and CD163 have been done on prostate Cancers and published in Indian Literature so far. Hence the study is undertaken to determine the expression of CD68 and CD163 in prostate Carcinomas.

AIMS AND OBJECTIVES:

- To determine the expression of M1 (CD68)and M2 (CD163) in prostate cancer.
- To find association between M1 , M2 Macrophage with Gleason's score & Stage of the disease in prostate carcinoma.

MATERIALS AND METHODS:

This is a retrospective observational study. All Transurethral resected Prostatic(TURP) Chips positive for prostate carcinoma, received in the Department of Pathology Sri Devaraj Urs Medical College, Tamaka, and Kolar . from December 2019 to October 2021 and also the paraffin blocks taken from all cases of Prostate cancer retrieved from Archives of Department of Pathology from the year January 2015 to November 2019 were included in the study. All Transurethral resected prostatic(TURP) Chips positive for carcinoma prostate confirmed by histopathological examination was included in the study. Data regarding the clinical details (age, Stage of the disease) was collected. H and E slides was reviewed for Histopathological types and Gleason's score of the tumor. Radiologic findings (USG,MRI or CT Findings) with respect to stage of disease, size of lesion, was noted. The CD 68 and CD 163 immuno stained slides were examined under low power (10X) and was looked for areas with maximum expression of CD 68 and CD 163 by two observers and were called as" Hot spots" . These hotspots were then viewed under higher magnification (40X) and CD 68 and CD 163 positive cells were counted and scoring was done on number of macrophages expressed by IHC.

RESULTS:

A total of 62 cases were studied and majority of the patients were in-between age of 61-70 years. Highest number of cases were in Gleason's score 8,9, 10 (62%), PSA levels 20-80ng/ml (64%), Tumor Size 3-6 cm (51.6%), T3 stage (40.3 %) , N1 lymph node stage (70.9%). M1 stage of (31%). CD 68 and Cd163 expression was analyzed with Gleason's score, TNM stage and PSA levels. CD68 score 3 was associated with low nodal and distant metastasis 6.8%) and 6.2% respectively.

CD163 Score 3 was associated with high metastasis to lymph node and distant metastasis of 86.3% and 25% respectively. On further analysis, statistically significant association between the CD 163 expression and Gleason's score, PSA levels, nodal and distant metastasis was found.

CONCLUSION:

CD 68 expression was associated with better prognosis with less nodal and distant metastasis and Cd163 expression has poor outcome with increased chances of nodal and distant metastasis. Further exploration of TAM mechanisms and immune checkpoints in the prostate tumor microenvironment can provide new light and idea for the treatment of prostate carcinoma.

Key words- Prostate carcinoma(Pca), Tumor associated macrophages (TAM), CD68, CD163, Gleason's score, Prostatic specific antigen(PSA).

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INTRODUCTION

INTRODUCTION

Prostate cancer is a disease of the elderly men above 65 years of age. Studies showed that prostate carcinoma is the second most diagnosed carcinoma in older men worldwide and the fifth most frequent cancer overall.[1] Prostate cancer has been projected to have the largest corresponding increase in men in the upcoming years.[1] Studies shows restricted data available on prostate carcinoma and showed very significant differences in, precipitating factors, incidence and disease characteristics of prostate carcinoma.[1] In India, restricted data available on true incidence of prostatic carcinoma as it is not a notifiable disease and there are few population-based cancer registries[PBCRs] in India[1]. Study done taking PBCRs, from 2009-2011 in different metro cities shows Annual Percentage Change of Karnataka 3.4%.[1] Study carried out in Kolar on prostate cancer cases showed 2.58% out of all other cancers[2,3]

Recurrent inflammation of prostate has been associated with high risk of cancer. Tumor associated macrophages(TAM) are main component of inflammation along with leukocytes, vascular endothelial cells and fibroblasts together form a tumor microenvironment, with immune cells representing its vital component. Macrophages are innate immune cells, they have 2 main phenotypes—M1, M2 which correspond to T-Helper cells.[4] IFN-Gamma, activates M1 macrophages Secrete cytokines IL-12 & Tumor necrosis factor-alpha ,Support anti tumor response. IL-4, IL-10 & IL13, activates M2 Macrophages Secrete anti-inflammatory cytokines –TGF-beta & IL-10 & angiopoietin & VEGF, Promote tumor growth.[4] The pan macrophage marker is CD68 and plays the role of pro-inflammatory and anti-tumor response. CD163 is a scavenger receptor up-regulated by macrophages in an anti-inflammatory environment and regarded as a highly specific monocyte/macrophage marker for M2 macrophages.[5] Numerous studies have confirmed that TAMs are associated with poor

prognosis of human carcinoma such as hepatocellular cancer, gastric cancer, lung cancer & breast cancer .[6,7]

NEED FOR THE STUDY:

Studies proposed that TAMs cumulation in tumors correlates with a poor prognosis. In prostate cancer, TAMs can enhance cancer cell invasion by stimulating tumor angiogenesis, degrading the extracellular matrix, and also suppresses the anti-tumor functions of cytotoxic T cells resulting in poor prognosis. Therefore, TAMs are an alluring target for therapeutic intervention by targeting their various function. Hence the study is undertaken. On H and E section, it is difficult to differentiate M1 and M2 phenotypes. Hence Immunostaining is used to identify M1 and M2 sub population of macrophages. CD68 is marker for M1 macrophage and CD163 is a marker for M2 macrophage. Only few studies determining expression of CD68 and CD163 have been done on prostate Cancers and published in Indian Literature so far. Hence the study is undertaken to determine the expression of CD68 and CD163 in prostate Carcinomas.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE:

EMBRYOLOGY

The prostate is a walnut sized organ at the base of the urinary bladder. It is the seat of three major causes of morbidity; benign prostatic hyperplasia (BPH), prostate cancer and prostatitis.

Embryo has the capacity to develop towards a female or male phenotype. At conception this will be determined and the Mullerian ducts , Wolffian ducts, the urogenital sinus (UGS) and the fetal gonad will be formed. Under the influence of hormones produced by fetal gonads like androgens the male sexual differentiation will take place by regression of mullerian duct system.

The prostate form in 50mm human embryos from the walls of USG at the site of of the Mullerian tubercle epithelial buds growing laterally. Solid branching cords are formed by these buds and develop a lumen which will give rise to a network of tubules and alveoli. Some of the apical cells as the lumen forms, become structurally polarized and start some secretory activity. The organ develops the acini and ducts are lined with a layer of flat basal epithelium and a luminal layer of tall columnar secretory epithelium while stroma composed of smooth muscle.[8] The basal and luminal epithelial cells are distinguishable on the basis of morphology and functionally by expression of different cytokeratin classes (keratins 14 and 5 in basal cells, 18 and 8 in luminal).[9]

The growth and development of the prostate begins with formation of prostatic buds from the fetal UGS and are complete at sexual maturity.[10]

ANATOMY AND HISTOLOGY[11]

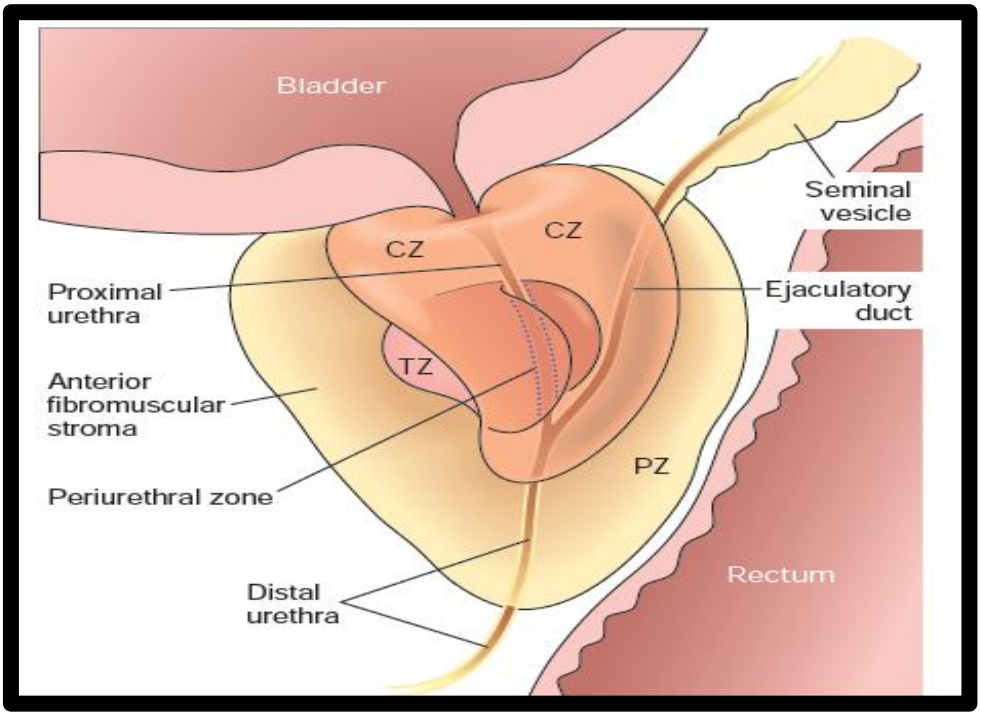


Figure:1 showing Adult prostate: Normal prostate contains 3 zones: Central Zone(CZ) Peripheral Zone (PZ), Transitional Zone (TZ). Most carcinoma arise from peripheral zone and Nodular hyperplasia arise from transitional zone causing obstruction

In normal adult male the prostate weighs up to 20 g and for subsequent growth and its differentiation it depends for and on androgenic hormones synthesized in the testis. It is divided into the fibro muscular stroma and three distinct glandular zones by McNeal: peripheral zone, transition zone, central zone.

The transition zone: surrounds the urethra in the mid portion of the prostate and is the anatomic region enlarged by benign prostatic hyperplasia.

The central zone: is shaped like an inverted pyramid at the base of the prostate and contains the ejaculatory ducts as they course to the prostatic urethra at the verumontanum, a posterior prominence in the prostatic urethra. The prostatic glands of the central zone may have a unique morphology with more deeply eosinophilic cytoplasm and more complex intraluminal architecture consisting of papillary infolding or epithelial bridges.

The peripheral zone: Envelops the transition zone (TZ) and caudally it extends to comprise most of the apex. Finally, the anterior tissues consist of smooth muscle, skeletal muscle from the anterior fibro muscular sling , and adipose tissue in the extra prostatic compartment.

HISTOLOGY:

The glandular component of the prostate is divided into acini and ducts. acini and ducts contain luminal secretory cells, a surrounding outer layer of basal cells, and scattered neuroendocrine cells. In luminal side secretory cells are present which, donate a vast variety of products to the seminal fluid. these cells form an undulating luminal surface and are characterized by relatively pale cytoplasm. They produce prostate-specific acid phosphatase (PSAP) and prostate-specific antigen (PSA),. The basal cells form a thin layer and separates the luminal secretory cells from the basement membrane.[11]

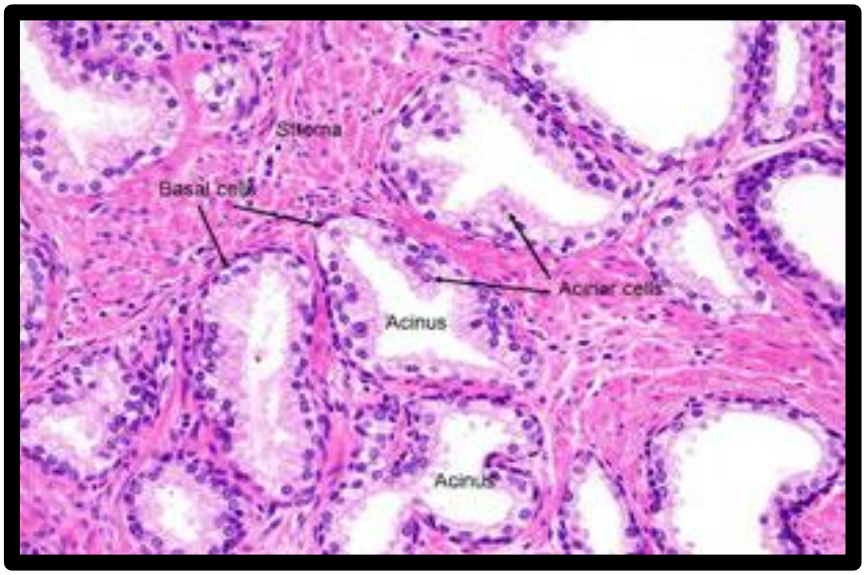


Figure 2 : Showing normal histology of prostate comprising of Glands lined by basal cells and acinar cells with lumen containing secretions. Stroma contains fibrocollagenous tissue and smooth muscle

INCIDENCE AND EPIDEMIOLOGY

- Prostate cancer is a disease of the elderly men above 65 years of age. Studies showed that prostate carcinoma is the second most diagnosed carcinoma in older men worldwide and the fifth most frequent cancer overall.[1] Prostate cancer has been projected to have the largest corresponding increase in men in the upcoming years.[1] Studies shows restricted data available on prostate carcinoma and showed very significant differences in, precipitating factors, incidence and disease characteristics of prostate carcinoma.[1] In India, restricted data available on true incidence of prostatic carcinoma as it is not a notifiable disease and there are few population-based cancer registries[PBCRs] in India[1]. Study done taking PBCRs, from 2009-2011 in different metro cities shows Annual Percentage Change of Karnataka 3.4%.[1] Study carried out in Kolar on prostate cancer cases showed 2.58% out of all other cancers[2,3] . Prostate carcinoma are common in developed countries North America, north-western Europe, Australia, and on Caribbean islands. It is less common in Asia, Africa, Central America, and South America [3] the reason behind this may be more intensive screening for prostate carcinoma in developed countries probably accounts for at least part of this difference, but other factors such as lifestyle differences (diet, etc.) are likely to be important as well. Based on GLOBOCAN 2018 estimates, 1,276,106 new cases of prostate cancer were reported worldwide in 2018.[12]

RISK FACTORS: [13,14,15,16,17,18,19,20,21,22,23,24,25,26]

1. Age: Prostate carcinoma is the most commonly seen in elderly males.
2. Ethnicity: Prostate carcinoma prevalence highly differs among different racial groups.
3. Family history and genetic factors: Several studies reported that inherited genetic background is associated with high risk for prostate cancer, contributing to about 5% of disease risks
4. Diet: Saturated animal fat, Red meat, Calcium, milk and dairy products, Vegetables like broccoli, Brussels sprouts, cauliflower, cabbage, and turnips(Crucifers or Brassica vegetables), Coffee, Low folate and vitamin B12, Obesity, insulin and physical activity, Cigarette smoking,
5. Sex hormones: data supporting a role for androgens in prostate cancer pathogenesis and progression, also known as the ~~–a~~ndrogen hypothesis
6. Chronic inflammation : There is a strong connection between prostate carcinoma and prostatitis
7. Sexually transmitted disease (STD): HPV & trichomonas vaginalis associated with prostate cancer.
8. Environmental carcinogens: The slow process of prostate carcinogenesis is also influenced by exposure to certain environmental factors that increase the risk of developing cancer. Bisphenol A (BPA), Agent orange (AO) & Chlordecone are associated with high risk of malignancy.

PROTECTIVE FACTORS[13]

Diet: Dietary soy and green tea, Tomatoes and lycopene, Vitamin E, Selenium

Ejaculatory frequency: The higher ejaculatory frequency may reduce the development of prostatic adenocarcinoma

SCREENING FOR PROSTATIC CARCINOMA:[27]

1. PSA,
2. Digital rectal exam (DRE): it has low sensitivity and specificity. A DRE checks for the consistency, size, and texture of the prostate gland. An abnormal DRE is any nodularity, induration, or asymmetry.

EXPERT RECOMMENDATIONS FOR PROSTATE CANCER SCREENING.[27]

The guidelines for prostate carcinoma screening are like: the American Urological Association, the American Cancer Society, and draft guidelines from the U.S. Preventive Services Task Force. Among which our institution follows American Urologic Association (AUA) Recommendations, in which there will be combined decision making between clinician and patient. Screening can be done for patients with age >40 yr. Discontinuation of screening can be done when the Life expectancy of the patient is <10 yr. Screening tests which can be used are PSA, digital rectal examination. Annual screening is done for patients >40 yrs.

Criteria for biopsy referral Age, family history, race or ethnic group, findings on digital rectal examination, total PSA, free PSA, PSA velocity, PSA density, previous biopsy findings, coexisting conditions

PSA AND ROLE OF OTHER ADJUNCTIVE PRE-BIOPSY TESTS.[27]

PSA is a glycoprotein secreted by prostatic secretory epithelium and seminal vesicles and is the most abundant protein in seminal plasma. PSA level increases in malignant as benign conditions of the prostate like BPH, prostatic inflammation or infection, perineal trauma, or sexual activity. Hence raised PSA level is not specific for prostate cancer. Normal PSA value does not elimate prostate carcinoma. Despite the lack of specificity, PSA remains the single most widely recruited test for early detection of prostate cancer. The normal value of PSA is considered to be less than or equal to 4 ng/ml.

CLINICAL FEATURES: Urinary retention. Increased frequency, hesitancy, nocturia, weight loss, impotency and hematuria in few patients.[28]

CATEGORY CRITERIA[29,30]

"Clinical (cT)

T -category

TX- Primary tumor cannot be assessed

T0- No evidence of primary tumor

T1 -Clinically inapparent tumor that is not palpable

T1a -Tumor incidental histologic finding in 5% or less of tissue resected

T1b -Tumor incidental histologic finding in more than 5% of tissue resected

T1c -Tumor identified by needle biopsy found in one or both sides, but not palpable

T2 -Tumor is palpable and confined within prostate

T2a -Tumor involves one-half of one side or less

T2b -Tumor involves more than one-half of one side but not both sides

T2c -Tumor involves both sides

T3 -Extraprostatic tumor that is not fixed or does not invade adjacent structures

T3a -Extraprostatic extension (unilateral or bilateral)

T3b -Tumor invades seminal vesicle(s)

T4- Tumor is fixed or invades adjacent structures other than seminal vesicles, such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall

Pathologic (pT)

T -category

T2- Organ confined

T3 -Extraprostatic extension

T3a -Extra prostatic extension (unilateral or bilateral) or microscopic invasion of bladder neck

T3b -Tumor invades seminal vesicle(s)

T4 -Tumor is fixed or invades adjacent structures other than seminal vesicles, such as external

sphincter, rectum, bladder, levator muscles, and/or pelvic wall

N- category

NX- Regional lymph nodes were not assessed

N0 -No positive regional lymph nodes

N1 -Metastases in regional lymph node(s)

M -category M criteria

M0- No distant metastasis

M1 -Distant metastasis

M1a -Nonregional lymph node(s)

M1b -Bone(s)

M1c -Other site(s) with or without bone disease”

Table:1 Stage Grouping of prostate carcinoma[31]

Stage 0	Tis	N0	M0
Stage 1	T1a-2a	N0	M0
Stage 2	T2b-c	N0	M0
Stage 3	T3	N0	M0
Stage 4	T4 Any T Any T	N0 N1 Any N	M0 M0 M1

Table 2: American Joint Committee on Cancer Prognostic Stage Grouping of prostatic carcinoma (AJCC)[32]

T Stage	N Stage	M Stage	PSA (ng/dL)	Grade Group	Stage Group
cT1a-c, cT2a	N0	M0	<10	1	I
pT2	N0	M0	<10	1	I
cT1a-c, cT2a	N0	M0	≥10, <20	1	IIA
pT2	N0	M0	≥10, <20	1	IIA
cT2b-c	N0	M0	<20	1	IIA
T1-2	N0	M0	<20	2	IIB
T1-2	N0	M0	<20	3 ~ 4	IIC
T1-2	N0	M0	≥20	1 ~ 2	IIIA
T1-2	N0	M0	≥20	3 ~ 4	IIIA
T3-4	N0	M0	Any	1 ~ 4	IIIB
Any T	N0	M0	Any	5	IIIC

"WHO Classification of prostate tumors 2016[32]

Epithelial tumours

Glandular neoplasms

Acinar adenocarcinoma

Atrophic

Microcystic

Foamy gland

Mucinous (colloid)

Signet ring-like cell

Pleomorphic giant cell

Sarcomatoid

Prostatic intraepithelial neoplasia,

high-grade

lntraductal carcinoma

Ductal adenocarcinoma

Cribriform

Papillary

Solid

Urothelial carcinoma

Squamous neoplasms

Adenosquamous carcinoma

Squamous cell carcinoma

Basal cell carcinoma

Neuroendocrine tumours

Adenocarcinoma with neuroendocrine

Well-d ifferentiated neuroendocrine tumour

Small cell neuroendocrine carcinoma

Large cell neuroendocrine carcinoma

Mesenchymal tumours

Angiosarcoma

Stromal tumour of uncertain malignant potential

Stromal sarcoma

Leiomyosarcoma

Rhabdomyosarcoma

Leiomyoma

Synovial sarcoma

Inflammatory myofibroblastic tumour

Osteosarcoma

Undifferentiated pleomorphic sarcoma

Solitary fibrous tumour

Solitary fibrous tumour, malignant

Granular cell tumour

Haematolymphoid tumours

Diffuse large 8-cell lymphoma

Chronic lymphocytic leukaemia

Small lymphocytic lymphoma

Follicular lymphoma

Mantle cell lymphoma.

Acute myeloid leukaemia

B lymphoblastic leukaemia/lymphoma

Miscellaneous tumours

Pseudohyperplastic Cystadenoma

Nephroblastoma

Rhabdoid tumour

Germ cell tumours

Clear cell adenocarcinoma

Melanoma

Paraganglioma

Neuroblastoma

Metastatic tumours

Tumours of the seminal vesicles Solid

Epithelial tumours

Adenocarcinoma

Squamous cell carcinoma

Mixed epithelial and stromal

tumours Cystadenoma

Mesenchymal tumours differentiation

Leiomyoma

Schwannoma

Mammary-type myofibro blastoma

Gastrointestinal stromal tumour, NOS

Leiomyosarcoma

Mesenchymal tumours

Angiosarcoma

Liposarcoma

Solitary fibrous tumour

Haemangiopericytoma

Miscellaneous tumours

Choriocarcinoma

Seminoma

Wel l-differentiated neuroendocrine tumour

Lymphomas

Ewing sarcoma

Metastatic tumours"

CD 68(Cluster of Differentiation 68)

Also known as Macrosialin, CD 68 is a 110 kD transmembrane glycoprotein containing 354 amino acids and an important member of scavenger family. It is encoded by CD 68 gene on

chromosome 17. Normally it stains cells of macrophage, lineage including Kuffer cells and osteoclasts.

The binding of CD 68 to selectins and organ specific lectins is mediated by Glycosylated extra cellular domain on its surface. Its functions include activation and recruitment of macrophages in a specific site, phagocytosis of dead cells and foreign bodies.

The lysosomes and late endosomes of the macrophages express CD 68 antigen in the granules thus giving a cytoplasmic staining.

CD 68 positive TAM s in the tumor microenvironment are associated with increased serum and stromal levels of VEGF .This not only promotes angiogenesis inside the tumour but also reduces the efficacy of radiotherapy.[33] There is an established fact that increased macrophage index and high vascular grade is associated with decreased relapse overall survival and free survival and accounts as a poor prognostic factor. [34]

CD 163 (Cluster of differentiation 163)

CD 163 is a member of scavenger receptor family and a resident tissue macrophage. It acts a receptor for hemoglobin haptoglobin complex and has a pivotal role to play in body s immune mechanism in response to hemolysis and bacterial infections.[33]

It has a molecular size of 130k Da and has 1048 amino acid residues in extracellular domains.

A dissolved form of CD 163 is seen in cerebrospinal fluid and is called CD 163 represents shedding of receptor and functional modulation of CD163.

CD 163 is up regulated in disease likes Diabetes, Gauchers disease, Rheumatoid arthritis and Hodgkin's lymphoma.

Numerous studies have shown than higher expression of CD 163 molecule in prostatic cancers associated with increased chances of lymph node metastasis, higher grade, increased tumour size and higher chances to tumor recurrence. [34,35,36,37]

MATERIAL & METHODS

MATERIALS AND METHODS

STUDY DESIGN – Retrospective observational study

SOURCE OF DATA: Transurethral resected prostatic(TURP) Chips positive for prostate carcinoma, received in the Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, and Kolar . from December 2019 to October 2021 and also the paraffin blocks taken from all cases of Prostate cancer retrieved from Archives of Department of Pathology from the year January 2015 to November 2019 were included in the study.

STUDY COURSE- January 2019 to October 2021

DURATION OF STUDY – 2 years

METHODOLOGY

All Transurethral resected prostatic(TURP) Chips positive for carcinoma prostate confirmed by histo-pathological examination was added in the study. Data regarding the clinical details (age, Stage of the disease) was collected from Medical Record Department. H and E slides were reviewed for Histopathological types and Gleason's score of the tumor. Radiologic findings (USG,MRI or CT Findings) with respect to stage of disease, size of lesion, were noted.

TUMOR SIZE : Recent studies showed that tumor size along with other parameters like PSA and Gleason's score contributes in tumor progression and patient prognosis[57]

In this study we have divided the tumor size into 3 groups by MRI results taking the largest dimensions on MRI

Size
1-3 cm
>3- 6 cm
>6 cm

Table3: 2014 modified Gleason grading and Grade Group comparison[17]

Grade	GLEASON SCORE	HISTOLOGIC FEATURES
1	≤3 + 3 = 6	Only individual discrete well-formed glands
2	3 + 4 = 7	Predominantly well-formed glands with lesser component of poorly formed glands, fused glands, glomerations, or cribriform glands
3	4 + 3 = 7	Predominantly poorly formed glands, fused glands, glomerations, or cribriform glands with lesser component of well-formed glands (if >5%)
4	4 + 4 = 8 3 + 5 = 8 5 + 3 = 8	glands, glomerations, or cribriform glands Predominantly well-formed glands with lesser component of sheets, cribriform glands with comedonecrosis, or single cells. Predominantly sheets, cribriform glands with comedonecrosis, or single cells with lesser component of well-formed glands (if >5%
	≥4 + 5 = 9	Only sheets, cribriform glands with comedonecrosis, or single cells

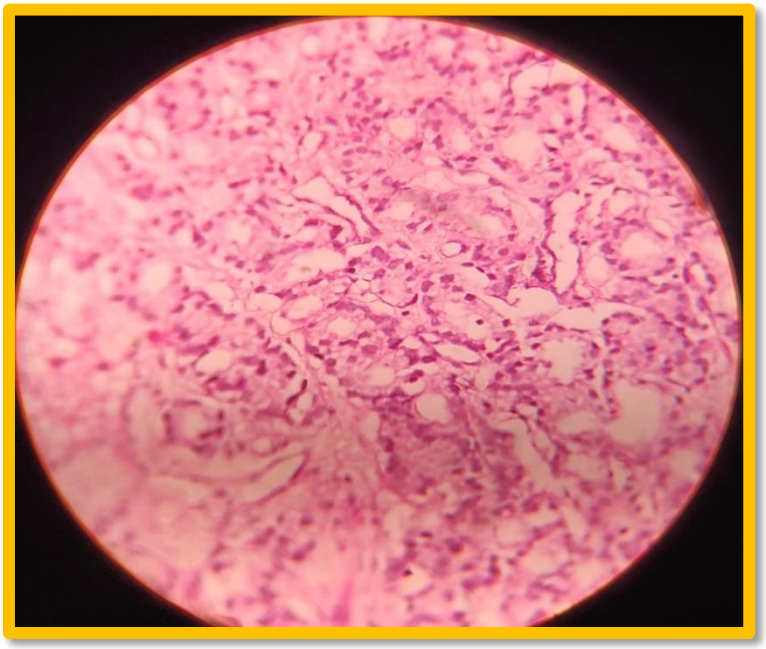


Figure3: Shows H and E stained slide with 100x power Gleason's score:3+3=6 Only individual discrete

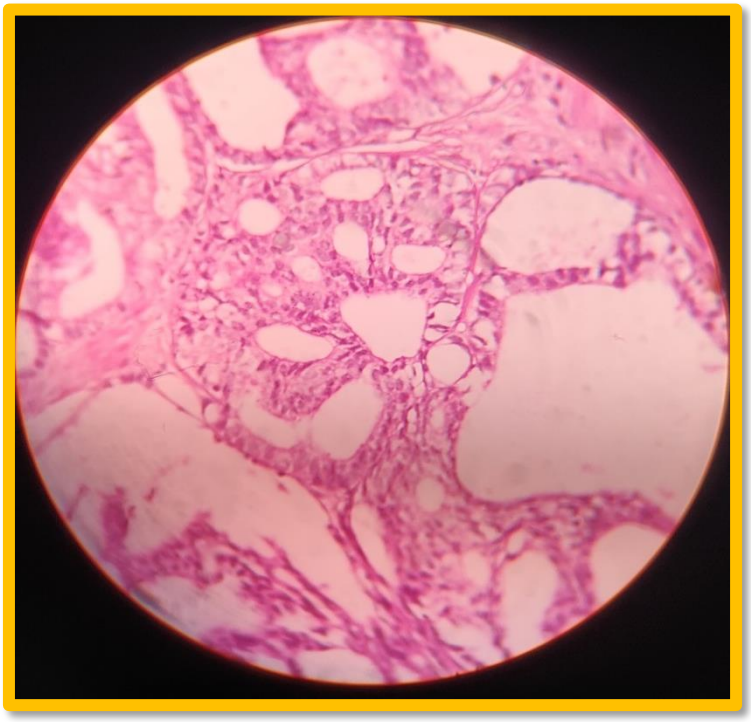


Figure4: Shows H and E stained slide with 100x power Gleason's score:3+4=7 Predominantly well-formed glands

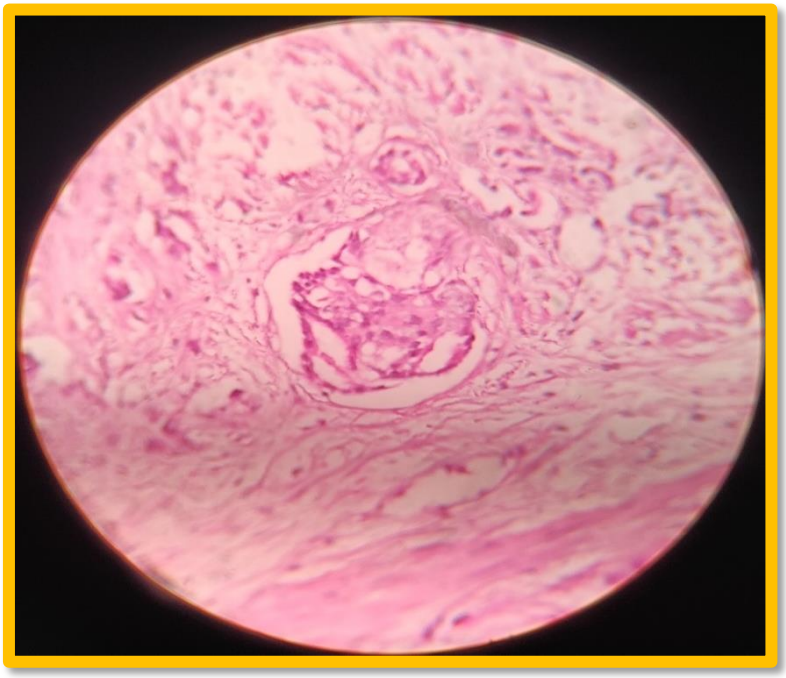


Figure 5: Shows H and E stained slide with 100x power Gleason's score:3+4=7

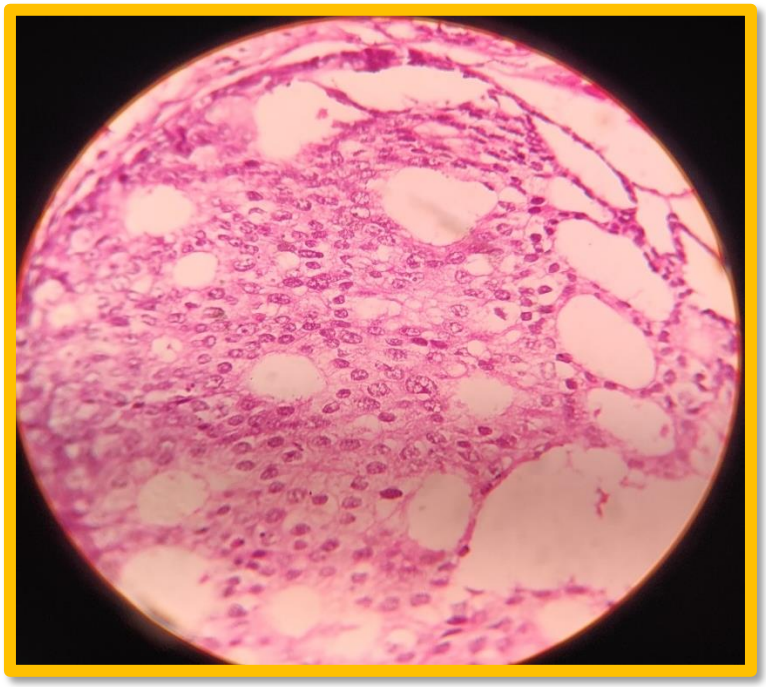


Figure 6: Shows H and E stained slide with 100x power. Gleason's score:4+3=7 Predominantly poorly formed glands,

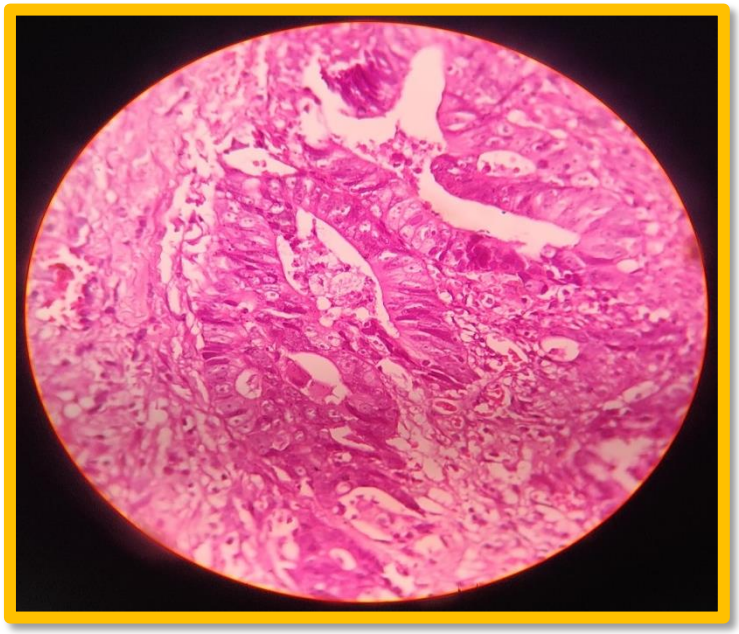


Figure 7: Shows H and E stained slide with 400x power Gleason's score:4+3=7 Predominantly poorly formed glands,

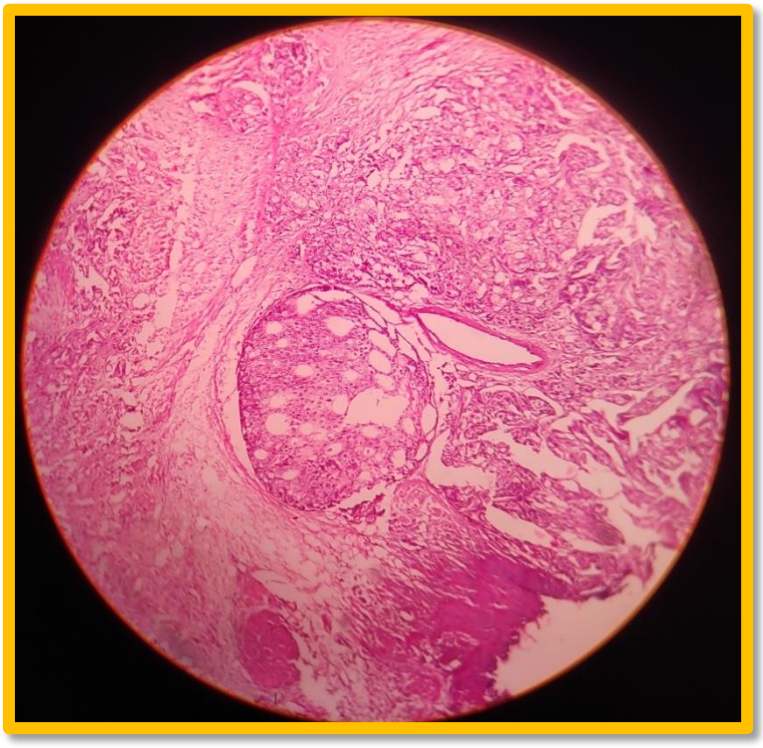


Figure 8: Shows H and E stained slide with 40x power Gleason's score: 4+4=8 glands, glomerations, or cribriform

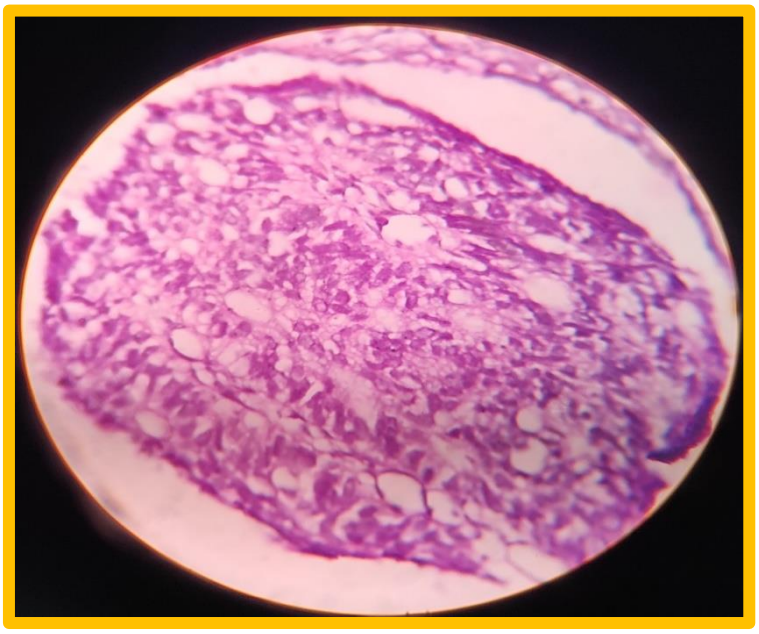


Figure 9: Shows H and E stained slide with 400x power Gleason's score: 4+4=8 glands, glomerations, or cribriform

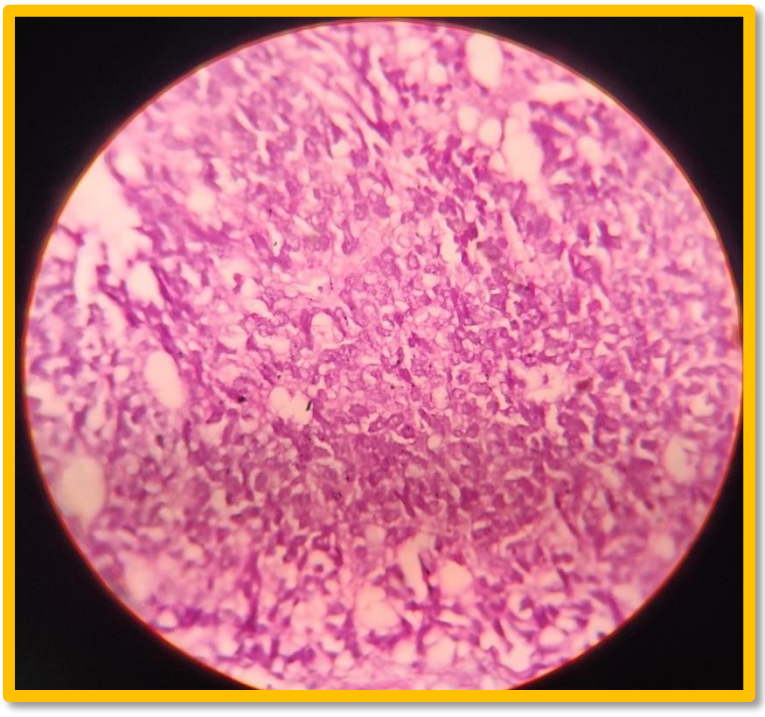


Figure 10: Shows H and E stained slide with 400x power Gleason's score: 5+5 showing tumor cells predominantly arranged in sheets .

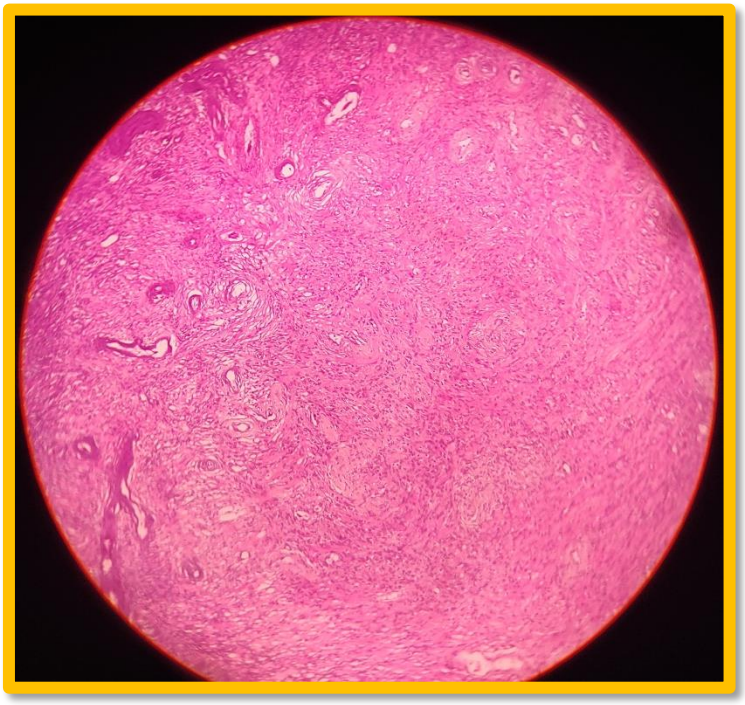


Figure 11: Shows H and E stained slide with 40x power Gleason's score: 5+5=10 showing tumor cells in low power, predominantly tumor cells arranged in sheets.

IMMUNOHISTOCHEMISTRY ANTIBODIES DETAILS

Immunostaining for CD68 and CD163 (Biocare mouse antibody & secondary antibody from Dako) was performed on all cases of Prostatic adenocarcinoma using appropriate positive and negative controls by peroxidase and anti peroxidase method.

IHC PROTOCOL

1) Section Cutting: Sections are cut at approximately 3-4 µ m, floated on to positive charged slides and incubated at 37^{o} C for one day and further incubated at 58° C overnight.

2) Deparaffinization and Dexylinisation

Xylene –I - 15 mins

Xylene –I I - 15 mins

Ab alcohol – I - 1min

Ab alcohol – II - 1min

90%Alcohol – 1min

70%Alcohol -1min

3) Tap water – 10 min washing

4) Distilled water – 5 min rinsing

5) Antigen Retrieval

Microwave at power 10 for 2 cycles of 6 minutes each in TRIS EDTA BUFFER of PH 9.0. Later Slides were cooled to room temperature.

6) Transfer to TBS buffer

7) Peroxidase block

8) TBS buffer

9) Power block

10) Drain and cover section with TARGET Ab

11) TBS buffer

12) Probe

13) TBS buffer

14) Super sensitive polyp –HRP

15) TBS buffer

16) DAB Color development

17) TBS buffer

18) Tap water

19) Hematoxylin Counter stain

20) Tap water

21) 90%Alcohol

22) Absolute Alcohol

23) Alcohol: Xylene 1:1

24) Xylene

25) Mount with DPX

POSITIVE CONTROL- Tonsil tissue containing macrophages were taken as positive control

Immunohistochemistry Scoring:

The CD 68 and CD 163 immuno stained smears were examined under low magnification (10X) and was looked for areas with maximum expression of CD 68 and CD 163 by two observers and were called as" Hot spots" . These hotspots were then viewed under higher magnification (40X) and CD 68 and CD 163 positive cells were counted and scoring was done on number of macrophages expressed by IHC.[53]

CD-68 and CD 163 scoring:

- 0-30 macrophages- Score1
- 31-60 macrophages- Score 2
- >60 macrophages- Score 3

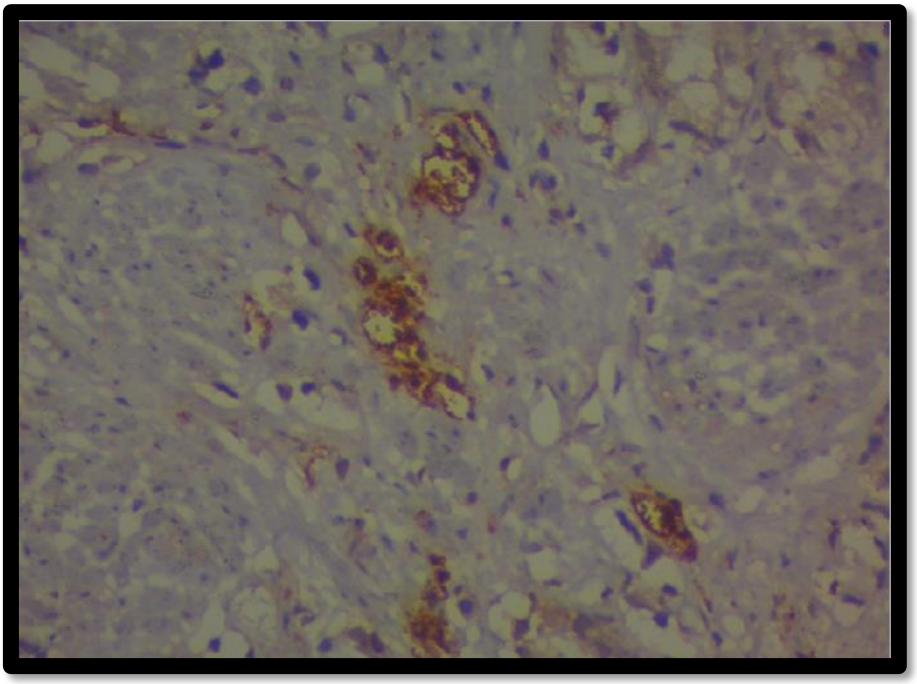


Figure 12: Showing IHC staining in 400x with CD68 expression - Score 1 (0-30 macrophages)

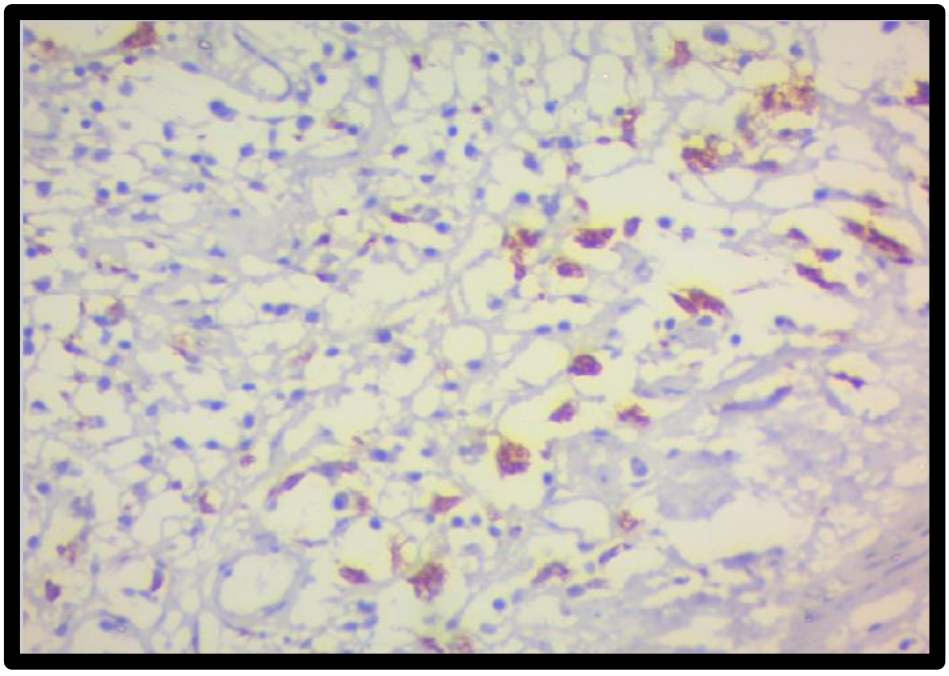


Figure 13: Showing IHC staining in 400x with CD68 expression - Score 1 (0-30 macrophages)

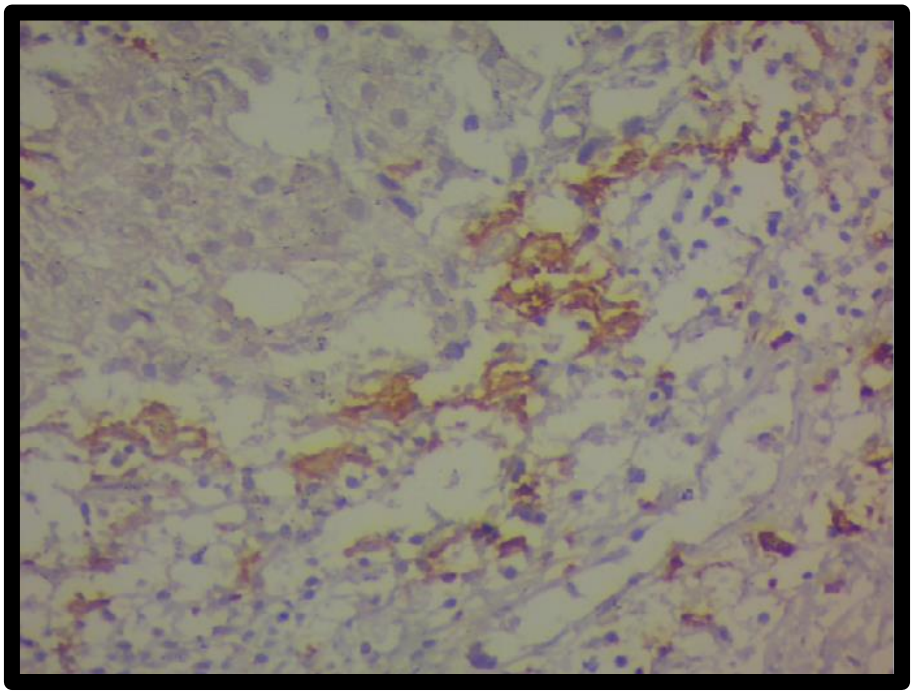


Figure 14: Showing IHC staining in 400x with CD68 expression Score 2 (31-60) macrophages)

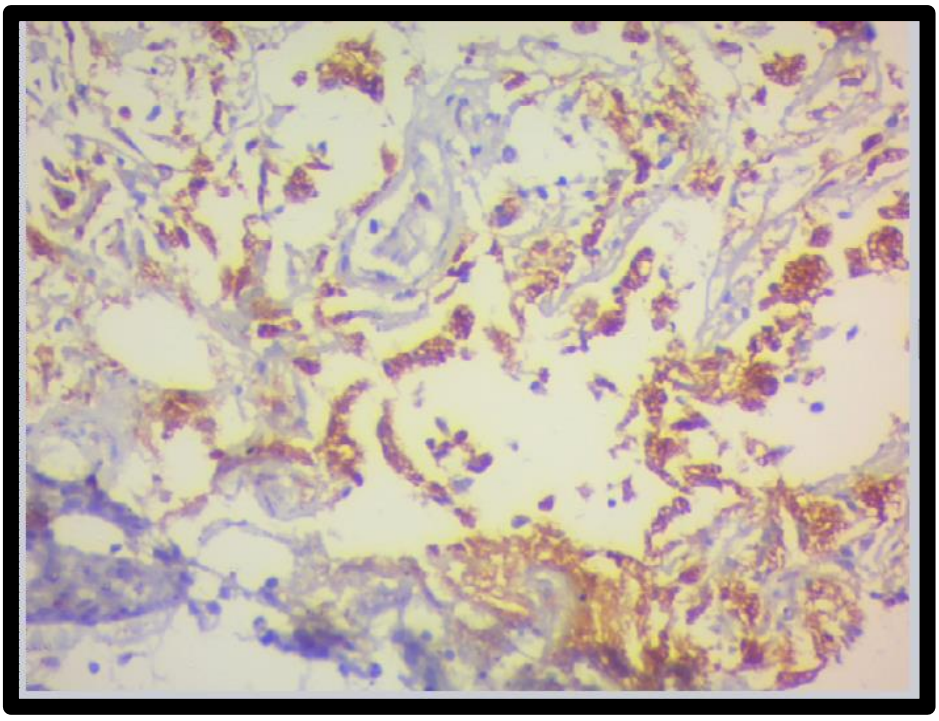


Figure 15: Showing IHC staining in 400x with CD68 expression Score 3(>60 macrophages)

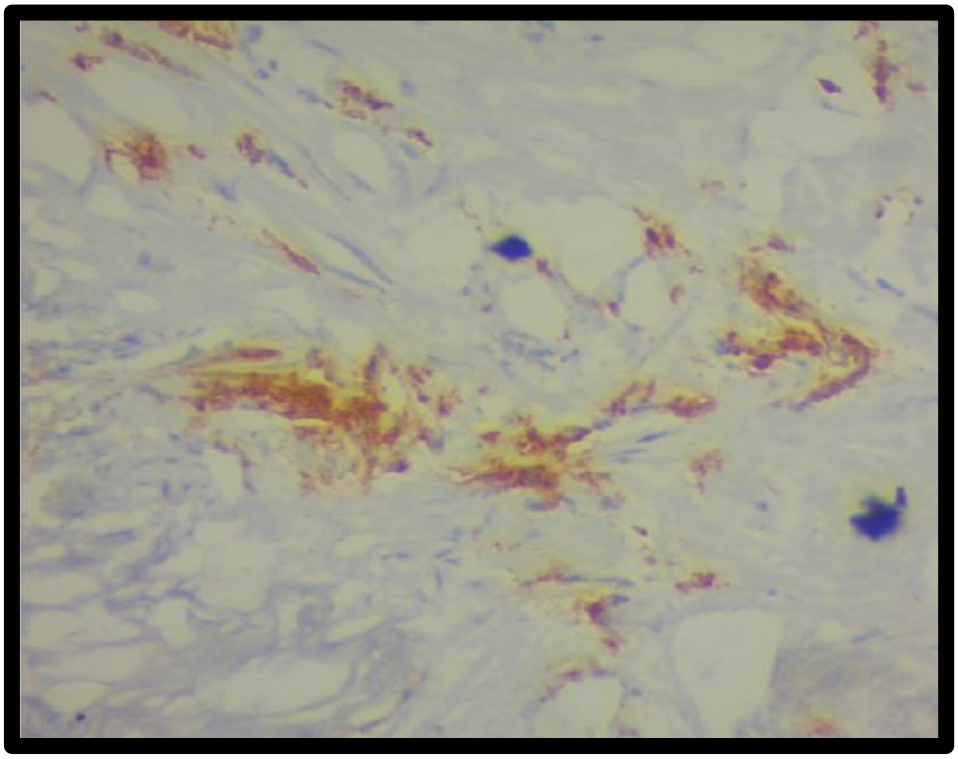


Figure 16: Showing IHC staining in 400x with CD163 expression Score 1 (0-30 macrophages)

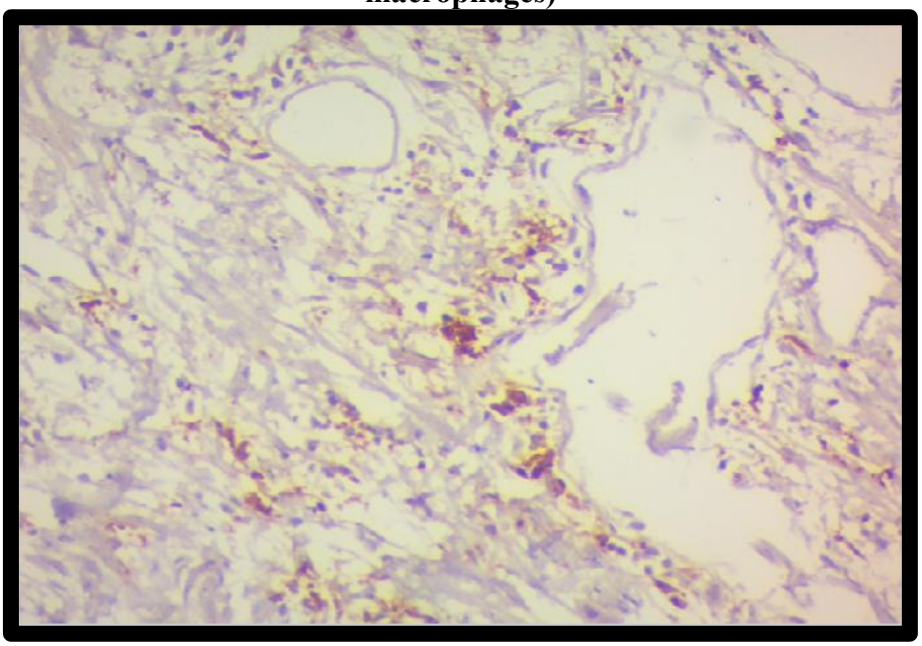


Figure 17: Showing IHC staining in 400x with CD163 expression Score 2(31-60) macrophages)

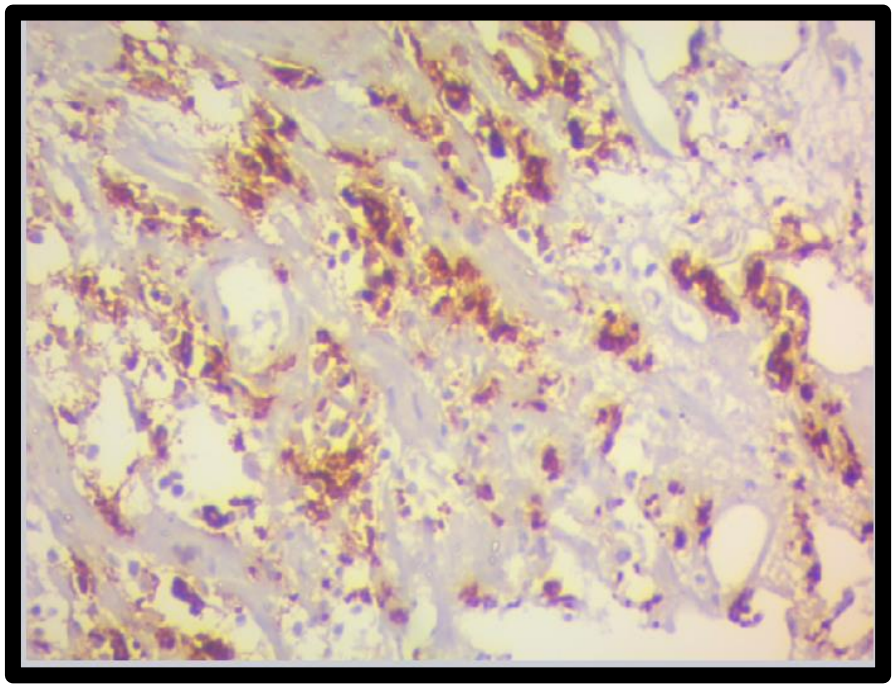


Figure 18: Showing IHC staining in 400x with CD163 expression - Score 3(>60 macrophages)

All the scoring was done by two pathologists independently & both were unaware of clinical data. All the decision were taken by both the pathologist based on the consensus. In case of any discrepancies the case was referred to a third pathologist for final decision which was acceptable to both.

SAMPLE SIZE:

Sample size was estimated by using the proportion of CD163 marker positivity in prostate cancers which was 12% by using Lancotti etal.[50] by using the formula

Equation sample size is = $\frac{Z_{1-\alpha}^2 p(1-p)}{d^2}$

Here $Z_{1-\alpha}$ = Standard normal variant

P = Expected proportion in population based on previous studies

d = Absolute error of 12%

P= 63.4

q = 36.6

d = 12%

Using the above values at 95% Confidence level a sample size of 62 subjects with prostate cancer will be included in the study.

Statistical analysis:

Data was analyzed using SPSS 22 version software from Microsoft excel data sheet. Categorical data was represented in the form of Proportions and Frequencies. Qualitative data for test of significance was done by utilizing Chi-square test or Fischer's exact test (for 2x2 tables only).

Continuous data was represented as standard deviation and mean.

To identify the mean difference between two quantitative variables independent t test was used as test of significance. Graphical representation of data, MS Excel and MS word was used to obtain various types of graphs.

p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyze data

INCLUSION CRITERIA:

All Adenocarcinoma cases which are diagnosed on Histopathology were included in the study.

EXCLUSION CRITERIA:

1. Patients subjected for Chemotherapy and Radiotherapy.
2. Secondary metastasis to prostate.
3. Recurrent lesion
4. Urothelial carcinoma involving prostate

RESULTS

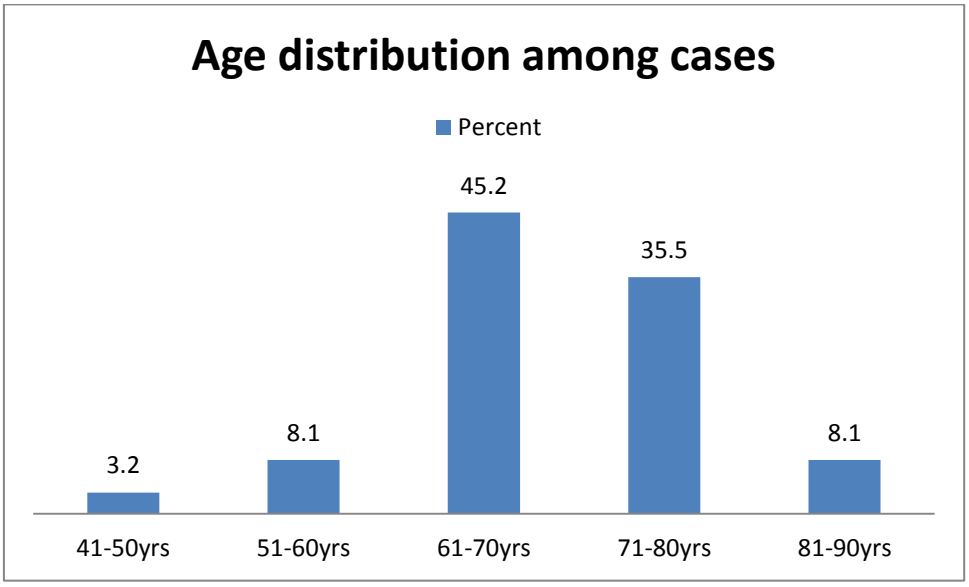
RESULTS

A total of 62 patients (19 with distant metastasis and 43 without metastasis) diagnosed with prostate adenocarcinoma were included in the study. PCa with metastasis was defined as invasion of other organs, including bone, visceral (such as bladder, testis, and other organs) or lymph nodes. To assess if the PCa patients were metastatic, bone scans, ultrasound, MRI scans (or CT or PET scans) were conducted

Table 4:- Distribution of subjects according to age group.

Age group	Frequency	Percentage(%)
41-50yrs	2	3.2
51-60yrs	5	8.1
61-70yrs	28	45.2
71-80yrs	22	35.5
81-90yrs	5	8.1
Total	62	100.0

Figure 18:- Bar diagram showing Distribution of subjects according to age group.



In the present study more number of patients were in the age group of 61-70 yr with mediam age group of 70 yrs.

Table: 5 Distribution of cases with different clinico-pathological parameters

Symptoms	Cases	Percentage(%)
Urinary retention	49	79.03
Burning micturation	5	8.06
Urinary hesitation & dribbling	8	12.9

In Present study 79%(49 cases) of patients came with chief complaints of urinary retention, 12.9% (8 cases) with urinary hesitation and 8% (5 cases) of patients with burning micturition.

Figure19:Graph showing cases with different clinico-pathological parameters

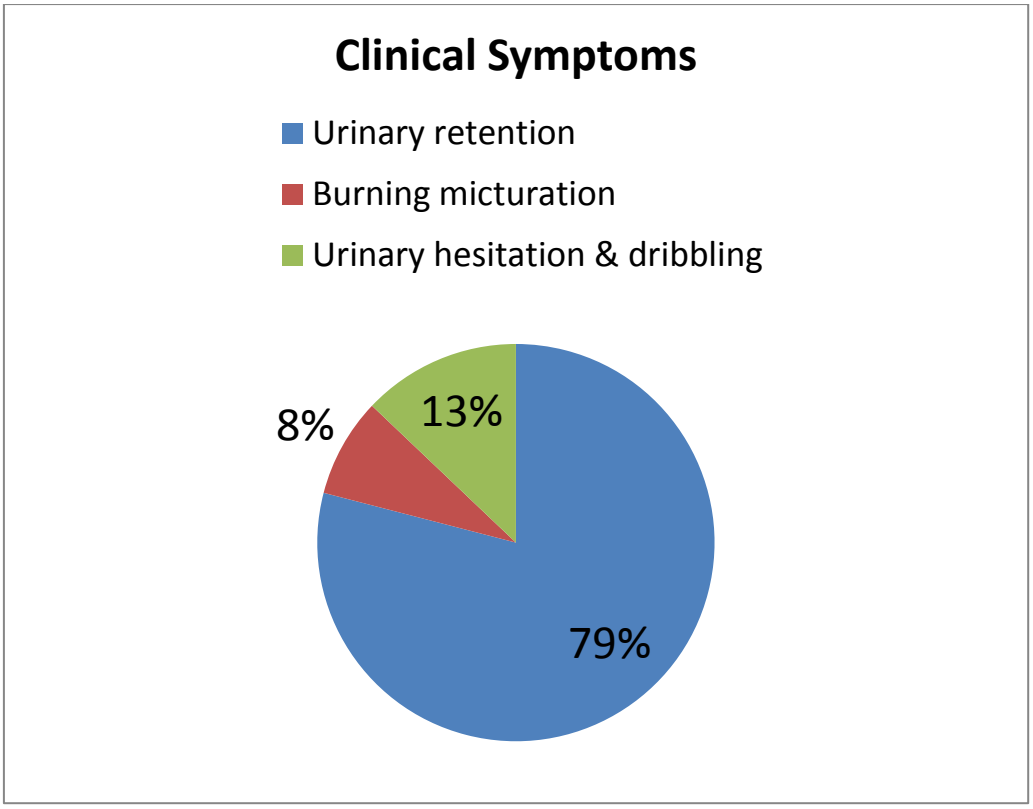


Table 6:- Distribution of subjects according to Gleason's Score.

Gleason's Score	Frequency	Percentage (%)
6	9	14.5
7	15	24.2
8	6	9.7
9	21	33.9
10	11	17.7
Total	62	100.0

In the present study more number of patients had Gleason's score of 9, 33.9% (21 cases), followed by Gleason's score 7, 24.2%(15 cases)

Figure 20:- Graph showing Distribution of subjects according to Gleason's Score.

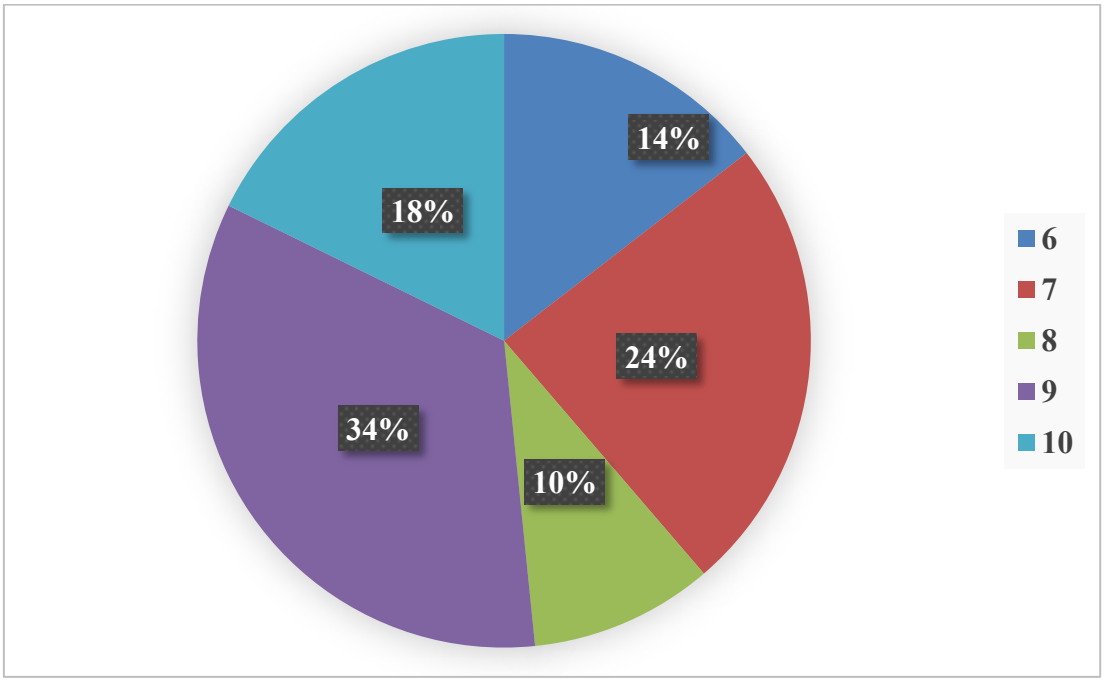


Table 7:- Distribution of subjects according to Gleason's Grade Grouping

Gleason's Grade Grouping	Frequency	Percentage(%)
1 (3+3=6)	3	4.8
2 (3+4=7)	11	17.7
3 (4+3=7)	8	12.9
4 (4+4=8)	8	12.9
5 (5+5=10)	32	51.6
Total	62	100.0

In this study 51.6%(32 cases) were in Grade 5 then 17.7% (11 cases) were in Grade 2.

Figure 21:- Graph showing Distribution of subjects according to Gleason's Grade Grouping.

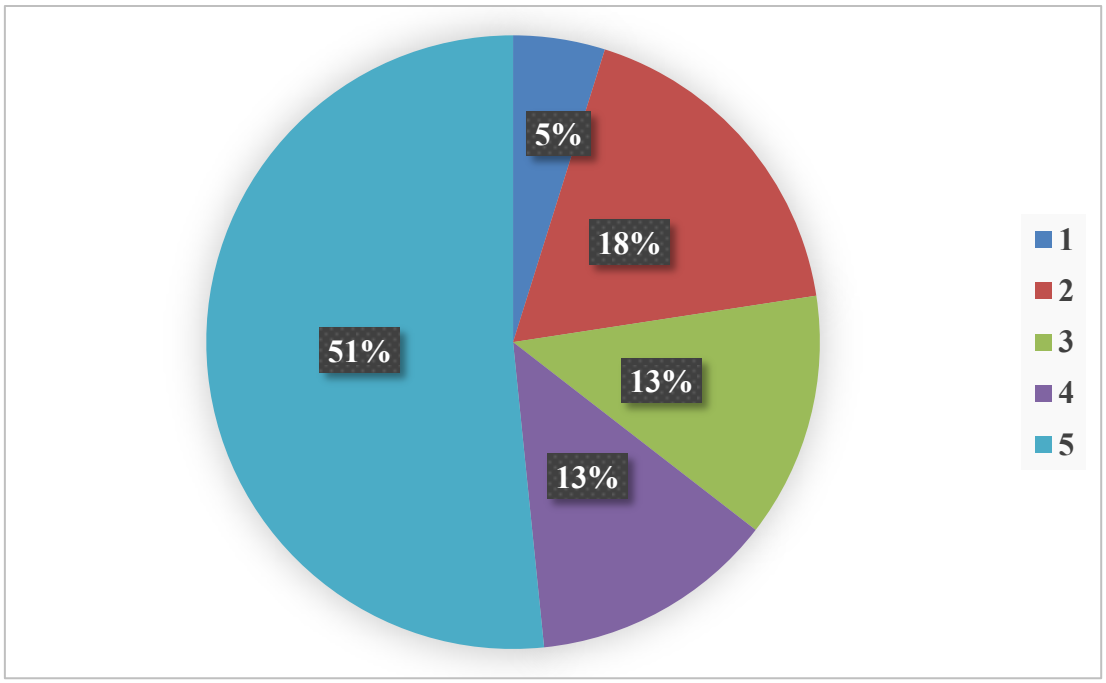


Table 8:- Distribution of subjects according to PSA levels

PSA levels(ng/ml)	Frequency	Percentage (%)
4-19	10	16.1
20-39	13	21.0
40-59	13	21.0
60-79	14	22.6
80-99	7	11.3
≥100	5	8.1
Total	62	100.0

In this study 65% (40) patients were showing elevated levels of PSA between 20-79 ng/ml

Figure 22:- Graph showing Distribution of subjects according to PSA levels.

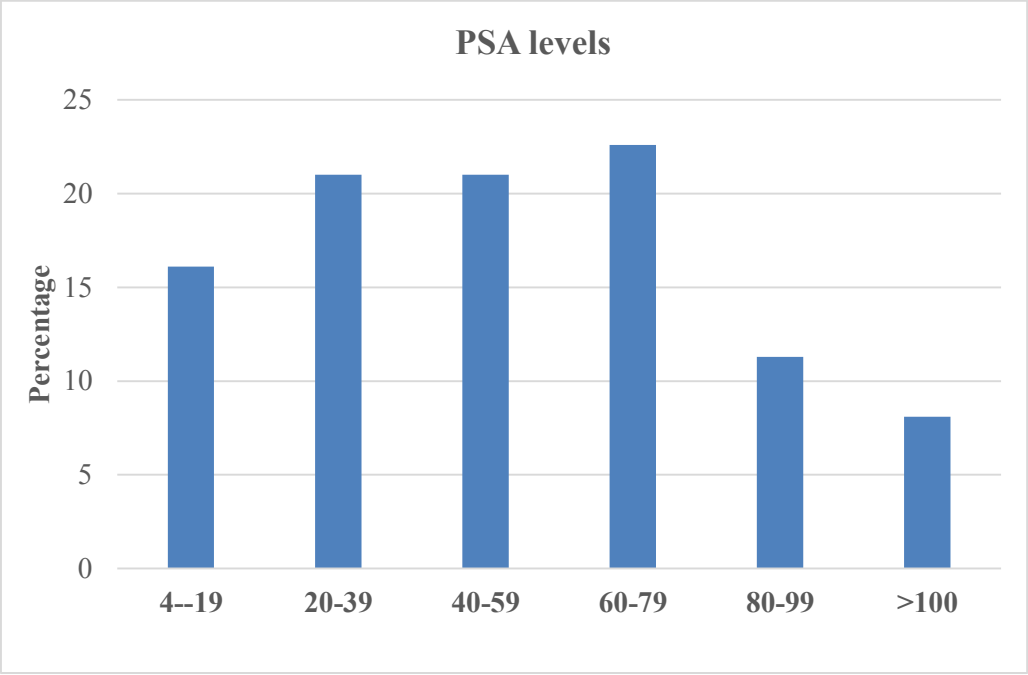
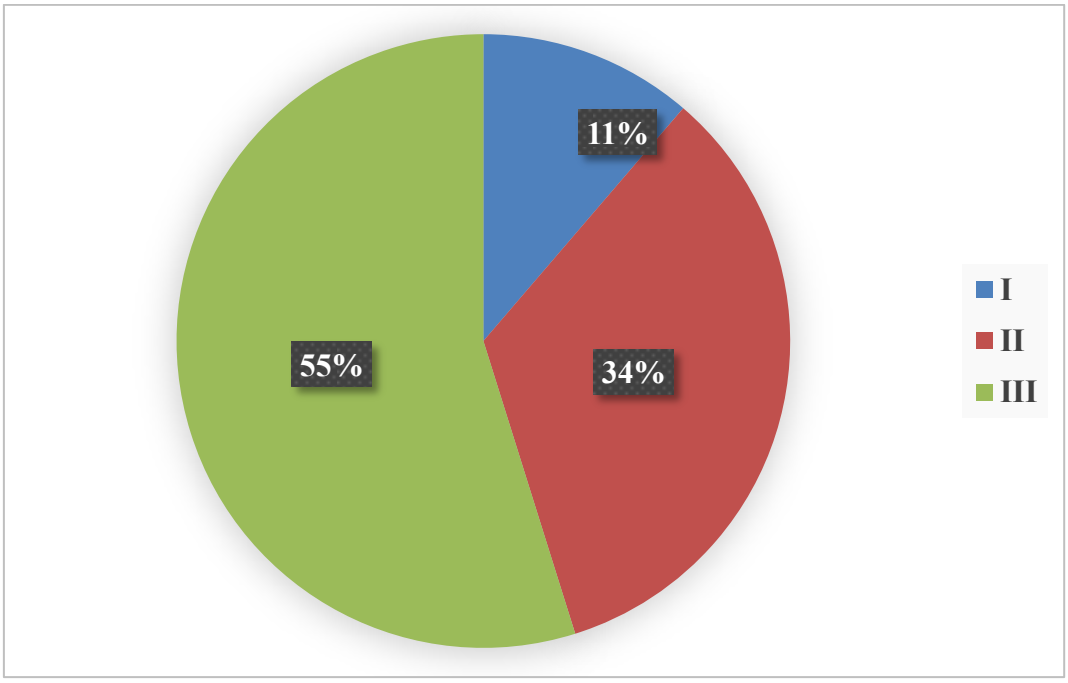


Table 9:- Distribution of subjects having prostatomegaly on USG

USG Grading	Frequency	Percentage (%)
I	7	11.3
II	21	33.9
III	34	54.8
Total	62	100.0

In this study 54.8%(34 cases) of cases were showing USG grade-III, followed by 33.9% in USG grade-II and 11.3% (7 cases) in USG grade-I.

Figure 23:- Graph showing Distribution of subjects having prostatomegaly on USG



Tumor size, tumor staging, lymph node and distant metastasis was taken into consideration by MRI, Bone scan, USG and PET scan and analyzed

Tumor size on MRI of all the patients was considered and largest dimension were taken into consideration and Tumor size has been divided into 3 categories and were analyzed.

Table 10:- Distribution of subjects according to tumor size on MRI

Tumor Size	Frequency	Percentage(%)
1-3cms	6	9.7
3-6cms	32	51.6
>6cms	24	38.7
Total	62	100.0

In the above table ,we can see that 51.6%(32) of cases were having tumor size of 3-6cm, 38.7% (24 cases) of >6 cm of tumor size and 9.7% (6 cases) of tumor size 1-3 cms at the time of diagnosis by MRI.

Figure 24 :- Graph showing Distribution of subjects according to tumor size.

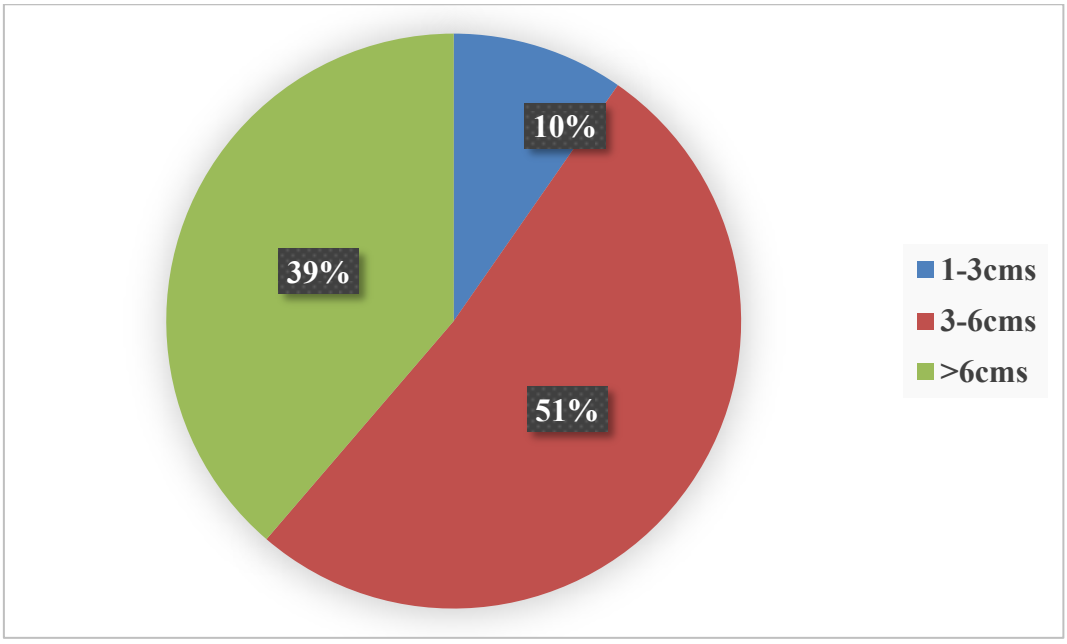


Table 11:- Distribution of subjects according to tumor staging

Clinical Staging	Frequency	Percentage(%)
T2	18	29.0
T3	25	40.3
T4	19	30.7
Total	62	100.0

In the present study 40.3% (25) of the cases were in clinical stage of T3, 30.6% (19) cases in T4 stage and 29% (18 cases) in T2 stage.

Figure 25:- Graph showing Distribution of subjects according to tumor staging.

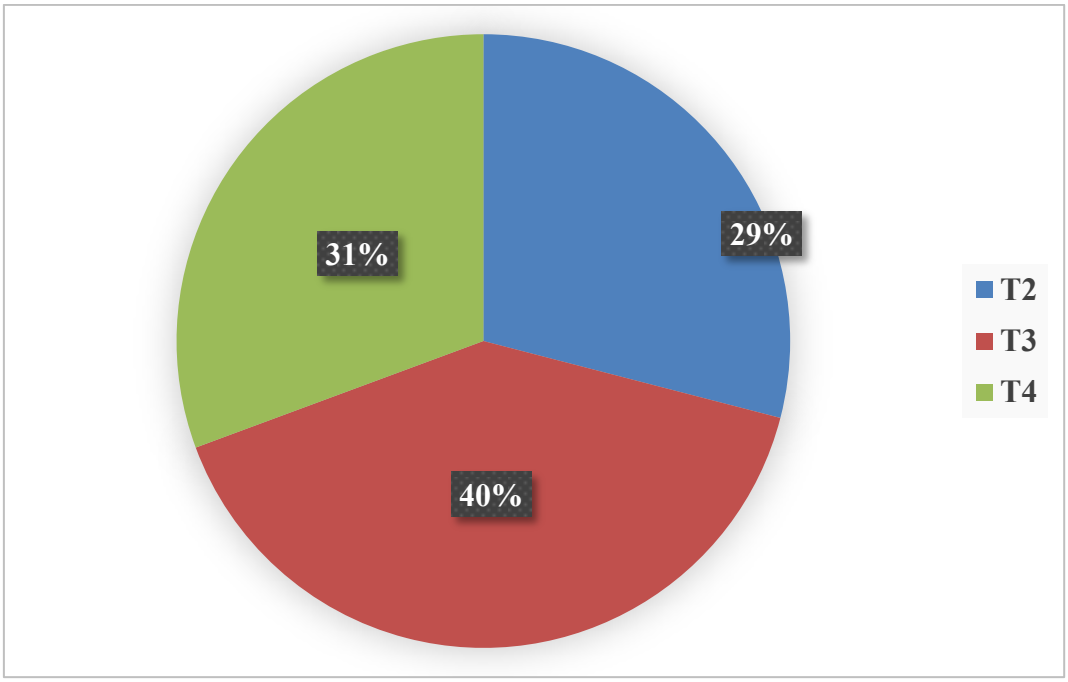


Table 12 :N staging distribution among subjects

		Frequency	Percentage(%)
N Staging	N0	18	29%
	N1	44	70.9%

In this study maximum cases were in N1 stage 70.9% (44 cases), followed by N0 29% (18 cases).

Figure 26: N staging distribution among subjects

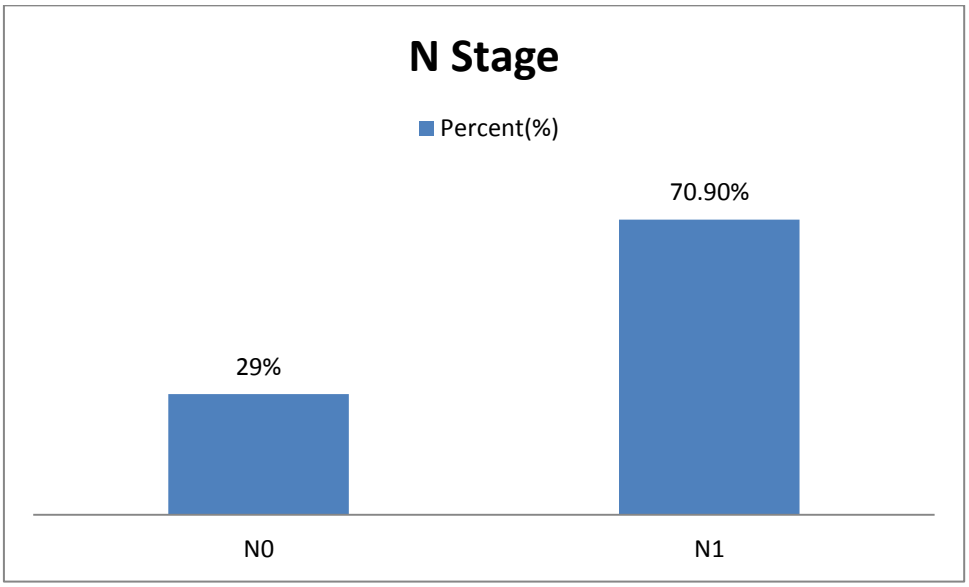


Table 13: Metastasis staging among subjects

		Frequency	Percent(%)
M stage	M0	43	69.3%
	M1a	16	25.8%
	M1b	2	3.2%
	M1c	1	1.7%
	Total	62	100.0

In the present study 69.3%(43) of cases were not showing distant metastasis, 19 cases were showing distant metastasis with 25.8% in M1a, 3.2% and 1.6% in M1b and M1c respectively

Figure 27: Graph showing staging of metastasis among subjects

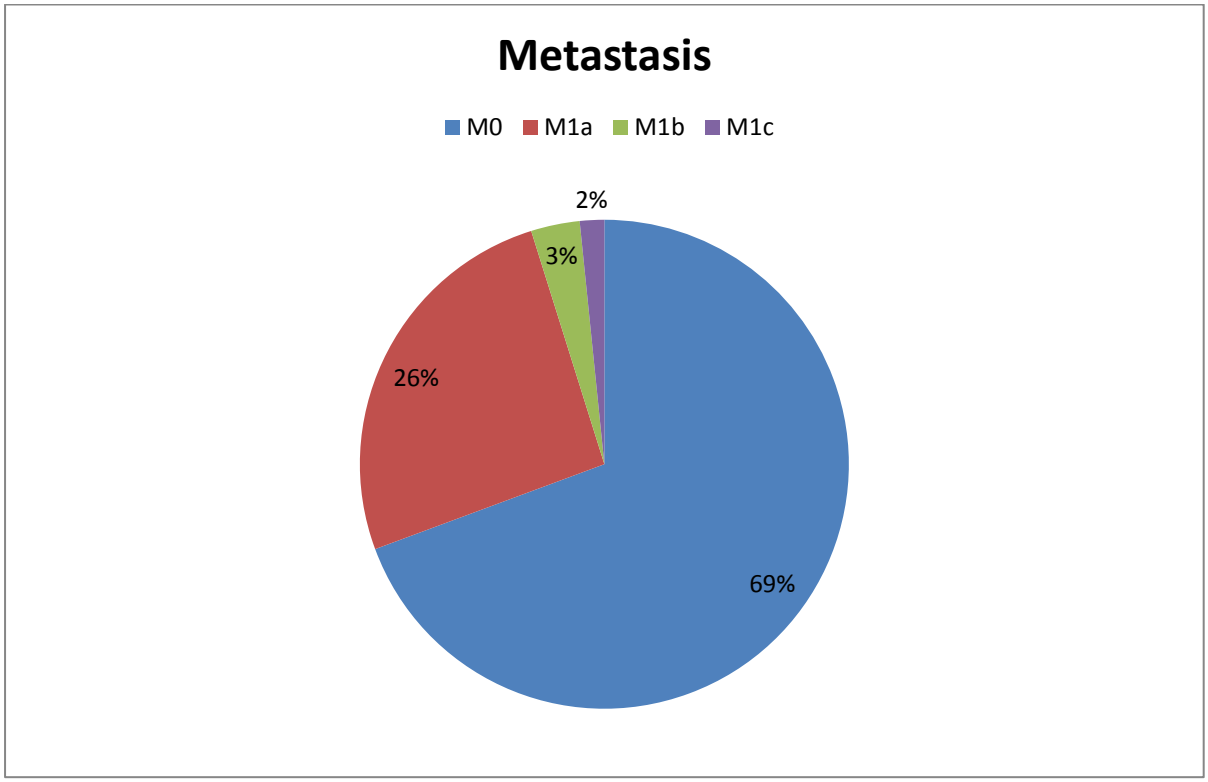


Table 14:- Distribution of subjects according to CD68 Expression

CD68 Score	Frequency	Percentage (%)
Score 1(0-30)	4	6.5
Score 2(31-60)	22	35.5
Score 3(>60)	36	58.0
Total	62	100.0

In this study 58.1% (36) of cases were expressing the CD68 in > 60 macrophages with score 3, 35.5%(22) followed by Score 2 and 6.5% (4) with Score 1.

Figure 28:- Graph showing Distribution of subjects according to CD68 Expression.

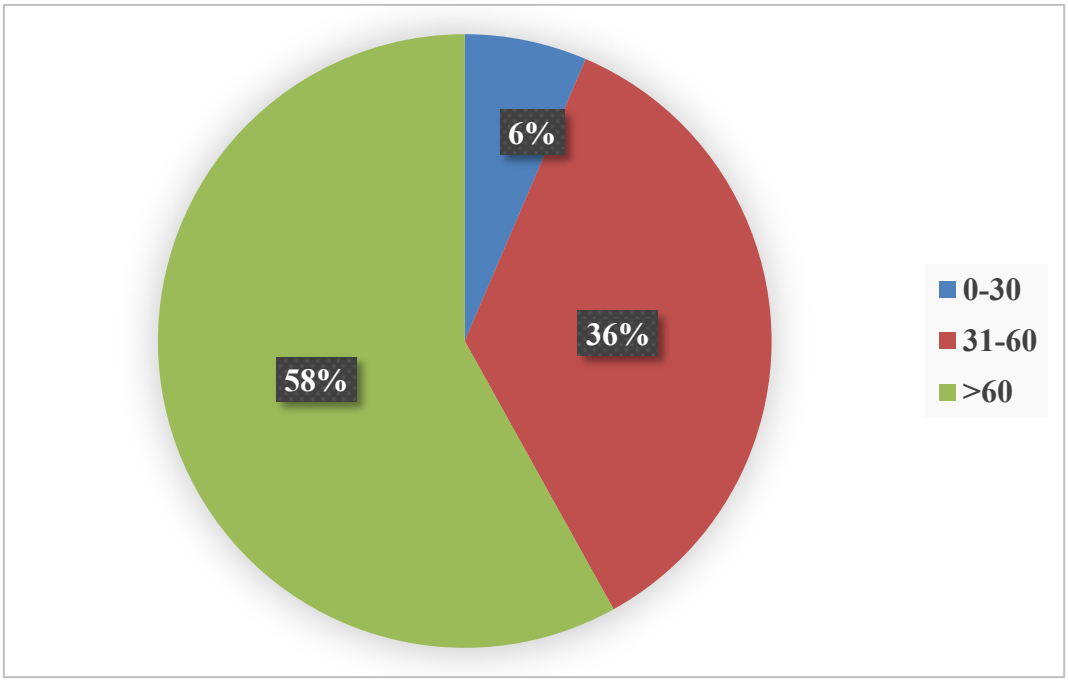


Table 15:- Distribution of subjects according to CD 163 expression

CD 163 Score	Frequency	Percent
Score 1(0-30)	12	19.4
Score 2(31-60)	12	19.4
Score 3(>60)	38	61.2
Total	62	100.0

In the present study 61.3%(38) of the patients expressed CD 163 macrophages >60 cells with Score 3, 19.4%(12) cases with Score 2 and 1 respectively.

Analyzing both CD68 and CD 163 expression more number of macrophage were expressed as score 3. Many studies scrutinized that expression of CD 68 has good prognosis and expression of CD 68 has poor prognosis with lymph node metastasis and distant metastasis.

Figure 29:- Graph showing Distribution of subjects according to CD 163 expression

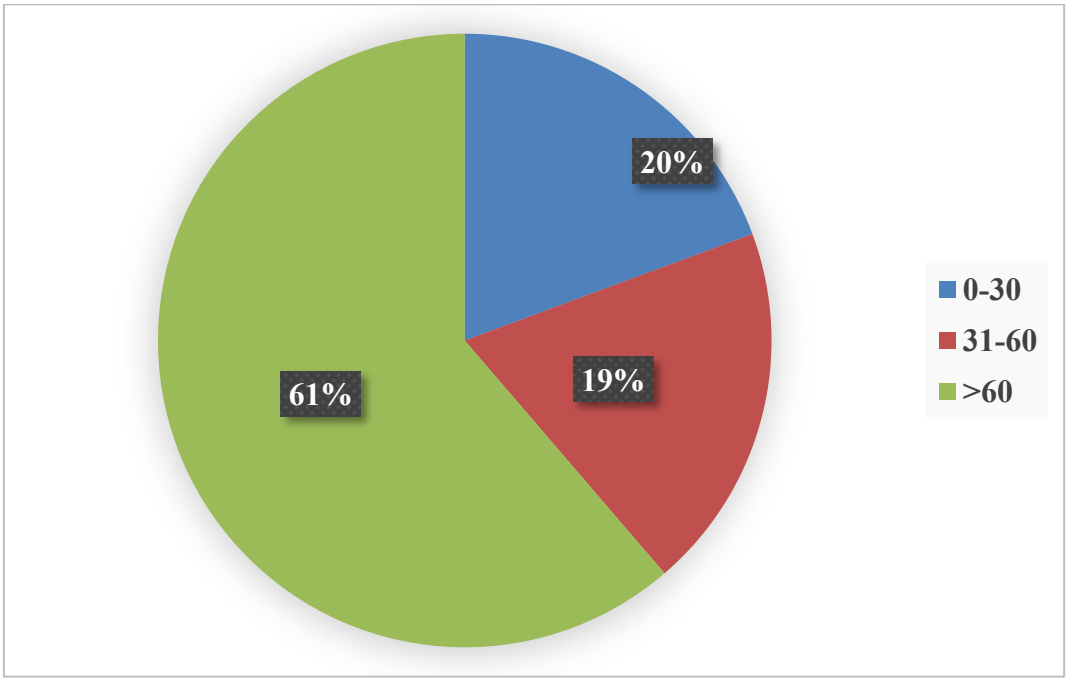


Table 16:- Distribution of subjects according to Gleason's Score and TUMOR SIZE

Gleason's Score	TUMOR SIZE		
	1-3cms	**3-6cms**	**>6cm**
6	4	4	1
	44.44%	44.44%	11.12%
7	2	12	1
	13.3%	80.0%	6.7%
8	0	3	3
	.0%	50.0%	50.0%
9	0	10	11
	.0%	47.6%	52.4%
10	0	3	8
	.0%	27.3%	72.7%

p value of 0.001, showed statistically significant difference between tumor size and Gleason's Score. In the present study we can see that 80% (12 cases) with Gleason's score 7 have tumor size of 3-6 cms, followed by 47.6%(10 cases) and 52.4%(11 cases) with tumor size of 3-6 and >6 cms respectively of Gleason's score 9 and 72.7% (8 cases) with Gleason's score 10. So, this study shows Gleason's score is directly proportional to tumor size.

Figure 30:- Distribution of subjects according to Gleason's Score and TUMOR SIZE.

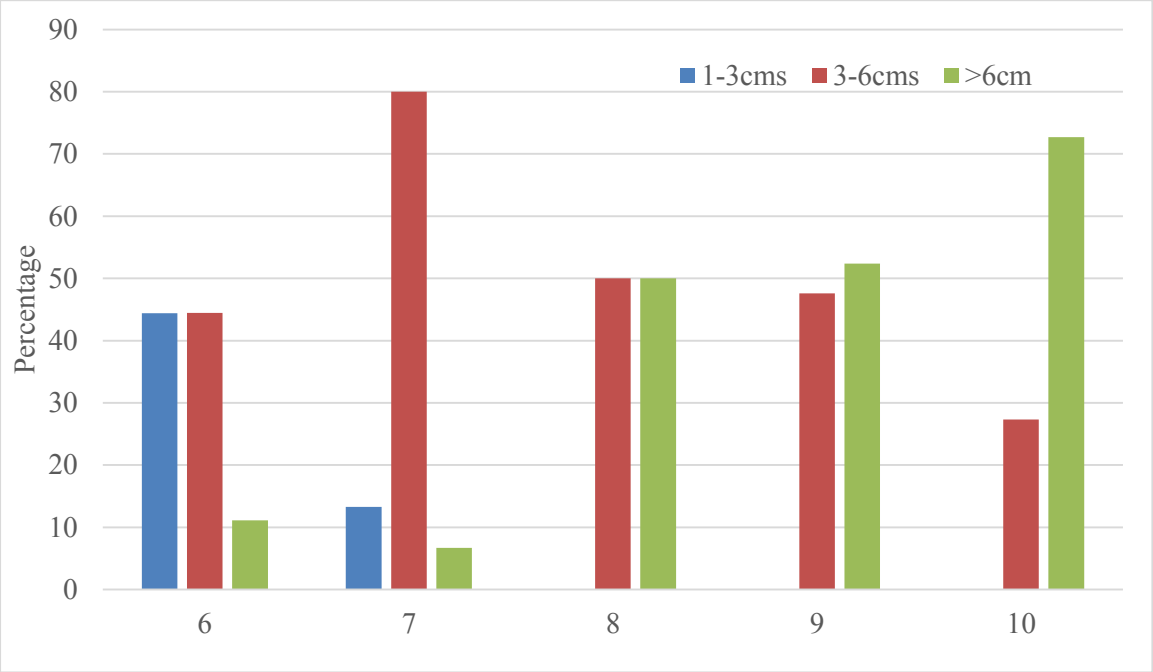


Table 17:- Distribution of subjects according to PSA levels and TUMOR SIZE

PSA levels	TUMOR SIZE		
	1-3cms	3-6cms	>6cm
4-19	4	6	0
	40.0%	60.0%	.0%
20-39	1	8	4
	7.7%	61.5%	30.8%
40-59	1	8	4
	7.7%	61.5%	30.8%
60-79	0	9	5
	.0%	64.3%	35.7%
80-99	0	1	6
	.0%	14.3%	85.7%
≥100	0	0	5
	.0%	.0%	100.0%

p value < 0.001, showed statistically significant difference between tumor size and PSA levels. By the above table we conclude that PSA level is directly proportional to tumor size

Figure 31- Distribution of subjects according to PSA levels and TUMOR SIZE.

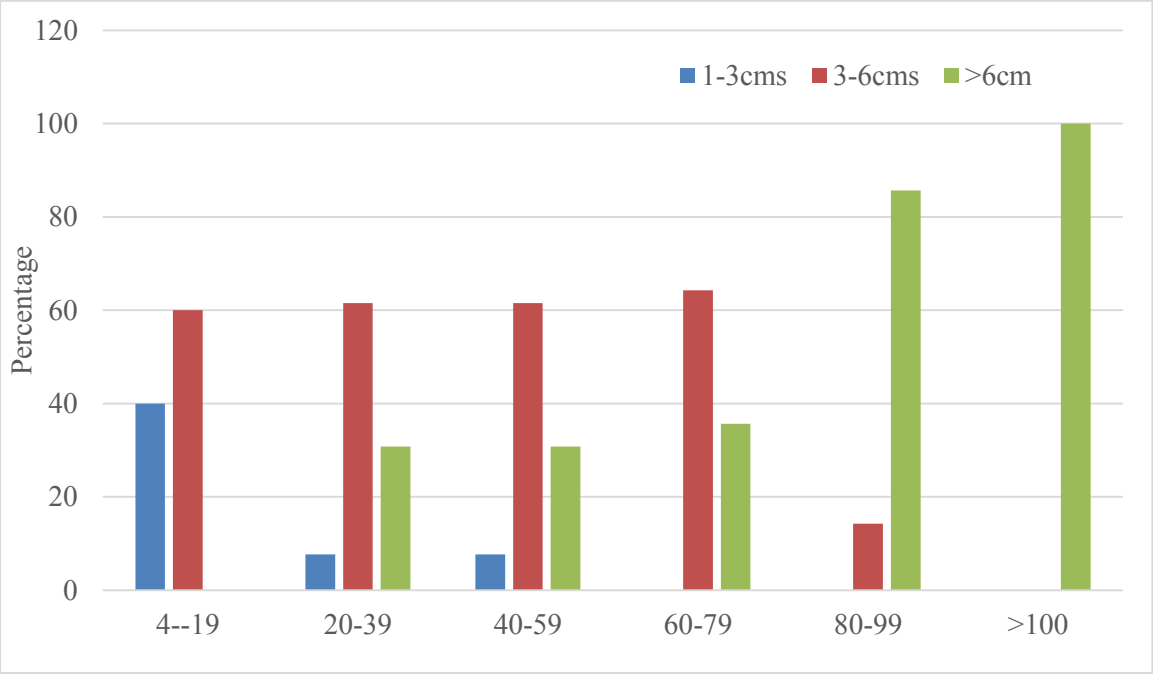


Table 18:- Distribution of subjects according to Gleasons Score and PSA levels

Gleasons Score	PSA					
	4-19	20-39	40-59	60-79	80-99	≥100
6	6	0	2	0	0	1
	66.7%	.0%	22.2%	.0%	.0%	11.1%
7	4	8	2	1	0	0
	26.7%	53.3%	13.3%	6.7%	.0%	.0%
8	0	2	2	1	0	1
	.0%	33.3%	33.3%	16.7%	.0%	16.7%
9	0	2	5	9	4	1
	.0%	9.5%	23.8%	42.9%	19.0%	4.8%
10	0	1	2	3	3	2
	.0%	9.1%	18.2%	27.3%	27.3%	18.2%

p value of 0.003, showed statistically significant difference between Gleason's Score and PSA levels. Above table show that Gleason's score is directly proportional to PSA levels.

Figure 32:- Graph showing Distribution of subjects according to Gleason's Score and PSA levels

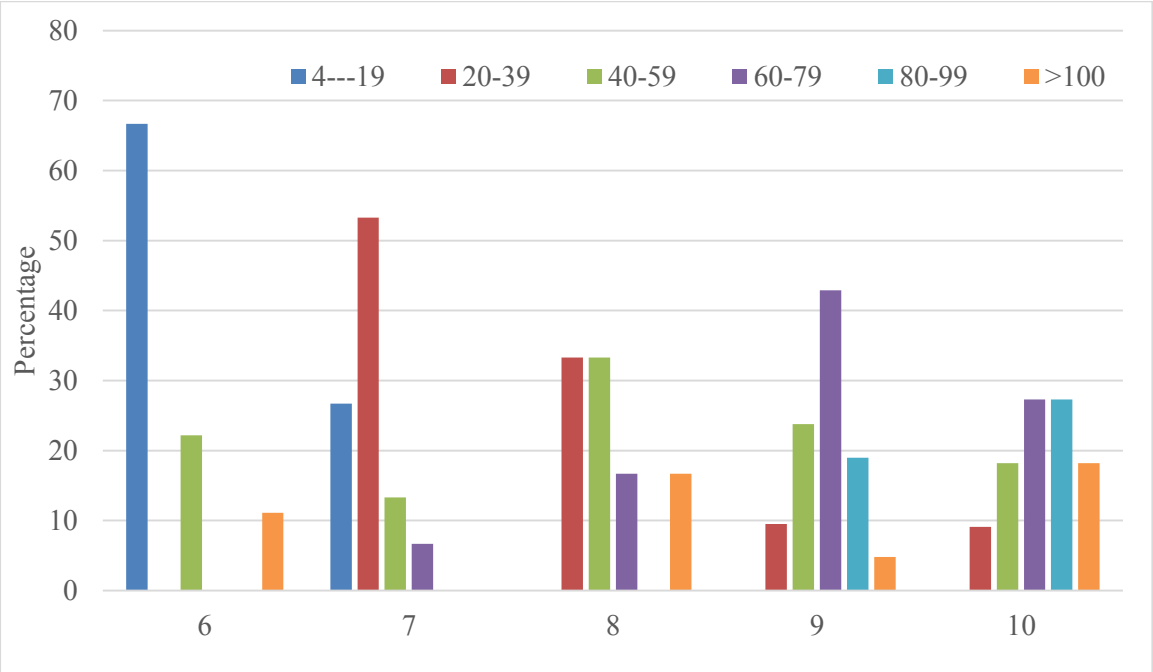


Table 19:- Distribution of subjects according to Gleason's Score and CD68 Expression

Gleason's Score	CD68 expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
6	1	6	2
	11.1%	66.7%	22.2%
7	1	4	10
	6.7%	26.7%	66.7%
8	1	1	4
	16.7%	16.7%	66.7%
9	1	8	12
	4.8%	38.1%	57.1%
10	0	3	8
	.0%	27.3%	72.7%

P value >0.005, showed statistically significant difference between Gleason's Score and CD 68. Here we can see that as the Gleason's score increases there is increase in the expression of CD68. Studies show that if the expression of CD68 is more than the prognosis is good with less lymph node metastasis.[40]

Figure 33:- Graph showing Distribution of subjects according to Gleason's Score and CD68 expression.

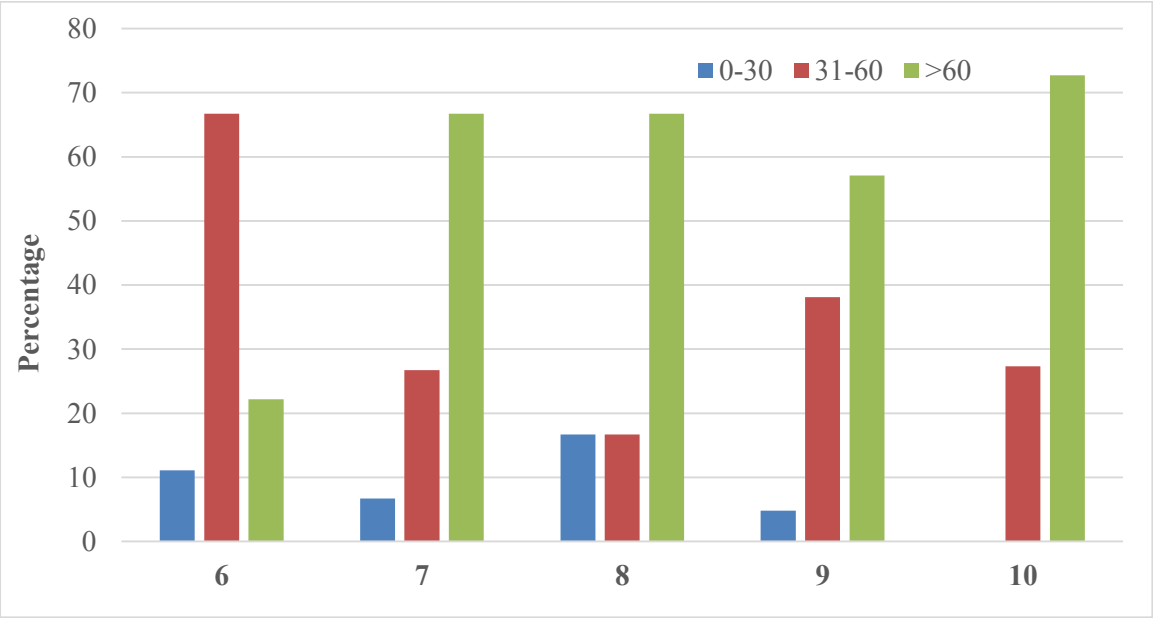


Table 20:- Distribution of subjects according to PSA levels and CD68 expression

PSA levels(ng/ml)	CD68 expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
4-19	1	5	4
	10.0%	50.0%	40.0%
20-39	2	4	7
	15.4%	30.8%	53.8%
40-59	1	2	10
	7.7%	15.4%	76.9%
60-79	0	5	9
	.0%	35.7%	64.3%
80-99	0	5	2
	.0%	71.4%	28.6%
≥100	0	1	4
	.0%	20.0%	80.0%

p value >0.005, showed no statistically significant difference between CD 68 expression and PSA levels. This table shows that more number of cases were between the PSA levels of 20-80 ng/ml with more number of cases seen with Score 2 and score 3

Figure 34:- Graph showing Distribution of subjects according to PSA levels and CD68 expression

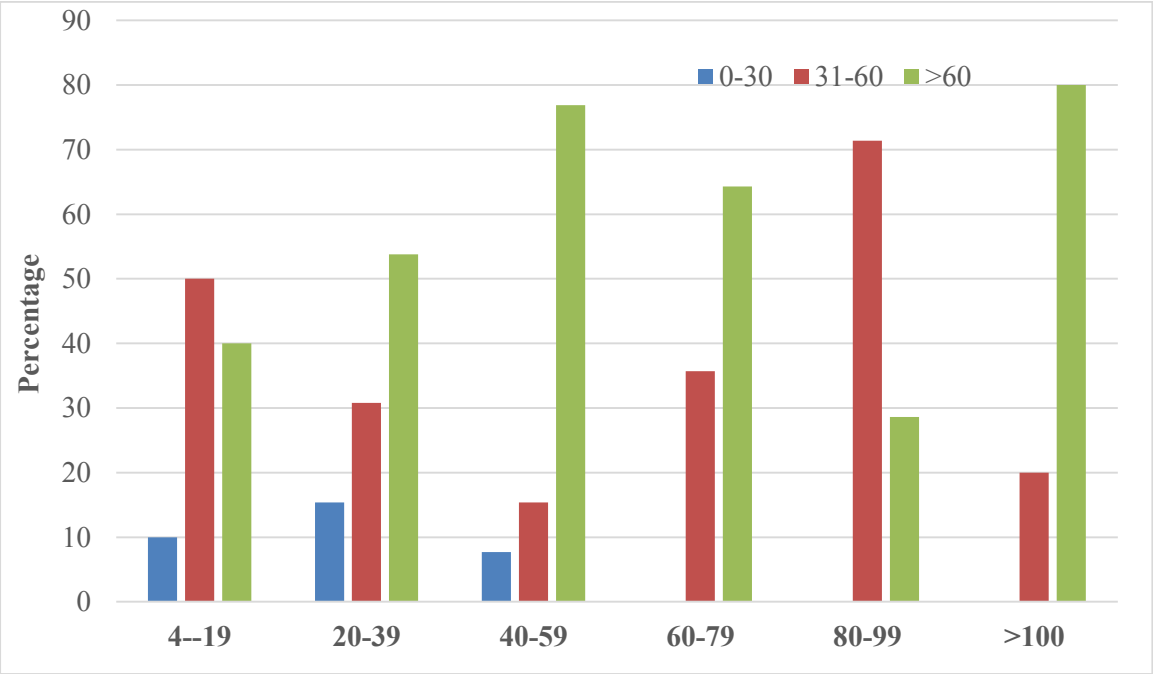


Table 21:- Distribution of subjects according to Tumor size and CD68 expression

Tumor size	CD68 expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
1-3cms	0	3	3
	.0%	50.0%	50.0%
3-6cms	3	11	18
	9.4%	34.4%	56.3%
>6cms	1	8	15
	4.2%	33.3%	62.5%

p value of 0.814, showed statistically significant difference between Tumor size and CD 68 expression. In this table maximum number of cases were of tumor size 3-6 with score 2, in which 34.4 %(11 cases) and 56.3%(18 cases) with score 3. Tumor size of >6 cms Score 2 in which 33.3% (8 cases) and 62.5% (15) with score 3.

Figure 35:- Graph showing Distribution of subjects according to Tumor size and CD68 expression

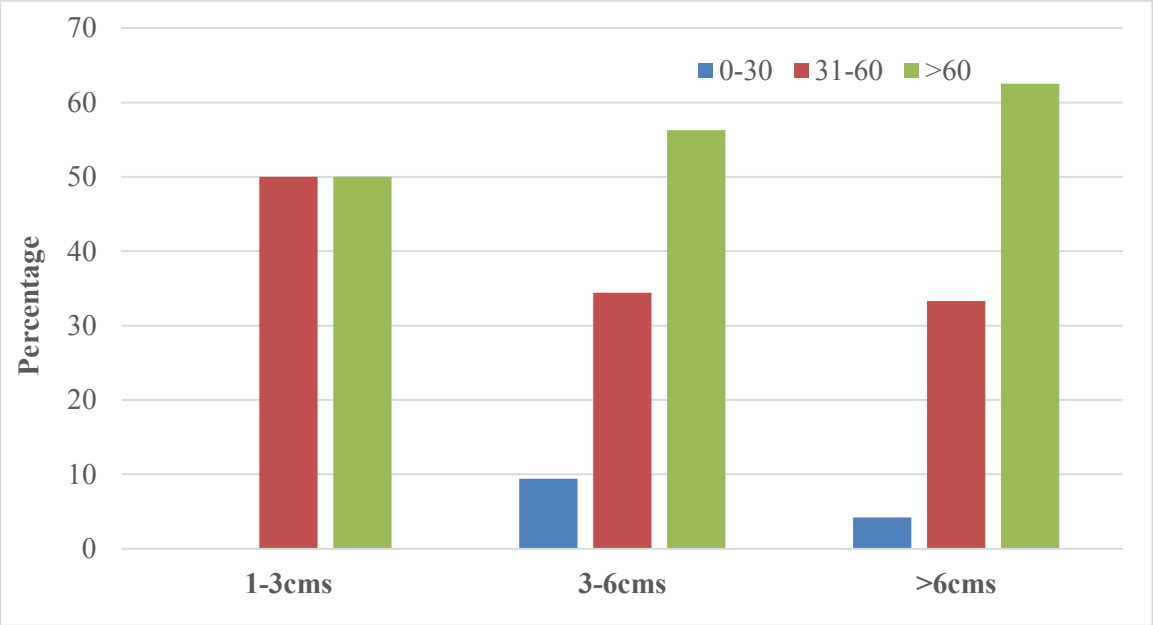


Table 22:- Distribution of subjects according to staging and CD68 expression

Clinical staging	CD68 expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
T2	3	7	8
	16.7%	38.9%	44.4%
T3	1	10	14
	4.0%	40.0%	56.0%
T4	0	5	14
	.0%	26.3%	73.7%

P value of 0.170, showed statistically significant difference between clinical staging and CD 68e expression. Maximum number of CD 68 were expressed in T3 and T4 with Score 2 being 40%(10 cases)and 26.3 % (5 cases) respectively and Score 3 being 56%(14 cases) and 73.7% (14 cases) respectively.

Figure 36:- Graph showing Distribution of subjects according to staging and CD68 expression

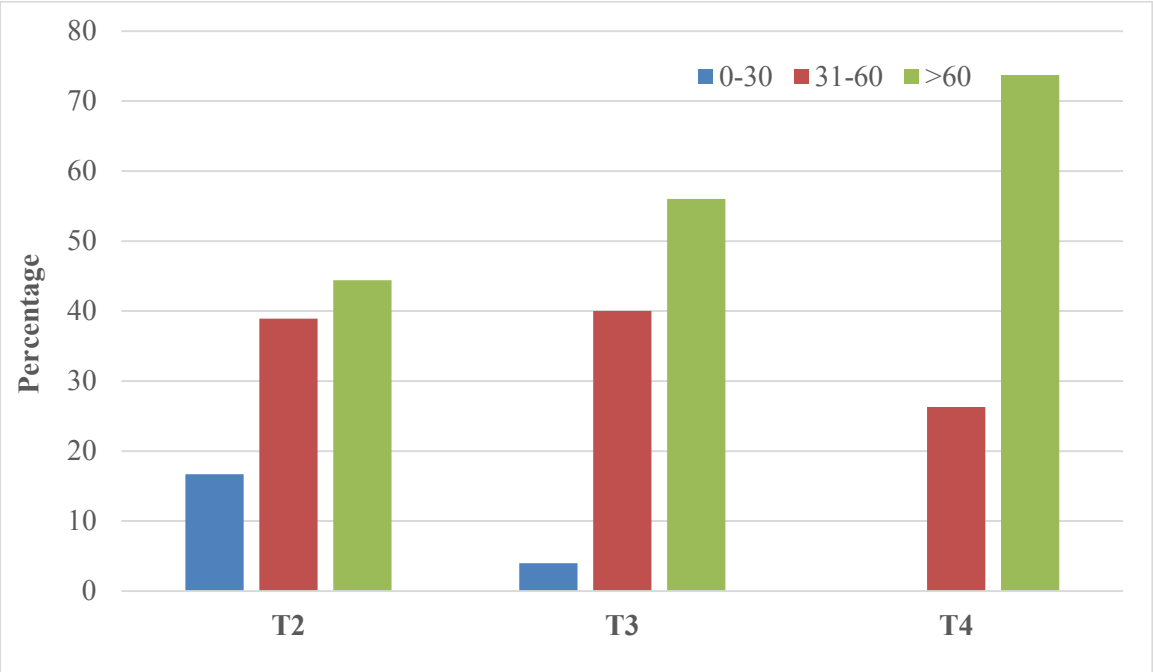


Table 23 : Association between N Stage and CD 68 expression.

N stage	CD 68 expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
N0	02 (11.1%)	02 (11.1%)	14(77.7%)
N1	35 (79.5%)	06 (13.6%)	03 (6.8%)

In the study there was no significant association between N Stage and CD 68 expression with P value of 0.137. Lymph node metastasis was seen in total of 44 cases of which 79.5%(35) cases having score 1, 13.6%(6) having score 2, 6.8% (3) with score 3. The present study concludes that as CD 68 expression Score increases there was no lymph node metastasis and patient has better prognosis.

Figure 37 : Association between N Stage and CD 68 expression

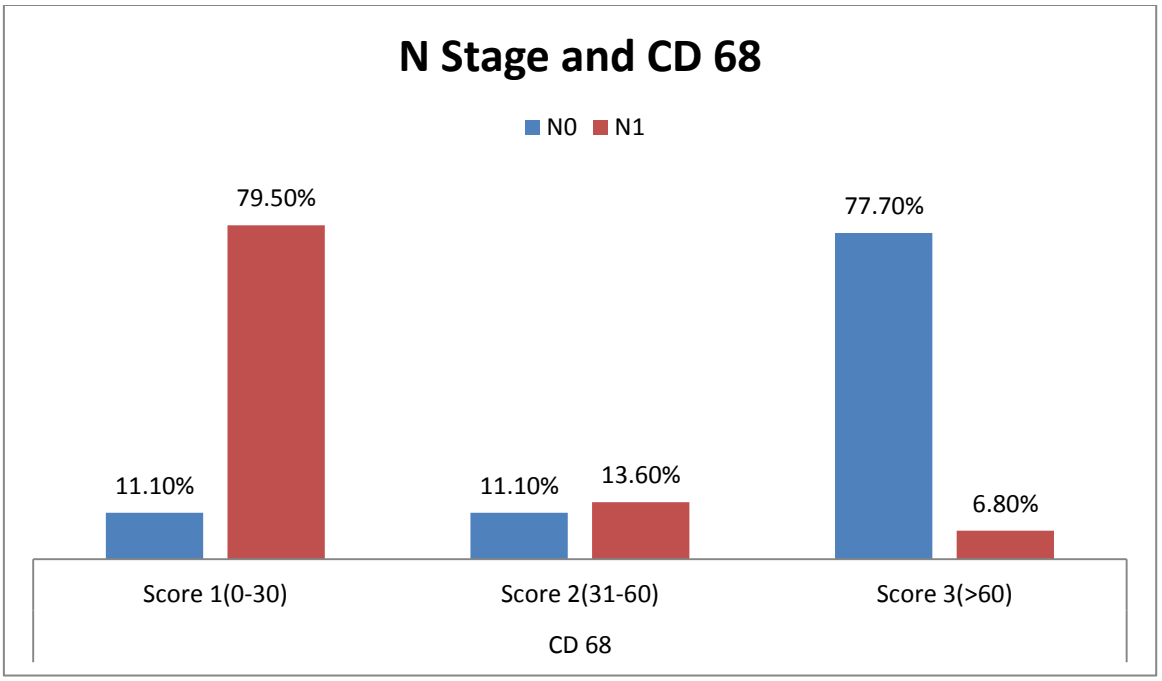


Table 24 : Association between M Stage and CD 68 expression.

Metastasis	CD 68 expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
M0	3 (6.9%)	05(11.6%)	35 (81.3%)
M1a	12 (75%)	02(12.5%)	01(6.2%)
M1b	02 (100%)	00	00
M1c	02 (100%)	00	00

In the study there was no significant association between M stage and CD 68 expression with P value of 0.102. In the present study 44 cases shows no distant metastasis with 81.3% (35) cases having score 3 in M0 . Distant metastasis was seen in 18 cases in which 75% (12) cases seen in M1a stage with score 1. This study concludes that as the metastasis increases the expression of CD 68 decreases and CD 68 macrophages have no role in metastasis and provides protection against cancer spread.

Figure 38 : Association between M Stage and CD 68 expression.

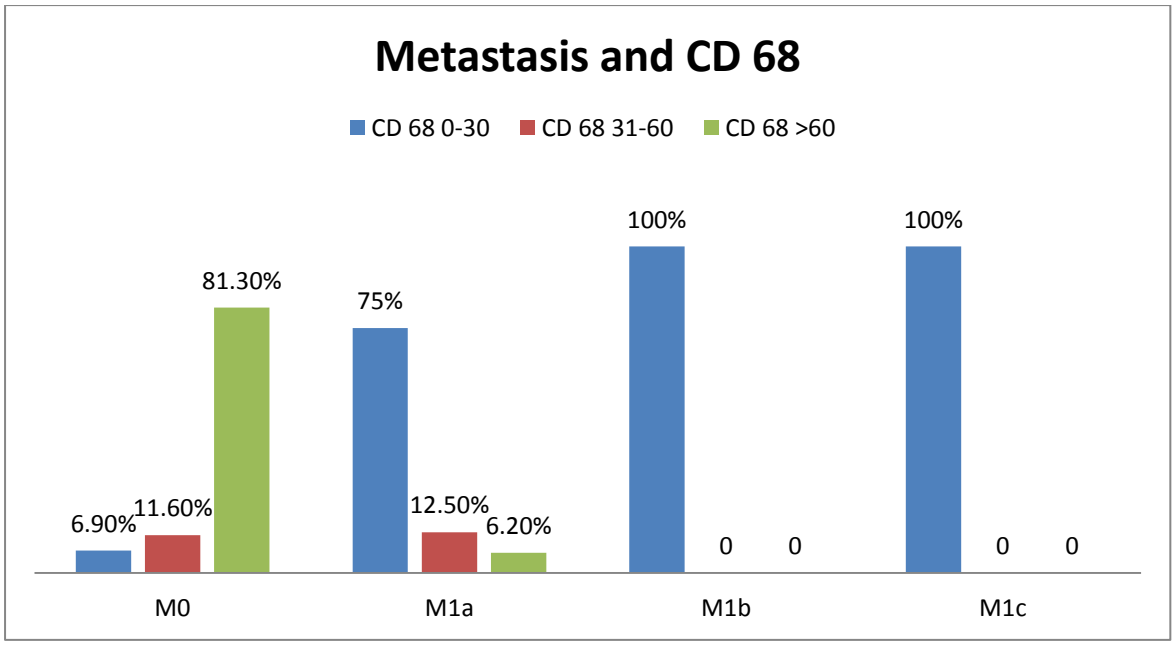


Table 25:- Distribution of subjects according to Gleason's Score and CD163 expression.

Gleason's Score	CD 163 Expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
6	7	1	1
	77.8%	11.1%	11.1%
7	4	7	4
	26.7%	46.7%	26.7%
8	0	0	6
	.0%	.0%	100.0%
9	1	4	16
	4.8%	19.0%	76.2%
10	0	0	11
	.0%	.0%	100.0%

p value of 0.001,showed statistically significant difference between CD 163 expression and Gleason's Score. Here we can see that the Gleason's score is directly proportional to the expression of CD163. Studies show that if the expression of CD163 is increased than the prognosis is bad with lymph node and distant metastasis.[40]

Figure 39:- Graph showing Distribution of subjects according to Gleason's Score and CD163 expression

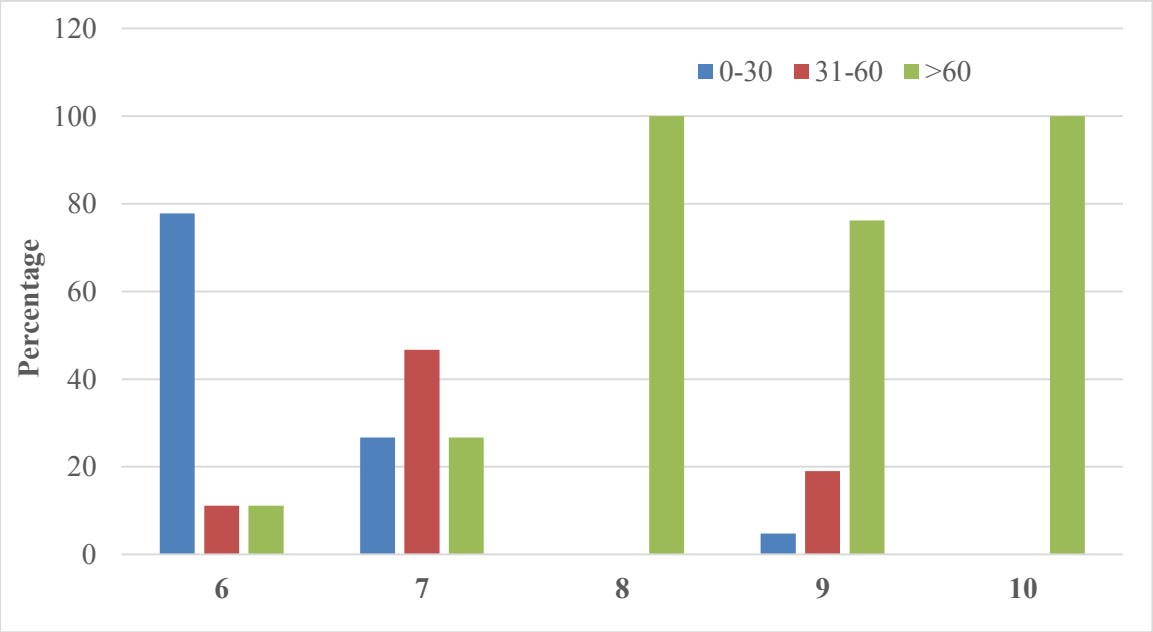


Table 26:- Distribution of subjects according to PSA levels and CD163 expression

PSA levels	CD163 Expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
4-19	6	4	0
	60.0%	40.0%	.0%
20-39	2	3	8
	15.4%	23.1%	61.5%
40-59	4	3	6
	30.8%	23.1%	46.2%
60-79	0	1	13
	.0%	7.1%	92.9%
80-99	0	1	6
	.0%	14.3%	85.7%
≥100	0	0	5
	.0%	.0%	100.0%

p value of 0.001, showed statistically significant difference between CD 163 and PSA levels. This table shows that maximum number of cases were between the PSA levels of 20-80 ng/ml with more number of cases seen with Score 2 and score 3

Figure 40:- Graph showing Distribution of subjects according to PSA levels and CD163 expression

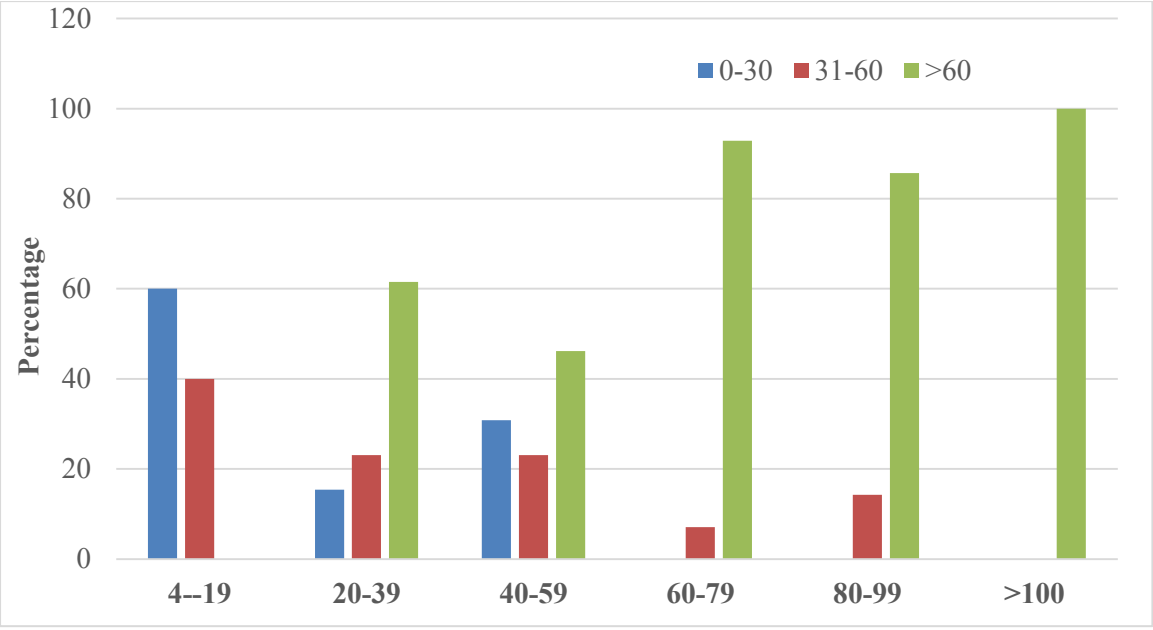


Table 27:- Distribution of subjects according to Tumor size and CD163 expression

Tumor size	CD163 expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
1-3cms	3	3	0
	50.0%	50.0%	.0%
3-6cms	9	7	16
	28.1%	21.9%	50.0%
>6cms	0	2	22
	.0%	8.3%	91.7%

P value of 0.001 showed statistically significant difference found between Tumor size and CD 163. In this table maximum number of cases were of tumor size 3-6, 28.1% (9) cases with score 1, 21.9%(7) cases with score 2 and 50% (16) cases with score 3, followed by tumor size >6 cms having 91.7% (22) cases with score 3. This study concludes that as the tumor size increases the CD 163 expression increases and has poor prognosis.

Figure 41:- Graph showing Distribution of subjects according to Tumor size and CD163 expression

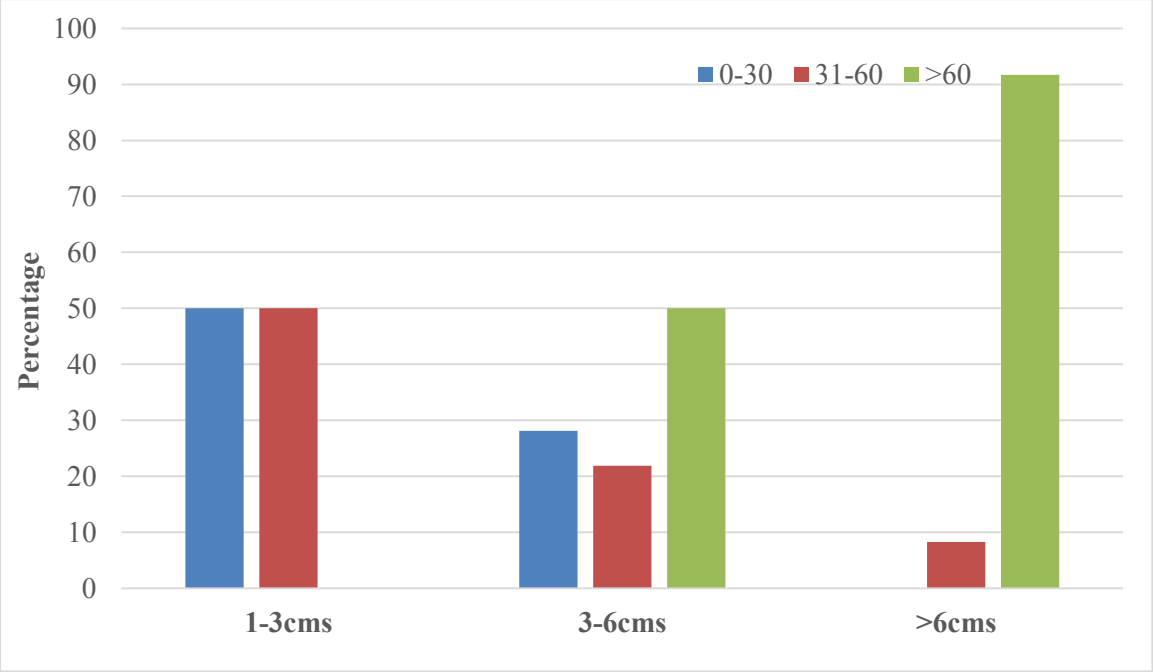


Table 28:- Distribution of subjects according to tumor staging and CD163 expression

Clinical staging	CD163 expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
T2	8	6	4
	44.4%	33.3%	22.2%
T3	3	4	18
	12.0%	16.0%	72.0%
T4	1	2	16
	5.3%	10.5%	84.2%

p value 0.001, showed statistically significant difference between tumor staging and CD 163. In the present study majority of the cases seen in T3 stage with 72% (18 cases) expressing CD163 score 3 and 84.2% (16) cases seen in T4 stage with score 3 concluding the tumor stage is directly proportional to the CD163 expression, showing the association of CD163 expression cases having poor prognosis.

Figure 42:- Graph showing Distribution of subjects according to tumor staging and CD163 expression

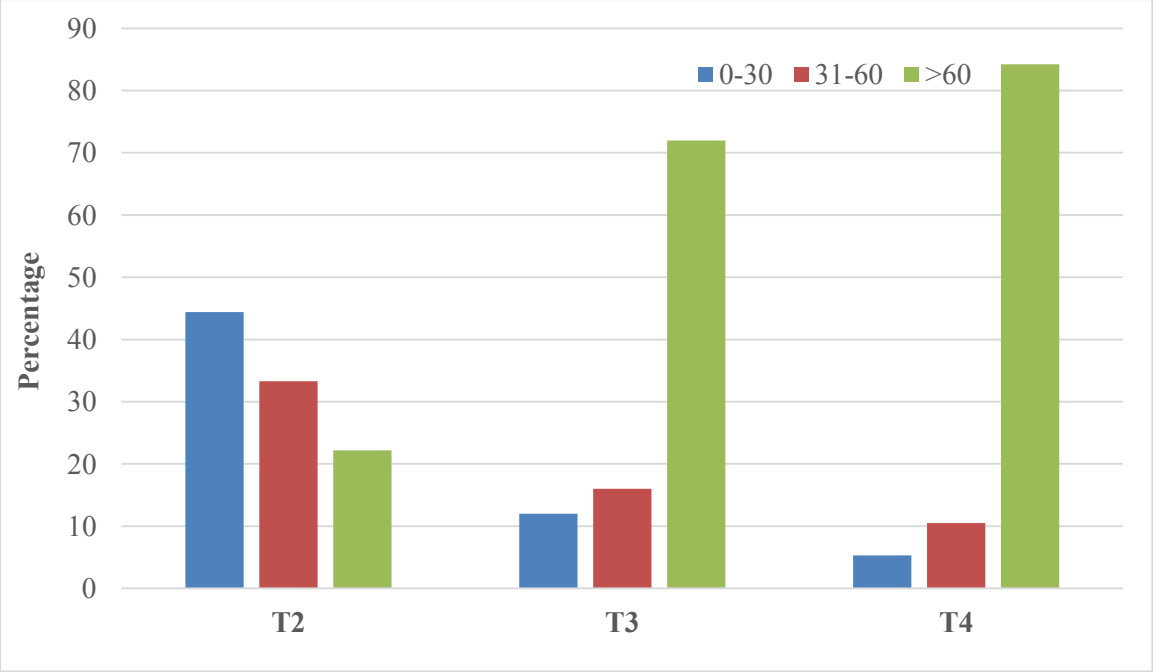


Table 28: Association between N Stage and CD 163 expression.

N stage	CD 163 Expression		
	Score 1(0-30)	Score 2(31-60)	Score 3(>60)
N0	15 (83.3%)	01 (5.5%)	02(11.1%)
N1	02 (4.5%)	04(9%)	38 (86.3%)

In the study, significant association between N Staging and CD 163 expression was found with p value 0.004* . Lymph node metastasis was seen in total of 44 cases of which 86.3%(38) cases having score 3, 9%(4) having score 2 and 4.5% (2) of cases with score 1. Hence, this study concludes that as CD 163 expression Score increases there was more number of cases with lymph node metastasis and patient has poor prognosis.

Figure 43: Association between N Stage and CD 163 expression.

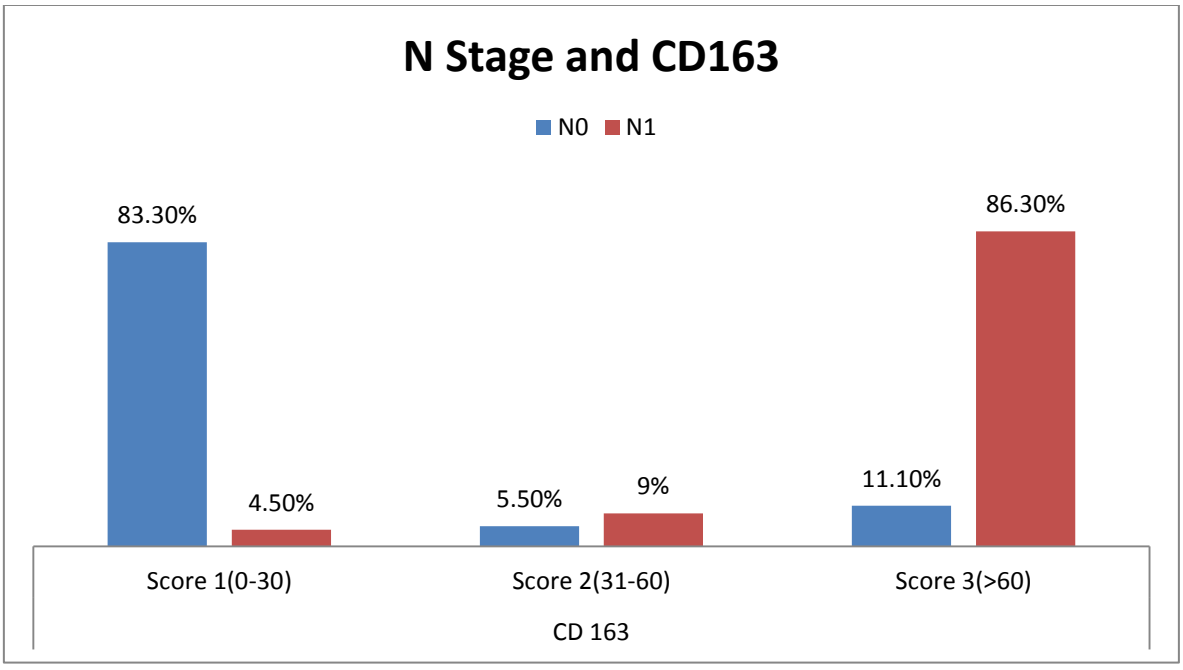
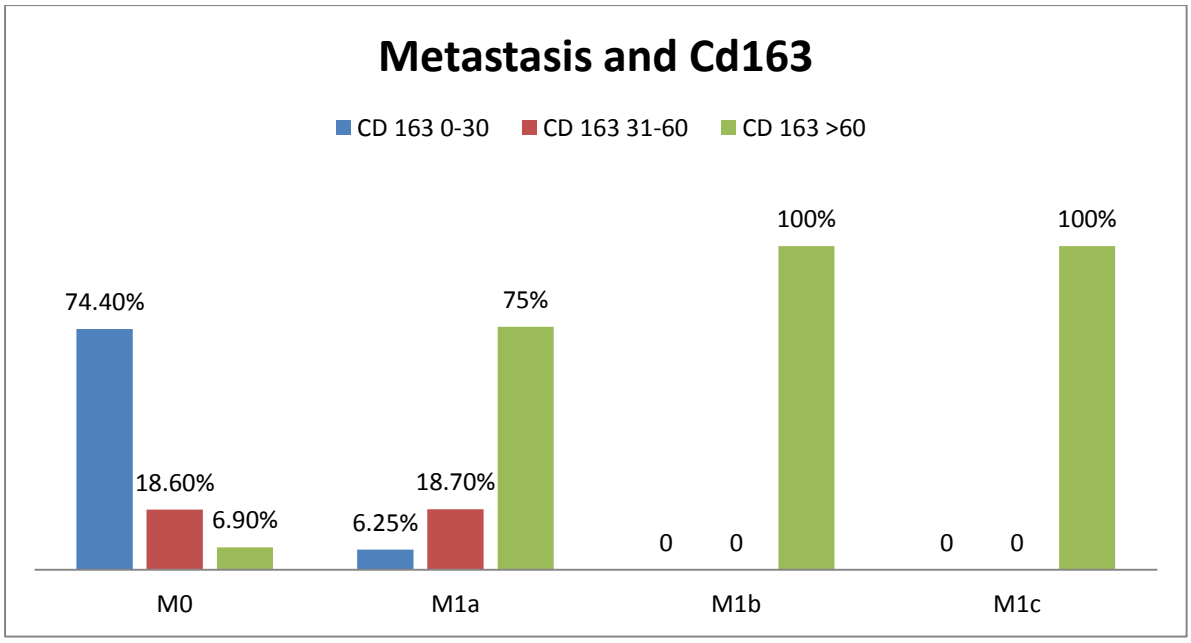


Table 29: Association between M Stage and CD 163 expression

Metastasis	CD 163 Expression		
	0-30	**31-60**	**>60**
M0	32 (74.4%)	08 (18.6%)	03 (6.9%)
M1a	01(6.25%)	03(18.7%)	12 (75%)
M1b	0	0	02 (100%)
M1c	0	0	01 (100%)

In the present study 44 cases had no distant metastasis with 74.4% (32) cases having score 1, 18.6% (8) cases having score 2 and 6.9% (3) cases having score 3 in M0 stage . Distant metastasis was seen in 18 cases of which 75% (12) cases seen in M1a stage with score 3. So, this study concludes that as the metastasis increases the expression of CD 163 increases and CD 163 macrophages have a role in metastasis and increases cancer spread with poor prognosis.

Figure 44: Association between M Stage and CD 163 expression



DISCUSSION

DISCUSSION:

Chronic inflammation of prostate is associated with high risk of cancer.[33] Inflammation comprises of leukocytes, vascular endothelial cells , fibroblasts , Tumor associated macrophages(TAMs) like M1 & M2 which are 2 main phenotypes of macrophages which forms tumor microenvironment.[37,38] M1 macrophage is activated by IFN-gamma (Interferon-gamma) which secrete cytokines IL-12(Interleukin) & TNF-alpha (Tumor Necrosis Factor-Alpha which support anti-tumor activity.[38] M2 macrophage is activated by IL-4, IL10, IL 13 which secrete anti-inflammatory cytokines like- TGF-beta(Tumor Growth Factor- Beta), IL-10, angiopoietin & VEGF (Vascular Endothelial growth Factor) which promote tumor growth.[39] The macrophage marker CD68 is expressed in M1 and plays the role of pro-inflammatory and anti-tumor response.[40] CD163 is expressed mainly by M2 macrophages, in an anti-inflammatory environment and is highly specific macrophage marker for M2 macrophages.[40] Studies have confirmed that TAM, mainly M2 are associated with poor prognosis of human cancer such as hepatocellular cancer, gastric cancer, lung cancer & breast cancer[41] Studies show that in prostate tumor microenvironment immune cells act as a double-edged sword. At different prostate carcinoma stages, tumor-associated macrophages like M1, M2 may involve in initiation and progression of carcinoma prostate, by interacting with tumor cells or by secreting cytokines.[42] Study shows that M2 macrophages & regulatory T-cells Infiltration in Prostatic Carcinoma patients contribute tumor progression. So possibility of these inflammatory cells creating an immunosuppressive environment is taken into consideration.[43,44] Many studies show that TAMs mainly M2 phenotype aggregation in tumors correlates with a poor clinical outcome.[43,45,46,47] Few studies show that targeted blockade of interleukin-6 receptor(IL-6R) & high mobility protein-1(HMGB-1) resulted in improvement of enzalutamide therapeutic effect in carcinoma prostate.[45] Therefore, TAMs are an appealing target for therapeutic intervention. The specific macrophage subtypes

present in a prostatic adenocarcinoma have prognostic value, suggesting that the relative proportions of these populations are related to patient outcome. Understanding the relative contributions of these subtypes will not only inform patient prognostication, but will help in immunotherapeutic strategies [48,49,50,]

Table: 30 Comparison of age distribution with other studies

Age	Lancotti.M et al[50]	Erlandsson. A etal[40]	Present Study
Median age group	67 yrs	74. 8 yrs	70 yrs

In the study the median age group of subjects were 70 yrs which was similar to the study done by Lancotti. M et al. and Erlandsson. A etal.[50,40]

Clinical symptoms: In the study Most common clinical symptom was urinary retention with 79.03% (49) cases shown in Table 5 and Figure 19 which is in contrast with study done by Kitagawa Y etal. which showed 22.2 % of patients with urinary retention and Hamilton W etal. showed 33.3% with urinary retention and 47% of patients came with increased urinary frequency .[51,52]

Table 31: Comparison of Gleason's score with other studies.

Gleason's Score	Lancotti.M et al[50]	Erlandsson. A etal[40]	Present study
6	30 (32.2)	45 (20.0)	9 (14.5%)
7	40 (43)	44 (19.6)	15 (24.2%)
8-10	23 (24.8)	100 (44.4)	38(62%)

Gleason's Score: In the present study maximum number of patients have high Gleason's score of 8-10, 62% (38 cases), followed by Gleason's score 7, 24.2%(15 cases) as shown in the Table 6 and Figure 20. which is in disconcordant with the studies done by Lancotti. M et al. and Erlandsson. A etal.[50,40] This difference can be because of early presentation of the patient and good screening facilities in the western countries.

PSA Levels: In the present study 64% (40 cases) were showing PSA levels between 20-79 ng/ml which is similar to study done by Lovely Jose etal[53] with 66.5% (40) cases and Zivkovic etal.[54] 52.5% cases showing PSA levels >20 ng/ml. Any PSA levels above 20 ng/ml above 70 yrs should be suspected of prostate carcinoma and should undergo thorough screening.

In the present study there was significant correlation of PSA levels with tumor size with p value <0.0001 as shown in Table 8 and Figure 22 which is similar to study done by Gustavo F etal. Kavita Kumari et al.[55,56] with statistical significance of <0.004 and < 0.021 respectively.

Tumor Size:

In the present study we can see that 51.6%(32) of cases were having tumor size of 3-6cm, 38.7% (24 cases) of >6 cm of tumor size and 9.7% (6 cases) of tumor size 1-3 cms at the time of diagnosis by MRI as shown in Table 10, Figure 24. When Tumor size was analyzed with PSA levels and Gleason's score we can see that 80% (12 cases) with Gleason's score 7 have tumor size of 3-6 cms, followed by 47.6%(10 cases) and 52.4%(11 cases) with tumor size of 3-6 and >6 cms respectively of Gleason's score 9 and 72.7% (8 cases) with Gleason's score 10 as shown in Table 16,17 Figure 30,31. So, this study shows as Gleason's score increases the tumor size increases, which is similar to study done by C. Gaffney et al in which most of the cases were showing tumor size of >5 cm in 45% (101 cases) with Gleason's score 8,9,10

and Gleason's Grade Group 3, 4 and 5.[57] Present study also concludes that tumor size cannot be a single useful parameter as predictor of histopathology and genomic aggression , but including tumor size as a useful additional parameter of genomic risk prediction with other pathologic and clinical measurements can be considered.

PSA levels and Gleason's score: In the present study there was significant correlation of PSA levels with Gleason's score with p value <0.0003 as shown in Table 18 Figure 32 . Which is similar to study done by Lovely Jose etal. With significant statistical difference of <0.031. [53]

Table 32: Comparison of Gleason's score and TNM stage with CD 68 Macrophage prevalence score 3 >60 cells with other studies.

	CD68 expression (Present study) >60 (Score-3)	**Lanciotti etal.[50] M1 prevalence**
Gleason's Score		
6	12 (19.3)	19 (55.9)
7		12 (35.3)
8-10	24 (38.7%)	3 (8.8)
TNM staging		
T2	8(12.9%)	19 (55.9)
T3	14(22.5%)	7 (20.6)
T4	14(22.5%)	8 (23.5)
N1	03 (6.8%)	2 (5.9%)
M1	01(6.2%)	2 (5.9%)

Many studies have concluded that CD 68 expression has better prognosis with less extra capsular invasion, lymph node metastasis and distant metastasis. In the present study we analyzed CD 68 expression score 3 association with Gleason's score, TNM Staging. We saw that as the Gleason's score increased the CD 68 expression in the tumor cells increased, when this was compared to TNM stage number of cases was 14 (22.5%) in T3 and T4 but lymph node metastasis and distant metastasis was seen in 3 (6.8%) and 01 (6.2) respectively. So we concluded that as the CD68 expression increases there was less lymph node and distant metastasis providing the protection against cancer spread, which was similar to study done by Lancotti et al.[50] Where they have come to the inference that M1 or CD 68 expression was having better prognosis without extra capsular invasion. In this study 31(91%) cases were found to be of Gleason's score 6 and 7 In contrast with the present study, This can be because of early screening facilities in the western countries.

Table 33: Comparison of Gleason's score and TNM stage with CD 163 Macrophage prevalence score 3 >60 cells with other studies

	CD163 expression (Present study) >60(Score-3)	**Lanciotti etal.[50] M1 prevalence**
Gleason's Score		
6	5 (8.06)	31 (52.6)
7		16 (27.1)
8-10	33 (53.2)	12 (20.3)
Tumor staging		
T2	4 (6.4%)	14 (23.8)
T3	18 (29.0%)	43 (47.4)
T4	16 (25.8%)	2 (3.4)
N1	38 (86.3%)	3 (5.1)
M1	15(25%)	9 (15.2)

Many studies have concluded that CD 163 expression has poor prognosis with extra capsular invasion, lymph node metastasis and distant metastasis. In the present study we analyzed CD 163 expression score 3 association with Gleason's score, TNM Staging. We saw that as the Gleason's score increased the CD 163 expression in the tumor cells increased, when this was compared to TNM stage number of cases was 18 (29%) in T3 and 16 (25.8%) in T4, but lymph node metastasis and distant metastasis was seen in 38 (86.3%) and 15 (25%) respectively. So we concluded that as the CD163 expression increases there was more number of lymph node and distant metastasis. CD163 acts as pro inflammatory cells and promotes cancer spread and If CD163 expression in the tumor cells are more than the patient have poor prognosis having lymph node metastasis, bone metastasis and distant metastasis.[58, 59] This is similar to the study done by Lancotti etal.[50] Which showed that expression of CD163 is associated with extra capsular invasion and lymph node and distant metastasis.

CD163 TAM with various mechanism in the tumor microenvironment promotes the proliferation and metastasis of prostate carcinoma and also involves in the regulation of neuroendocrine differentiation and androgen deprivation therapy resistance in prostate cancer.[58]

TAM mechanisms in the regulation of tumor proliferation and progression of prostate carcinoma is very complex and many theories has been proposed on this So, Further exploration of TAM mechanisms and immune checkpoints in the prostate tumor microenvironment can provide new light and idea for the treatment of prostate carcinoma.[60]

CONCLUSION

CONCLUSION:

This study concludes that both M1 and M2 macrophages expressed in prostatic adenocarcinoma was associated with high Gleason's score. CD 163 expression was expressed more in cases with lymph node and distant metastasis. This also showed statistical significance with the different parameters which were assessed. As tumor micro environment is considered as double edge sword and molecular changes at different stages of carcinoma cannot be simply assesed, more studies are needed to establish the results. To the best of our knowlegde , during review of literature did not reveal any Indian study on Tumor associated macrophages on prostatic carcinoma. This study is a maiden attempt to understand the pathogenesis of TAM on Pca along with standardization of the scoring system of CD 68 and CD 163 in order to assess the effect of targeted immunotherapy on long term prognosis of the patients.

SUMMARY

SUMMARY

1. The present study was conducted in the Department of Pathology , Sri Devaraj Urs Medical College, Tamaka, Kolar from December 2019 to October 2021. Also retrospective cases from January 2015 to November 2019 were included in the study.
2. A total of 62 cases were studied and majority of the patients were in-between age of 61-70 years.
3. Highest number of cases were in Gleason's score 8,9, 10 (62%), PSA levels 20-80ng/ml (64%), Tumor Size 3-6 cm (51.6%), T3 stage (40.3 %) , N1 lymph node stage (70.9%). M1 stage of (31%).
4. CD 68 and Cd163 expression was analyzed with Gleason's score, TNM stage and PSA levels. CD68 score 3 was associated with low nodal and distant metastasis 6.8% and 6.2% respectively.
5. CD163 Score 3 was associated with high nodal and distant metastasis of 86.3% and 25% respectively.
6. On further analysis, there was a statistically significant association between the CD 163 expression and Gleason's score, PSA levels, nodal and distant metastasis.
7. CD 68 expression was associated with better prognosis with less nodal and distant metastasis and Cd163 expression has poor outcome with increased chances of nodal and distant metastasis.
8. Further exploration of TAM mechanisms and immune checkpoints in the prostate tumor microenvironment can provide new light and idea for the treatment of prostate carcinoma.

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ANNEXURE

ANNEXURE-I

INFORMED CONSENT FORM

STUDY TITLE: Immunohistochemical Evaluation of Tumor Associated Macrophages (M1 & M2) In Carcinoma Prostate: An Institutional study.

I, _____have read or have been read to me the patient information sheet and understand the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information will be collected and disclosed during the study.

I have had my opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction.

I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature / thumb impression Date:

(subject) Place:

Name and signature / thumb impression Date:

Place:

(Witness/Parent/ Guardian/ Husband)

ANNEXURE-II

PATIENT PROFORMA SHEET

STUDY TITLE: ―Immunohistochemical Evaluation of Tumor Associated Macrophages (M1 & M2) In Carcinoma Prostate: An Institutional study"

PLACE OF STUDY: Sri Devaraj Urs Medical College attached to R.LJalappa Hospital and Research,Tamaka, Kolar.

To determine the expression of M1 (CD68)and M2 (CD163) in prostate cancer.

To find association between M1 , M2 Macrophage with Gleason's score & Stage of the disease in prostate carcinoma.

You are requested to participate in a study conducted by the department of pathology as a part of dissertation. This study will be done on breast carcinoma specimens of the patients. The specimens will be collected from the department of pathology, SDUMC, Kolar.

This study will be approved by the institutional ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research.

This informed consent document is intended to give you a general background of study. Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study.

If you are willing to participate in the study you will be asked to sign an informed consent form by whichyou are acknowledging that you wish to participate in the study and entire

procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.

For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR : Dr. SOUMYA. M. HADIMANI

ANNEXURE-III

PATIENT PROFORMA

Name :
Age: Hospital Number:

Anonymised Sample No:

Chief complaint :

History of presenting illness :

Past history :

Personal history :

Local examination:

Biopsy Number:

Histopathological diagnosis :

Gross :

Microscopy :

Gleasons score:

Stage of disease:

KEY TO MASTER CHART

PSA –Prostate Specific Antigen

USG- Ultrasonography

MASTER CHART

Sl.No	Biopsy no	Age	Hospital number	Gleasons Score	Gleasons Grade	CD68 Hotspot	Mean	CD163 Hotspot	Mean	PSA Level 0-4 ng/ml	volume	USG Grade	MRI/CT TUMOR SIZE	Metestasis	Radiological staging PI-RADS	orchidectomy	Clinical staging	PSA density PSA/volume
1	B/269/2020	75	818677	2+3 = 5.	1	20	6.60%	10	3.3	7	30	II	5.2X3.5X2.2	-	Grade-2	-	T2	0.23
2	B-619-19	55	697122	3+4 = 7	2	66	22	41	13.2	18	38	II	4.2x4x3	+	4		T3	0.47
3	B-723-19	65	699647	5+5= 10.	5	52	17.3	95	31.6	90	40	II	8x5x4.5	+	5		T3	2.25
4	B-1392-19	65	590889	4+3=7	3	78	26	61	20.3	25	30	II	4.5x 4x 3	+	3		T2	0.83
5	B/1854/19	70	746780	5+5=10	5	81	27	105	35	55	36	I	3x2.5x4	+	4		T4	1.52
6	B/2141/19	80	750389	4 + 5 = 9.	5	67	22.3	34	11.3	44	26	III	6x5x5	+	4		T3	1.69
7	B-1998-19	82	752890	4+5=9	5	86	28.6	52	17.3	59	28	III	8x9x8	+	4		T3	2.1
8	B-2802-19	76	651408	5+5= 10.	5	72	24	110	36.6	77	30	III	7.5x6x5	+	4		T4	2.5
9	B-1615-19	65	736247	5+5= 10.	5	65	21.6	92	30.6	46	32	II	5.5x4x4	+	4		T4	1.4
10	B-599-19	61	RLJH0000688865	5+5= 10.	5	80	26.6	120	40	81	42	III	8.8X5.5X4	+	4	RP	T3	1.9
11	**B-1318-15**	80	1021298	4 + 3 = 7	3	74	24.6	44	14.6	62	45	II	4x2x3	+	3		T2	1.3
12	B-84-15	67	1020216	5+4=9	5	60	20	92	30.6	60	30	II	4.1x3.5x3	+	4		T3	2
13	B-310-18	74	538882	4 + 3 = 7	3	93	31	33	11	35	25	I		+	3		T2	1.4
14	B-331-17	64	390741	3+4=7	2	82	27.3	25	8.3	25	33	II	3.3x2.5x3	+	4		T3	0.75
15	B-369-15	70	1020454	5+4=9	5	87	27.3	25	8.3	58	31	III	5.8x6x4	+	43		T4	1.8
16	B-459-18	73	552521	3+4=7	2	72	24	38	12.6	9	25	I	2.5x2x2	-	3		T2	0.36
17	B-475-15	74	1020508	3+4=7	2	61	20.3	20	6.6	15	25	II	3.5x4x2.5	-	4		T2	0.6
18	**B/654/16**	40	261525	5+5=10	5	55	18.3	92	30.6	71	36	III	5x4.5x4	+	2		T4	1.9
19	B-688-15	80	1020650	3+4=7	2	32	10.6	55	18.3	16	30	II	4.5x4x3	+	4		T3	0.5
20	B-911-15	55	1020849	4+3=7	3	45	15	25	8.3	20	28	II	3.5x3.3x2.1	+	3		T3	0.71
21	B-930-16	75	274102	3+4=7	2	69	23	81	27	48	33	III	7.5x6x5.5	+	3		T2	1.4
22	**B-978-16**	83	275494	**3+3 = 6**	2	55	18.3	21	7	6	28	I	3.7x4x3.3	-	3		T2	0.21
23	B-1110-18	80	577423	3+3 = 6.	2	49	16.3	22	7.3	9	29	II	3.5x4x	-	3		T2	0.3
24	B-1135-18	75	579136	4+3 = 7	3	62	20.6	86	28.6	22	35	II	5.5x4x4	+	4		T3	0.6
25	**B-1149-16**	78	274815	**5+5=10**	5	84	28	130	43	25	38	III	6x7.5x5	+	4		T4	0.6
26	B-1202-15	70	121863	4+2	3	31	10.3	20	6.6	10	26	I	2.2x2x1.5	-	2		T2	0.38
27	**B-1203-16**	71	283731	3+4=7	2	47	15.6	30	10	29	36	II	5x4.2x3	-	3		T2	0.8
28	B-1392-18	60	590889	4+3=7	3	38	12.6	74	24.6	31	30	II	4.3x3x2.5	+	3		T3	1
29	B-1571-18	80	596575	5+5=10	5	88	29.3	97	32.3	69	30	III	7.5x8x6	+	4		T4	2.3
30	b-1675-16	82	298486	**4+4=8**	4	43	14.3	86	28.3	39	25	III	6.6x5.2x4	+	4		T3	1.5
31	B1727-15	70	142484	5+4=9	5	60	20	90	30	50	28	III	5.5x4.2x3	+	4		T3	1.7
32	B-1815-15	70	1021809	4 + 5 = 9.	5	88	29.3	65	21.6	28	25	III	8x8x7	+	4		T4	1.12
33	B-1891-16	79	307474	**4+5=9**	5	55	18.3	73	24.3	60	28	III	7.2x6x5	+	4		T4	2.14
34	**B-1967-16**	70	305802	3+5=8	4	81	27	101	33.6	25	30	II	4.9x5x3	+	3		T3	0.8
35	B-2023-16	70	311958	**3+5 = 8**	4	25	8.3	96	32	44	40	III	3.5x4x3	+	3		T2	1.1
36	**B/2059/15**	70	176183	3+3=6	2	60	20	30	10	17	25	I	2.5x1x1	-	2		T2	0.6
37	**B/2062/15**	75	176697	4 + 5 = 9.	5	23	7.6	77	25.6	32	30	III	7.8x6.2x5.2	+	5		T3	1.06
38	**B/2235/15**	69	179469	**2+3=5**	1	55	18.3	24	8	58	28	III	2.2x3x2	-	2		T2	2.07
39	B-2255-18	82	627884	4+5=9	5	68	22.6	32	10.6	90	35	III	3x2.4x2	+	3		T4	2.5

Sl.No	Biopsy no	Age	Hospital number	Gleasons Score	Gleasons Grade	CD68 Hotspot	Mean	CD163 Hotspot	Mean	PSA Level 0-4 ng/ml	volume	USG Grade	MRI/CT TUMOR SIZE	Metestasis	Radiological staging PI-RADS	orchidectomy	Clinical staging	PSA density PSA/volume
40	B/2466/16			4+5=9	5	61	20.3	88	29.3	76	28	III	6x3.5x5	+	5		T4	2.7
41	B-2517-17	75	505938	**3 +3=6.**	2	46	15.3	120	40	>100	48	III	8X4.6X5.6	+	2	+	T2	2.08
42	B-2563-16	80	340327	5+4=9	5	55	18.3	86	28.6	66	25	III	5.4x2x6	+	5		T3	2.64
43	B-2600-16	52	342124	5+5=10	5	74	24	99	33	>100	47	II	8x5x4.7	+	5		T4	2.1
44	B-2640-15	72	203353	1+2	1	66	22	10	3.3	10	20	I	2x2x2	-	2		T2	0.5
45	B-2717-16	68	340786	**5+4 =9**	5	82	27.3	105	35	69	32	III	3.1x2x4	+	5		T3	2.1
46	B-2719-16	70	344620	**4+4-8**	4	67	22.3	105	35	75	26	III	5.5x6x2.1	+	3		T3	2.8
47	B-3056-15	82	223685	**5+4 =9**	5	55	18.3	81	27	82	36	III	7.4x6x5	+	4		T4	2.27
48	B-3274-16	44	371445	**4+4 = 8**	4	72	24	105	35	48	28	II	6.4x6x2	+	4		T3	1.7
49	B-959-19	65	699647	4+3=7	4	68	22.6	26	8.6	59	38	II	4.5x4x3.2	+	4		T3	1.5
50	B-1174-19	68	721829	4+5=9	5	91	30.3	62	20.6	65	40	III	6.5x4.7x3.6	+	4		T4	1.6
51	b-1388-19	67	729116	5+4=9	5	86	28.6	55	18.3	54	31	III	6.7X4.2X3.2	+	5	+	T4	1.74
52	B-1399-19	80	724005	**4+4=8**	4	76	25.3	98	32.6	>100	42	III	9.2X7X8.8	+	4	+	T3	2.3
53	B-1452-19	65	728111	5+5=10	5	79	26.3	111	37	>100	36	III	8X5.5X3	+	5	+	T4	2.7
54	B-646-20	60	838031	4+5=9	5	30	10	71	23.6	85	30	III	7.5X4X3.3	+	5	+	T3	2.8
55	B-25-21	65	887220	3+3=6	3	64	21.3	20	6.6	52	25	II	3X2.5X1.5	-	2	-	T2	2.08
56	B-54-21	65	887098	3+4=7	4	26	8.6	45	15	31.6	30	III	5.5X4X2.5	+	3	-	T2	1.05
57	B-125-21	66	886479	4+5=9	5	73	24.3	88	29.3	>100	48	II	7X4.1X3.6	+	4	-	T3	2.08
58	B-353-21	68	897465	5+4=9	5	81	27	110	36.6	75	36	III	7.7x5.4x6	+	5		T4	2.08
59	B-2837-18	77	307474	5+5=10	5	60	20	79	26.3	83	40	III	8x4.5x6	+	5		T4	2
60	B1727-15	70	142484	5+4=9	5	60	20	90	30	91	48	III	7.4x6.7x5	+	4		T4	1.8
61	B-1815-15	70	1021809	4 + 5 = 9.	5	88	29.3	65	21.6	78	34	III	6x3x3	+	4		T3	2.2
62	B-1891-16	79	307474	**4+5=9**	5	55	18.3	73	24.3	63	41	III	5.5x4x4	+	4		T3	1.5