

## Mast Cell Profile In Benign Prostatic Lesions Sujoy Kumar De\*, Soumi Pradhan<sup>2</sup>, Shashikala P<sup>3\*</sup>, Chethan K<sup>4</sup>

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### Abstract

**Background:** The analysis of prostatic lesions is important as development of benign prostatic hyperplasia is a universal phenomenon in aging men and Prostate is one of the leading sites of cancer among Indian men. In the present study, the count, morphology and distribution of mast cells, the Master cells of inflammation in benign prostatic lesions were recorded. **Materials & Methods:** Prostatic chips obtained by transurethral resection of the prostate (TURP) and open prostatectomy specimens were included in this study, irrespective of age and clinical diagnosis. After adequate fixation, and gross examination tissue bits were taken for processing. Based on criteria mentioned in Ackerman text book, H&E stained sections were studied in detail and lesions were categorized. Sections were stained with 1% aqueous Toluidine Blue stain; then the sections were examined at 400X magnification (high power) in random 10 non overlapping high power fields to record counting, distribution, morphology, shape of metachromatic mast cells. **Results:** This study includes 92 cases of prostate lesions. There were 88 cases (95.8%) of benign prostatic hyperplasia and rest all four were malignant lesions (4.2%). Mean age of the study population was 66.86 years. Majority of cases (39.1%) were in 7th decade and least number of cases (4.4%) was observed in 5<sup>th</sup> decade. About 98.9% specimens were procured by TURP in multiple bits. Among 88 nodular hyperplasia cases 82.9% presented with prostatitis. Mast cell quantification revealed a mean mast cell count of 5.42 in 10 HPF. There was no significant difference of distribution of mast cells in between various nodular hyperplasia specimens relative to glandular and stromal component of specimens. Mean mast cell count in nodular hyperplasia was 5.5/10 HPF. On statistical analysis mast cell density in NH without prostatitis versus NH with prostatitis, result was non-significant (P value=0.498). **Conclusion:** This study shows the variations in mast cell distribution in commonly encountered prostatic lesions. There is paucity of such studies in the literature and the possible utility of mast cell count to differentiate malignant from benign and atypical conditions needs further evaluation.

**Keywords:** Prostate, prostatitis, benign prostatic lesions, mast cell, transurethral resection of the prostate (TURP), prostatectomy

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### Introduction

Prostate is a tubulo-alveolar organ, the primary

function of which is the production of prostatic fluid and delivery of sperm. About 50%-60% of men belonging to sixth and seventh decade are present with enlargement of prostate either due to benign hyperplasia or carcinoma. Among clinically diagnosed cases of benign prostatic hyperplasia, 20-40% harbor foci of prostatic intraepithelial neoplasia (PIN) and carcinoma. Carcinoma of the prostate is the most common malignant tumour among elderly men which

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is known for metastasis [1]. Biomedical literature has documented two types of basophilic leukocytes: mast cells and basophils. Mast cells, omnipresent cells are seen in between tissue environment and organisms. Mast cells are derived from hematopoietic progenitor cells; mature in the tissues and reside there [2]. Mast cells develop either in connective tissue or in serous cavities. Mast cells are found commonly in skin, gastrointestinal tract, and respiratory tract [3]. Mast cells have many similarities with basophils; both are derived from bone marrow cells. Both contain electron dense metachromatic granules, stained by basic dyes and yield numerous inflammatory mediators such as histamine and histidine. Both show high affinity to receptor for IgE and on activation by anti IgE antibodies, mediator secretions are induced which play prominent role in allergic inflammation and immune responses. Mast cell activation is the characteristic feature of chronic inflammation. Tryptase from activated mast cells may provide a signal for abnormal fibrosis in inflammatory disease [4]. Mast cell expresses FGF-2 to microenvironment which stimulates angiogenesis and promotes tumour growth. Mast cell is considered as an independent prognostic marker for prostate cancer. Presence of immunohistochemical mast cell markers tryptase and c-Kit indicate poor outcome in prostate cancer [5]. Degranulated mast cell indicates prostatitis [6]. In a study conducted by Amir T *et al.*, Toluidine Blue (0.1%) was used to demonstrate mast cells in prostate specimens and their count was expressed as cells in per sq. Mm [7]. Prostatitis is important to differentiate the true infectious processes of prostate from the insignificant mononuclear infiltrate regularly seen accompanying with nodular hyperplasia. This phenomenon is sometimes referred by terms such as 'chronic nonbacterial prostatitis' or 'lymphocytic prostatitis'. Gram-positive bacteria are the most common pathogens found in cultures from prostatic fluid [8,9]. In acute bacterial prostatitis, microscopically, there are sheets of neutrophils around prostatic glands, often with marked tissue destruction and cellular debris. The stroma is edematous and haemorrhagic, and micro abscesses may be present. Diagnosis is based on culture of urine and expressed prostatic secretions; biopsy is contraindicated because of the potential for sepsis [10]. In chronic bacterial prostatitis, it is more common than bacterial prostatitis and shadows infection elsewhere in the urinary tract. It is the common cause of relapsing UTI and is usually triggered by E.coli infection [11]. The present study aims at finding mast cells count and its variation in various benign pathological lesions of prostate, and its relation to various structures in inflamed and non-

inflamed prostate. This further helps to find the possible role of mast cells in the pathogenesis and outcome of different benign pathological lesions of prostate.

## Materials & methods

This was a descriptive and inferential study on mast cell profile in benign prostatic lesions undertaken in the Department of Pathology, SSIMS&RC over a period of 2 years from Dec,'13 to Nov,'15. Ethical permission to the study was obtained from Institutional Ethics Committee. Prostate specimens required for the study were obtained from patients who underwent TURP and open prostatectomy at SS Hospital attached to SSIMS&RC and other private hospitals in and around Davangere district; which were sent for histopathology to the Department of Pathology from December 2013 November 2014.

**Inclusion Criteria:** Prostatic chips obtained by transurethral resection of the prostate (TURP) and open prostatectomy specimens were included in this study, irrespective of age and clinical diagnosis.

### Exclusion Criteria:

1. Prostatic biopsy e.g. trucut, needle biopsy for histopathology examination.
2. Poorly preserved prostate specimens.
3. Autopsy specimens.

The specimens were procured in the department of Pathology with relevant data in 10% formalin solution. After adequate fixation over a period of 24 to 48 hours, samples were subjected to detail gross examination i.e. colour, consistency, weight, volume, presence of nodules, appearance on cut section etc. and representative tissue bits of TURP and prostatectomy were taken for processing, based on criteria mentioned in Ackerman [12]. Entire tissue bits were taken in four cassettes until it filled up, after that for each additional 10 gm. weight one extra cassette was taken. Those tissues were routinely processed and paraffin sections of 4 – 5 µm thickness were taken and stained with H&E and 1% aqueous Toluidine blue. H&E stained sections were studied in detail and lesions were categorized as inflammatory, non-inflammatory, neoplastic or nonneoplastic pathology. Relevant clinical data obtained from the request form, and other details were recorded in the proforma.

**Methods of Toluidine Blue Staining:** Sections were stained with Toluidine Blue by Modified Johnson's method [13].

**Solution:** 0.1% Aqueous Toluidine Blue solution (prepared as in Johnson's method)

<b>Procedure:</b> <input type="checkbox"/> <input type="checkbox"/> Deparaffinise the sections and hydrate to water <input type="checkbox"/> <input type="checkbox"/> Stain with Toluidine blue solution for 1 minute. Rinse in distilled water <input type="checkbox"/> <input type="checkbox"/> Air dry <input type="checkbox"/> <input type="checkbox"/> Clear with Xylol and Mount with D.P.X	<b>Results</b> <input type="checkbox"/> <input type="checkbox"/> Mast cells - purplish pink <input type="checkbox"/> <input type="checkbox"/> Corpora amylacea- purple (considered for metachromasia of mast cell granules) <input type="checkbox"/> <input type="checkbox"/> Back ground- blue
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After staining with 1% aqueous Toluidine Blue stain sections were initially checked under 4X and 10X objectives for staining characteristics and to select the proper area; then the sections were examined at 400X magnification (high power) in random 10 non overlapping high power fields for counting, distribution, morphology (either degranulated or intact), shape of mast cells. Relevant data were noted and entered in excel sheet for analysis. Statistical

analysis was done by SPSS 20 software. The data were presented in terms of numbers, percentages, and graphs. Results were presented as mean±SD and range. P value calculated wherever necessary.

#### Results

The present study includes 92 prostate specimens of which 91(98.9%) were obtained by TURP and one (1.1%) by open prostatectomy [Table 1].

**Table 1: Type of surgery and number of specimens**

Type of Surgery	No. of specimens	%
TURP	91	98.9
Prostatectomy	1	1.1
Total	92	100

Age of these patients who underwent surgery ranged from 46-85 years with a mean± SD of 66.86± 9.10 years. In this study 4 patients (4.4%) were in 5th decade, 7 patients (7.6%) were in the ninth decade; 81 patients (88%) were in 6<sup>th</sup> to 8<sup>th</sup> decade [Table 2].

**Table 2: Age distribution of the patients studied**

Age range	No. of patients	Percentage%
41-50	4	4.4
51-60	23	25
61-70	36	39.1
71-80	22	23.9
>80	7	7.6
Total	92	100

#### Macroscopic Appearance

**Weight-**Weight of the prostate specimens varied from 7 grams to 40 grams, with Mean± SD of 21.64±7.18

grams. Weight of the 39 samples (42.4%) was 11 grams to 20 grams and another 39 specimens weighing in between 21-30 grams [Table 3].

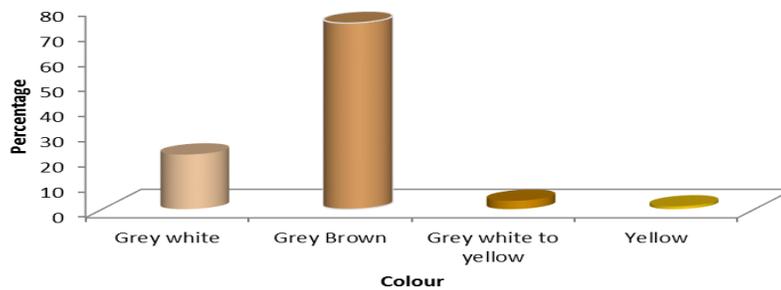
**Table 3: Weight of prostate specimens**

Weight (grams)	No. of samples	%
1-10	7	7.6
11-20	39	42.4
21-30	39	42.4
31-40	7	7.6
Total	92	100

**External Surface-**About 91 TURP specimens were in multiple bits, and one sample which was obtained by open prosectomy, measured 9x6x3 cms with gray

white nodular surface. Cut section showed nodularity, tiny cystic spaces oozing milky fluid [Fig. 2].

**Colour-**Colour of the specimens varied with 73.9% specimens gray brown in colour [Fig. 1].



**Fig 1: Column chart showing colour distribution of prostate specimens**

**Consistency**-Majority of specimens, eighty one (88%) were rubbery in consistency, followed by firm consistency in 10 specimens (10.9%). One specimen was hard in consistency.

**Histopathology Features**-Based on the histopathology 88 (95.8%) prostate specimens were classified as non-neoplastic i.e. benign prostatic hyperplasia (nodular hyperplasia) and four (4.2%) were neoplastic i.e. carcinoma. Of these 4 carcinoma cases 3 cases were prostatic adenocarcinoma and one was adenosquamous carcinoma. Among 88 specimens with nodular hyperplasia 73 specimens (79.5%) showed associated inflammation with prostatitis and there was no evidence of prostatitis in 15 (16.3%) cases [Fig. 3-6].

**Mast Cell Profile**-Irrespective of microscopic diagnosis all sections were stained by 1% Toluidine Blue and were observed under 400x magnification (HPF) for mast cell number, morphology and distribution in randomly selected 10 non overlapping fields. The data was recorded and entered in excel sheet for analysis.

**Number of Mast Cells and Total Count**-Mast cell count varied from nil to 29/ 10 HPF, with a range of 1-29/10 HPF with a Mean  $\pm$  SD of 5.42 $\pm$ 4.74. In 53 specimens (57.4%) count ranged from 0-4/10 HPF and in 22 specimens (23.9%) count ranged from 5-9/10 HPF; in 12, 3 and 2 specimens mast cell count in 10 HPF ranged from 10-14, 15-19, >19 as showed in table 4.

**Table 4: Total mast cell count/10 HPF (400X)**

Total mast cell/10 HPF	No. of specimens	%
0-4	53	57.4
5-9	22	23.9
10-14	12	13.2
15-19	3	3.3
>19	2	2.2
Total	92	100

**Benign Prostatic Hyperplasia and Mast Cell**-Among 88 benign hyperplasia, chronic prostatitis was observed in all 73 cases (82.94%) of which 15 cases (15/73) (20.5%) showed acute on chronic inflammation. Microscopically nodular hyperplasia was sub grouped on the basis of relative amount of glands and stroma

into three types: (1) equal amounts of glands and stroma, 54 cases (61.35%), (2) predominant stromal components, 22 cases (25%), (3) predominant glandular proliferation compared to stroma, 12 cases (13.64%) [Table 5, 6].

**Table 5: Mast cell distribution in nodular hyperplasia**

Component	No. of specimens	Percentage	Mast cell count range	Mean mast cell count $\pm$ SD/10 HPF
Gland = stroma	54	61.36	0-20	5.89 $\pm$ 4.68
Stroma>gland	22	25	0-10	4.41 $\pm$ 3.02
Gland>stroma	12	13.64	2-29	5.75 $\pm$ 7.41
Total	88	100	-	-

**Table 6: Class interval distribution of mast cell in various types of nodular hyperplasia**

Class interval	Gland = stroma	Stroma>gland	Gland>stroma
0-2	12 (nil:2)	5 (nil:3)	2
3-5	21	12	8
6-8	8	2	2
9-11	7	3	0
>11	6	0	0
Total	54	22	12

Mast cell distribution studied in relation to age and nodular hyperplasia. Mean mast cell count varied from 3.5-5.8/ 10 HPF and patients were ranged from 5<sup>th</sup> to 9<sup>th</sup> decade. Mean mast cell count was 6.1 for 7<sup>th</sup> decade

and 3.5 in 5<sup>th</sup> decade [Table 6]. In eighty eight BPH specimens mast cell count ranged from zero to 29 in 10 non-overlapping random HPF with mean  $\pm$  SD of 5.5 $\pm$ 4.79 [Table 7].

**Table 7: Mast cell distribution in relation to age in nodular hyperplasia**

Age range	No. of specimens	Mean $\pm$ SD count of Mast cells/10 HPF
41-50	43	5 $\pm$ 1
51-60	23	5.7 $\pm$ 4.4
61-70	33	6.1 $\pm$ 5.8
71-80	21	4.4 $\pm$ 2.3
>80	7	5.8 $\pm$ 6.7

Of those 88 specimens of prostate of nodular hyperplasia 73 specimens (82.9%) had associated prostatitis and 15 (17.1%) did not show significant distribution. Mast cell count without prostatitis

specimens ranged from 2-18/10 HPF with a mean of 5.73 and in nodular hyperplasia with prostatitis count varied from 0-29 with a mean of 5.45 [Table 8].

**Table 8: Mast cell count, range in relation to inflammation in nodular hyperplasia specimens**

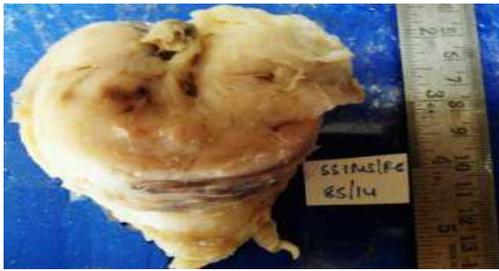
Specimen	Specimens No	Mast cell range	Mast cell mean count $\pm$ SD
NH without prostatitis	15	2-18	5.73 $\pm$ 4.15
NH with prostatitis	73	0-29	5.45 $\pm$ 4.9+3

**Morphology of Mast Cell by Toluidine Blue-**Mast cells were of different shape i.e. irregular, oval, round, elongated and kidney shaped. Oval mast cells were observed in 80 specimens (86.96%). In morphology mast cells were either intact or degranulated; degranulated mast cells were associated with inflammation. In a single case there was variable proportion of degranulated and intact mast cells with

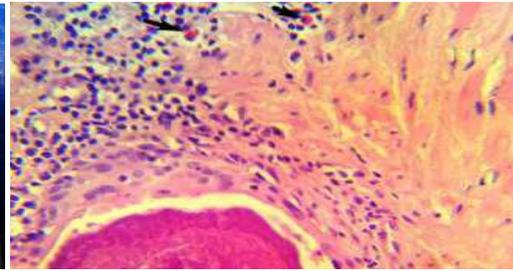
variable area of distribution. Out of the 92 specimens which showed mast cells i.e. n=85 (92.4%), intact mast cells were noted in 74 specimens (87.1%) and intact cells were absent in 8 cases; similarly ruptured mast cells were absent in 56 specimens (65.87%). Morphological distribution of cells was seen in below mentioned table 9.

**Table 9: Degranulated and intact type of mast cells with class interval**

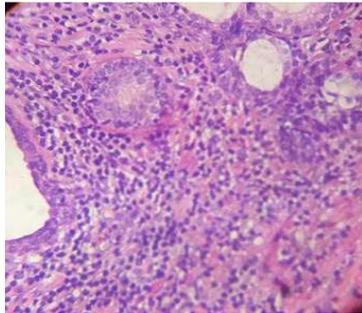
Type of cell	No. of specimens (n=92)	%
<b>Degranulated</b>		
0-1	66	71.6
2-3	17	18.5
4-5	3	3.3
6-7	3	3.3
>8	3	3.3
<b>Intact</b>		
0-1	18	19.4
2-3	30	32.5
4-5	27	29.5
6-7	4	4.4
8-9	3	3.3
>9	10	10.9



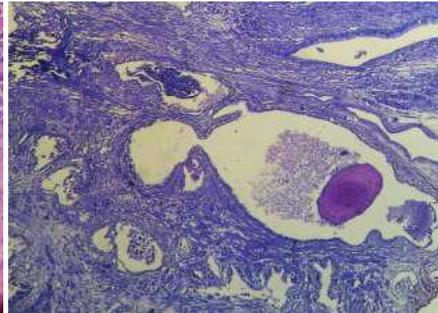
**Fig 2: Prostatectomy- grey white nodular surface**



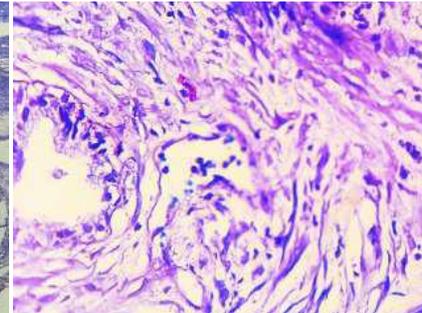
**Fig 3: Nodular hyperplasia with chronic prostatitis showing mast cells and corpora amylacea (arrow-mast cells) (H&E, High power)**



**Fig 4: Nodular hyperplasia with chronic prostatitis (H&E, High power)**



**Fig 5: Corpora amylacea, metachromatic purple colour (TB, High power)**



**Fig6:Perivascular meta chromatic elongated mast cell (TB, High power)**

## Discussion

In the present study, total 92 prostate specimens were examined. All the prostate specimens (98.9%) except one were obtained by TURP. It is a well-known fact that open prostatectomy for the treatment of bladder outlet obstruction is rarely performed today because of advances in TURP, anaesthetic procedure and  $\alpha$ -blocking agents for BPH [14]. There was no significant difference in mean age patients with benign prostatic lesions (66.79 years) compared to mean age of patients with carcinoma prostate (68.25 years). This is similar to study conducted by Amir *et al.* where mean age of patients with benign and malignant lesions were 63 and 64 years respectively [7]. Nodular enlargement of the gland is produced by hyperplasia of both glandular and stromal components; this result in an increase in the weight of the organ well beyond the 20 grams considered as normal for adult persons [15]. In present study, predominant specimens were procured as multiple grey white soft tissue bits; weight ranged from 7-40 grams with only one open prostatectomy specimen weighing 30 grams. Weight of the prostate specimens varied from 2-30 grams in a study on morphological spectrum of prostatic lesions by CN Anushree [16]. Of

the ninety two prostate specimens included for the study, majority 88 (95.8%) were benign, with a microscopic diagnosis of BPH and only four (4.2%) cases were prostatic carcinoma. BPH was the most common microscopic diagnosis on the TURP and total prostatectomy specimens, reported by various authors [17-19].

**Prostatitis and Mast Cell Profile:** All the 92 prostate lesions were sectioned and stained with toluidine blue for mast cell count. Mast cells count ranged from 0 to 29 in 10 non overlapping HPF and the mean mast cell count was 5.42/10 HPF. Mast cell count was zero in 7 specimens (7.61%). Of the 7 prostate specimens with zero count, most of them [n=5, (71.43%)] were nodular hyperplasia with chronic inflammatory cells in stroma; only two (28.57%) were from prostatic carcinoma. Amir *et al.* reported zero count in twenty two adenocarcinoma specimens [7]. Nodular hyperplasia lesions are histopathologically classified into three subtypes-a. An equal proportion of glands and stromal tissue, b. stroma>gland, c. glands>stroma; in this present study there was no significant difference between mean mast cell count and subtypes, which is similar to Amir et al study. [7]

**Mast cell distribution versus age in nodular hyperplasia:** Mean mast cell distribution in relation to age in nodular hyperplasia specimens were not statistically significant, p value=0.663 (one way ANOVA), similar to study carried by Amir *et al.* [7] and Aydin *et al.* [20]. Staemmler described that mast cells in human prostate increase with age in proportion to connective tissue stroma. In 1990, Stead RH *et al.* found that appendices with fibrosis having higher mast cell count and histamine which was present in the mast cells stimulate collagen synthesis [21].

**Nodular hyperplasia and inflammation -mast cell profile:** In comparison to mast cell density in NH

**Table 10: Nodular hyperplasia with and without prostatitis**

Diagnosis	Mean mast cell count /mm <sup>2</sup> ±SD by Amir <i>et al.</i> [7](N=no of specimens)	Mean mast cell count /10 random HPF present study (N=no of specimens)
NH without prostatitis	3.75±0.35 (101)	5.73±4.15 (15)
NH with prostatitis	1.7±0.31 (50)	5.45±4.9 (73)

In the present study, there was no significant difference between degranulated or intact morphology of mast cells with presence and absence of inflammation. There is paucity of study with similar observation.

**Table 11: Comparison of degranulated and intact mast cells with presence or absence of inflammation**

Variables	Inflammation [P value ( Chi-square test)]
Degranulated mast cells	0.685
Intact mast cells	0.574

### Morphology of Mast Cells

Gupta RK excluded TURP specimens in his study on mast cell variation in prostate and urinary bladder. He found them to be unsuitable because of deterioration of mast cell granules in tissues obtained after TURP [23]. In contrast in the present study intact mast cells were found in most of the NH specimens (81, 93.1%) of the 91 TURP specimens and degranulated mast cells were observed in 35 specimens (40.1%). Gupta RK in his study reported mean mast cell count of 40 and 210 in periacinar and stromal region in 10 HPF respectively, but in the present study Mean±SD cell count were 0.49±2.19, 2.35±2.55 respectively with total mast cell count in 10 HPF: 5.42±4.74. This variation probably is because of difference in the type of surgical specimens received for mast cell study. TURP specimens were excluded in Gupta RK study [23].

### Conclusion

It is important to know about mast cell profile in prostate lesions as because they may exert an unexpected role controlling prostate homeostasis through prostate stem cells. These help us to understand the course of these diseases, their trigger

without prostatitis versus NH with prostatitis, result was non-significant, p value=0.498 (Mann Whitney U test) contrasting with the study conducted by Amir *et al.* where p value was highly significant (p<0.0001) [7].

### Distribution of mast cells

In the present study majority of specimens showed stromal distribution (77.16%) of mast cells followed by perivascular distribution ( 71.74%). Focal increase in concentration of mast cells were seen around blood vessels and beneath prostatic urothelium. Globa *et al.* found mast cells distributed in small clusters along the blood vessels [22].

mechanisms and provide insights to formulate new agents which can modulate the function of mast cells and hence help us to develop new therapeutic approaches.

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