INTRODUCTION

Prostate cancer (CaP) is the second most widespread cause of cancer and the sixth foremost cause of cancer death among men worldwide. The sustained presence of particular hormones and growth factors stimulates the prostate, such as other sex accessory tissues, in its growth, maintenance, and secretory function. In the current study, we investigated the association of serum testosterone level and prostate-specific antigen (PSA) level before androgen deprivation therapy in metastatic PCa (mPCa) treated with hormone therapy.

Methods: This retrospective cohort was conducted from July 2017 to December 2019 in the Department of Urology, Post Graduate Institute of Medical Education and Research, Chandigarh on patients diagnosed with mPCa of age >40 years. Total serum testosterone was measured by Electro chemiluminescent immune assay before the treatment. PSA in serum specimens was evaluated.

Results: The mean age was 67.74±8.12 (55–80) years. Serum PSA levels at baseline, 3 months were 530.96±617.28 (ng/dL), and 56.31±99.04 (ng/dL), respectively. The baseline S. testosterone was 288.89±246.53 (ng/mL). Weak negative correlation between the two (p=0.051) As the serum testosterone levels decrease the PSA levels increase and vice-versa. Positive correlation between baseline testosterone and PSA decline (p<0.05). On histopathology, 58.7% (n=71) of the patients had perineural involvement, while 41.3% (n=50) did not have perineural involvement.

Conclusion: We conclude that the effect of testosterone might have a possible role in the assessment of treatment response as assessed by PSA. However, the exact implication and its role in disease assessment need to be examined in a larger prospective cohort.

Keywords: Prostate-specific antigen, S. testosterone, Metastatic prostate cancer, Androgen.
Moreover, clinical evidence supports testosterone replacement therapy in hypogonadal patients with erectile dysfunction, which is a frequent complication of treatment for PCs [8]. It is believed for a long time that higher TT contributed to PCs development and caused rapid.

In 1941, Huggins and Hodges showed rapid PCs progression in men who received testosterone and that metastatic carcinoma of the prostate was inhibited by eliminating androgens, through castration. They established PCs as “androgen-dependent.” This model suggests that high androgens cause de novo PCs or accelerate its growth and low levels are protective [7]. Although these findings appeared indisputable, some literature failed to support this concept. Multiple studies have shown no correlation between endogenous testosterone and PSA. Numerous studies revealed no relationship between TT and PCs risk [9]. Some studies even reported a relationship between low TT and increased risk or grade of PCs [10].

Metastatic disease and PSA
Serum PSA is a well-recognized, highly sensitive tumor marker for PCs. Preliminary data suggest that serum PSA levels measured soon after initiation of hormonal therapy appear to correlate well with clinical outcome [11]. Serial PSA determinations appear to be reliable since there is minimal random fluctuation between measurements in patients with metastatic tumor [12].

Based on unreliable evidence, it is believed that overall survival is poorer in these patients who have low PSA levels compared with patients who have PSA-positive metastatic prostate carcinoma [13]. The pattern of metastatic disease is considered to be unlike the pattern observed in tumors with high PSA levels, with increased numbers of visceral and soft-tissue metastases and a non-typical pattern of bone disease seen [14].

The oligometastatic disease can be defined as the development of three or fewer non-castrate lesions outside of the primary tumor [15]. These can be bone metastases or soft tissue. The concept of oligometastatic disease for all cancers is based on the order of a stepwise progression whereby cancer primarily metastasizes in a restricted way, before acquiring widespread metastatic behavior [16].

PSA with PCs
Data from the PCa Prevention Trial clearly show that the risk for PCs is continuous as PSA increase [17]. No significant differences were noted between cancer and benign groups with consideration to PSA level, PSA density, prostate volume, total testosterone level, or free testosterone level.

ADT role in PCs
ADT is well recognized as a backbone therapy for metastatic PCs (mPCa). Both research and development of increasingly precise assay technologies have enhanced our understanding of androgen production and signaling, and the recent data have suggested that the latest serum testosterone cut-off value of <0.7 mmol/L should be employed. Most clinical trials to date have used the historical 1.7 nmol/L cut-off, but the 0.7 mmol/L cut-off has been associated with improved patient outcomes.

The PSA level is the most valuable tool for monitoring disease status and the treatment response particularly in patients with advanced mPCa, as shown in numerous studies. However, the progression of PCs can sometimes arise in the presence of an undetectable or low serum PSA level.

Morote et al. in their recent study stated that serum testosterone has no association with the risk and tumor aggressiveness of PCs [7]. Other recent epidemiologic studies also have found no association between testosterone and PCs. The data are therefore conflicting, and on the clinical significance of testosterone level and development of PCs and more so there is a paucity of data in the Indian population.

In the current study, we investigated the association of serum testosterone level and PSA level before ADT in mPCa treated with hormone therapy.

**Aim of the study**
The role of serum testosterone level before ADT as a response marker in mPCa.

1. To evaluate the association of serum testosterone level before ADT and serum PSA response at 3 months after ADT
2. To see the association between serum testosterone and PSA response based on the different grade of PCs and modalities of treatment.

**METHODS**

After taking proper approval from the Institutional ethical committee this retrospective cohort was conducted from July 1st, 2017, to June 2018 and prospectively between July 1st, 2018, and December 31st, 2019, in the Department of Urology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh.

This study was conducted on patients diagnosed with mPCa and confirming inclusion and exclusion criteria of the study.

All patients of biopsy-proven adenocarcinoma of the prostate with metastasis and age >40 years were included in the study. Patients with a history of chronic liver disease, BMI >30, chronic kidney disease, having any known disease of the hypothalamo-pituitary axis and/or diabetes were excluded from the study.

**Methodology**

All patients of PCs underwent general physical examination, digital rectal examination, routine investigations including, serum PSA measurement. They under went stage evaluation which included contrast-enhanced MRI prostate, bone scan or prostate-specific membrane antigen positron emission tomography scan. Prostate biopsies were reported by the department of histopathology and the Gleason score was recorded.

**Measurement of serum testosterone level**
Total serum testosterone was measured by electrochemiluminescent immune assay before the treatment. In the morning at 8:00 am fasting blood sample (2 mL) was collected in EDTA vial and sent to the Endocrinology laboratory. The food and drug administration recently defined hypogonadism as a serum testosterone level of <300 ng/dL for clinical research trial [10].

**Measurement of serum PSA level**
PSA in serum specimens, at 8:00 am fasting blood sample (2 mL) was collected in plane vial and sent to Endocrinology laboratory. The normal reference range used for PSA is <4.0 ng/mL. Baseline testosterone and baseline PSA were done for all patients and PSA at 3 months after ADT, the association of serum testosterone with serum PSA response was assessed.

**Statistical analysis**
Data were statistically evaluated using SPSS v 22 software. The normality of data was checked by Kolmogorov-Smirnov test and expressed as mean plus/minus standard deviation. The proposed test was Student’s “t”-test, if given that data holds normality, otherwise Mann-Whitney test was applied. Wilcoxon matched pairs sign ranks test for pair-wise comparison of skewed data was used. To see the statistical difference in serum T level among different stages and Gleason grade of PCs, we used analysis of ANOVA. Correlation between variables was checked using the Pearson coefficient of correlation for normally distributed data and the Spearman correlation coefficient was used for skewed data. A p<0.05 was considered statistically significant.

**RESULTS**

This retrospective and prospective cohort study was conducted in the Department of Urology, Department of Endocrinology, PGIMER,
Chandigarh. During the study, a total of 121 patients were enrolled among which 60 patients were part of a retrospective cohort and 61 patients were in a prospective cohort.

The mean age of the patients with mPCa in the study was 67.74±8.12 years, with a range of 55–80 years. The majority of the patients were between 61 and 70 years of age (53%) followed by >70 years (31%).

The hormonal profile of the patients (n=121) who had mPCa indicated that the overall mean and SD values of serum PSA levels at baseline, 3 months were 530.9±617.28 (ng/dL), 56.31±89.04 (ng/dL) respectively. The baseline S. testosterone of the cohort was 288.89±246.53 (ng/mL) (Table 1).

There is a weak negative correlation between baseline testosterone and PSA decline (p<0.05) (Table 2). This means that patients with higher baseline testosterone had a better PSA decline following ADT (Table 2).

The GG groups were compared with respect to their baseline PSA, PSA change, and baseline serum testosterone. There was no significant difference between the two risk groups with respect to the above-mentioned parameters (Table 3).

### Table 1: Mean values of serum PSA levels and serum testosterone

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA baseline</td>
<td>530.98</td>
<td>333.00</td>
<td>617.28</td>
<td>4.07–3996</td>
</tr>
<tr>
<td>PSA at 3 months</td>
<td>56.31</td>
<td>12.00</td>
<td>89.04</td>
<td>0.02–567</td>
</tr>
<tr>
<td>PSA change</td>
<td>474.67</td>
<td>302.00</td>
<td>563.71</td>
<td>−483.1–3798</td>
</tr>
<tr>
<td>S. testosterone</td>
<td>288.89</td>
<td>246.00</td>
<td>246.53</td>
<td>2.50–1302</td>
</tr>
</tbody>
</table>

### Table 2: Comparative analysis of serum testosterone with PSA in metastatic prostate cancer

<table>
<thead>
<tr>
<th>Comparison of S. testosterone</th>
<th>Correlation coefficient (n=121)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA baseline</td>
<td>−0.178</td>
<td>0.051</td>
</tr>
<tr>
<td>PSA at 3 months</td>
<td>−0.564</td>
<td>0.000</td>
</tr>
<tr>
<td>PSA change</td>
<td>0.178</td>
<td>0.050</td>
</tr>
</tbody>
</table>

### Table 3: Comparative analysis of serum PSA and serum testosterone among PCa patients categorized based on Gleason grade

<table>
<thead>
<tr>
<th>Gleason grade</th>
<th>Intermediate (n=27)</th>
<th>High (n=94)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA baseline</td>
<td>658.00</td>
<td>376.50</td>
<td>0.241</td>
</tr>
<tr>
<td>IQR</td>
<td>804.97</td>
<td>487.00</td>
<td></td>
</tr>
<tr>
<td>PSA at 3 months</td>
<td>12.00</td>
<td>22.00</td>
<td>0.353</td>
</tr>
<tr>
<td>IQR</td>
<td>139.92</td>
<td>119.00</td>
<td></td>
</tr>
<tr>
<td>PSA change</td>
<td>394.00</td>
<td>252</td>
<td>0.084</td>
</tr>
<tr>
<td>IQR</td>
<td>825.08</td>
<td>332.80</td>
<td></td>
</tr>
<tr>
<td>S. testosterone</td>
<td>328</td>
<td>225</td>
<td>0.366</td>
</tr>
<tr>
<td>IQR</td>
<td>294</td>
<td>300</td>
<td></td>
</tr>
</tbody>
</table>

Our cohort of 121 patients, received various treatment modalities such as ADT alone, ADT with chemotherapy, ADT with chemotherapy, and palliative radiotherapy. The patients were grouped accordingly. There were 27 patients in ADT-only group, 88 in ADT with chemotherapy and 6 patients in ADT with Chemotherapy and Radiotherapy. Comparative analysis between these groups with respect to serum PSA levels and serum testosterone was performed. There was no significant difference in baseline PSA, PSA change, and S. Testosterone between the two groups (p=0.261, 0.471, and 0.583, respectively) (Table 4).

Patients with <4 metastasis and no visceral metastasis were considered an oligometastatic group. 53/121 were in this group. We did a comparative analysis to assess whether there was the difference between the baseline serum testosterone, baseline PSA, PSA response between the two groups. We found that there was no significant difference in baseline PSA, PSA at 3 months, PSA change, and S. Testosterone between the two groups (p=0.149, 0.627, 0.300, and 0.407, respectively) (Table 5).

**DISCUSSION**

To examine the same we studied patients of mPCa and analyzed their baseline serum testosterone with their PSA at baseline, 3 months, and change in PSA. We found that patients with higher baseline testosterone had less baseline PSA level before hormonal therapy. It is logical that if low serum testosterone levels are associated with a higher burden of disease which would be reflected in their PSA values, then there should have been a positive correlation. However, although Androgens, PSA, and PCa have been shown to be intimately associated, clinically, neither the total, free nor percent free T values enhance the ability of serum PSA to predict the pathologic tumor volume or stage of disease on an individual basis [19,20]. This finding needs to be validated in a larger prospective cohort. Although majority of our patients were in their 7th decade the baseline testosterone values for the Indian population in the age group in our cohort are not defined.

Another observation in this study was that higher testosterone levels had more PSA decline after ADT (correlation coefficient = 0.178 and p<0.05). According to one hypothesis, PCa cells that develop in the presence of a high level of testosterone may contain a high level of AR, which would make them very sensitive to androgen ablation [21]. A cohort study demonstrated that high levels of total serum testosterone levels at the time of diagnosis were associated with aggressive features of PCa, which is consistent with the results of previous studies [22]. ADT to lower the serum testosterone level remains a standard treatment for advanced disease to the present day. A study found that 3 months after the start of endocrine therapy, the PSA value in 26 patients had decreased to below the normal limit, whereas that in 14 patients was above 4.0 ng/mL. Concluding that there was a statistically weak positive significant association between serum testosterone and PSA response [23]. The demonstration that androgen suppression efficiently treats advanced PCa's and the fact that eminent serum androgen levels might predispose people to PCa, have attracted persistent interest. Our findings are in accordance with these studies.

We further examined weather serum testosterone levels were any different between various ISUP risk groups, the presence or absence of perineural invasion as found on histology. We did not find any significant difference in testosterone levels, serum PSA levels at baseline, 3 months between them. A study examining 455 men observed that low serum testosterone was not prognostic of biochemical recurrence, tumor volume, or disease progression, but that it was allied with Gleason 4–5 disease (odds ratio [OR] 2.4, 95% CI: 1.0–5.7; p=0.048) [24]. Our findings are in contrast with this study, however, we had not specifically defined or analyzed based on low vs high testosterone.

Other comparative analysis based on oligometastasis and non-oligometastasis PCa, visceral and bone metastasis and with various treatment modalities such as ADT only, ADT with Chemotherapy and ADT
Our study has various limitations.
1. Small sample size of our study (121 patients)
2. Retrospective cohort and the inherent bias associated with it
3. Shorter follow-up period (follow-up period of 13 months is too short to assess the true oncological outcomes).

**Table 4: Comparative analysis of various treatment modalities versus serum PSA level and serum testosterone in metastatic prostate cancer**

<table>
<thead>
<tr>
<th>Treatment modalities</th>
<th>PSA baseline</th>
<th>PSA at 3 months</th>
<th>PSA change</th>
<th>S. testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>ADT only (n=27)</td>
<td>299.92</td>
<td>629.00</td>
<td>45.00</td>
<td>113.00</td>
</tr>
<tr>
<td>ADT+Chemotherapy (n=88)</td>
<td>379.50</td>
<td>589.01</td>
<td>12.00</td>
<td>116.50</td>
</tr>
<tr>
<td>ADT+Chemo+Radiotherapy (n=6)</td>
<td>846.96</td>
<td>466</td>
<td>220.00</td>
<td>210.0</td>
</tr>
</tbody>
</table>

CONCLUSION

We conclude that the effect of testosterone might have a possible role in the assessment of treatment response as assessed by PSA. However, the exact implication and its role in disease assessment need to be examined in a larger prospective cohort.

REFERENCES

**Table 5: Comparative analysis of oligometastasis and non-oligometastasis prostate cancer with serum PSA and serum testosterone**

<table>
<thead>
<tr>
<th></th>
<th>Oligo metastasis (n=53)</th>
<th>Non-oligo Metastasis (n=68)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA baseline Median</td>
<td>331</td>
<td>433</td>
<td>0.149</td>
</tr>
<tr>
<td>PSA baseline IQR</td>
<td>475</td>
<td>642</td>
<td></td>
</tr>
<tr>
<td>PSA at 3 months</td>
<td>15</td>
<td>68</td>
<td>0.627</td>
</tr>
<tr>
<td>PSA at 3 months IQR</td>
<td>107.00</td>
<td>138.50</td>
<td></td>
</tr>
<tr>
<td>PSA change Median</td>
<td>217</td>
<td>273</td>
<td>0.300</td>
</tr>
<tr>
<td>PSA change IQR</td>
<td>288.20</td>
<td>390.42</td>
<td></td>
</tr>
<tr>
<td>S. testosterone Median</td>
<td>280</td>
<td>323</td>
<td>0.407</td>
</tr>
<tr>
<td>S. testosterone IQR</td>
<td>258</td>
<td>332</td>
<td></td>
</tr>
</tbody>
</table>

PSA: Prostate-specific antigen

along with Chemo as well as Radiotherapy then it was observed that there was no association found between serum PSA and serum testosterone. In 2016, Porcaro et al. found that high testosterone levels predicted an increased risk of Gleason score up grading (OR, 1.06; p=0.027) [25]. An animal study by Morgentaler and Trish stated that “Beyond a certain serum testosterone concentration, androgens have a restricted ability to stimulate PCa growth. Subsequent increases in serum testosterone levels beyond that concentration did not stimulate the prostate because the binding capacity of the intra-prostatic ARs had been saturated” [26].

Mikkola et al. studies have demonstrated that low, rather than high, testosterone levels at diagnosis were associated with various markers of poor prognosis, including an advanced pathological stage, higher Gleason scores, higher PSA levels, seminal vesicle invasion, and positive surgical margins [17]. They also reported that pre-treatment testosterone level in patients without mPCA was not higher than those with metastasis [17]. A pre-existing low serum testosterone level might selectively affect the growth of less androgen-dependent cancer cells, which might be more resistant to subsequent androgen withdrawal. To our knowledge, reports of the association between serum testosterone and the disease prognosis besides those of the western countries are very limited [27]. Our study further adds to the controversial effects of serum testosterone on PCA treatment and progression.