

## Association of Plasma Interlukin-6 in Metastatic & Non-Metastatic Prostate Cancer Patients presented to a Tertiary Care Hospital in Lahore Pakistan.

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### ABSTRACT

**Background:** Prostate cancer (PC) is a complex, multi-factorial disease. In spite of its high prevalence, the Pathophysiology of the PC progression is poorly understood. Cytokines play important role in immune regulation. Cytokines (IL-6) regulates growth of many tumor cells including prostate cancer, therefore it can be used as a marker for PC.

**Objectives:** To measure and compare the levels of IL-6 between metastatic and non- metastatic prostate cancer patients.

**Material and Methods:** This study was a Cross-sectional analytical and was conducted in the Department of physiology Federal Postgraduate Medical Institute (FPGMI) Lahore with collaboration of Urology department of Sheikh Zayed hospital Lahore. Patients with prostate cancer registered with Urology department, Sheikh Zayed Hospital Lahore were recruited for the study. Patients with inflammatory conditions, auto-immune diseases, obesity and Alzheimer's disease were excluded. A standard Elisa kit (Thermo scientific made in USA) was used for estimation of serum IL-6 levels. Data was recorded on a structured checklist, which contained basic demographic data along with finding of prostate specific antigen (PSA), prostatic biopsy, bone scan and serum IL-6 levels. Data was analyzed through SPSS version 20 for descriptive statistics.

**Results:** A total of 50 (25 metastatic and 25 non-metastatic) prostate cancer patients were being part of the present study. The mean PSA level was  $364.42 \pm 86.7$  among metastatic prostate cancer patients as compared to  $30.71 \pm 23.48$  non- metastatic, indicating significant difference ( $p = 0.001$ ). Similarly mean Gleason score was high ( $7.16 \pm 0.85$ ) among metastatic prostate cancer patients as compared to  $6.28 \pm 0.54$  among non-metastatic one. Significant difference ( $p = 0.014$ ) was observed regarding IL-6. It was  $25.73 \pm 80.48$  among metastatic patients in comparison with  $1.56 \pm 1.38$  among non-metastatic patients. There was strong correlation between PSA and Gleason score ( $r = 0.479$ ,  $p < 0.001$ ). Also positive association was found between IL-6 and PSA, ( $r = 0.285$ ,  $p = 0.045$ ).

**Conclusion:** Serum IL-6 might be considered as a predictor for metastatic prostate cancer. Along with PSA, serum IL-6 levels could be a strong predictor for the diagnosis of metastatic condition of prostate cancer.

**Key Words:** IL-6 levels, prostate specific antigen, metastatic and non- metastatic prostate cancer.

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### INTRODUCTION

Prostate gland has the size and shape of a walnut, is part of the male reproductive system that makes and store fluid which constitute part of the semen<sup>1</sup>. The constituents of Prostate gland are various cells like epithelial cells, glandular cells that are responsible for secretions, stromal cells and muscular cells that are capable for holding the epithelial cells. The stromal cells also help the prostate to grow by springing many growth factors in both normal and cancerous conditions<sup>2,3</sup>. PC is among the most commonst cancers around the world, although the rates vary widely across different countries. It is less common in Southeast Asia and more common in Europe and USA.<sup>4</sup> It is the second commonest factor responsible for death in U.S and U.K after lung cancer.<sup>5</sup> According to the first report from Karachi Cancer Registry prostate cancer ranks at number six among

cancers in males in Pakistan with incidence rate of 5.4/100,000 per year.<sup>6</sup> PC is a complex, multi-factorial disease still, the pathophysiology of PC progression is poorly understood. Tumor genesis is generally believed to be a stepwise procedure that is usually associated with alteration in the genetic makeup eventually causing variations in the expression and function of genes.<sup>7</sup> Prostate cancer (PC) arise predominantly from Androgen dependent secretory epithelial cells, therefore the androgen receptor (AR) signaling is one common factors that affects both the development and progress of PC.<sup>8,9</sup> It is suggested that AR is also altered in several ways during the progression of hormone independent, castration resistant prostate cancer (CRPC).<sup>8,9</sup> The two common type of carcinomas associated with prostate gland are Adenocarcinoma and Squamous cell carcinoma starting as carcinoma in Situ or P.I.N (Prostatic Intraepithelial Neoplasia) which ultimately spreads to the surrounding prostatic tissue and stroma forming tumors. Management is planned on the basis of PSA levels, histological grading (Gleason Score).<sup>10,11</sup> Cytokines are hormone like molecules that act as pro- inflammatory and anti-inflammatory in a paracrine and autocrine fashion. They are expressed from immune, epithelial, and

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stromal cells.<sup>12,13</sup> There are more than 100 different cytokines other than lymphokines discovered till now. Cytokines (e.g. IL-6) not only regulate immune and inflammatory response but also regulates growth of many tumor cells including prostate cancer.<sup>14</sup> IL-6 has a predominant role in the development and progress of CRPC. It may induce Prostate cancer in various ways. It is concerned with different steps in PC such as the neuroendocrine differentiation, androgen production and also in the activation process of androgen receptors.<sup>15</sup> As IL-6 plays a vital role in the stimulation and enhancement of the differentiation of prostate cancer cells from an androgen dependent to an androgen independent phenotype, it might serve as a useful tool for diagnosing the Prostate cancer<sup>16,17</sup>. IL-6 cause growth of these cells by both autocrine and paracrine fashion but in LNCaP cell lines (derived from distant lymph nodes) the role of IL-6 is controversial. Some studies reveal that IL-6 increase the growth of LNCaP cells while some studies claim that IL-6 inhibits the growth of LNCaP cells.<sup>18,19</sup> Based on all these functions of IL-6 that allows it to play various roles in the pathophysiology of PC it is important to validate and target this cytokine in prostate Ca which might be of some help in the therapy of advance androgen independent prostate cancer alone or in combination with chemotherapeutic agents.

## MATERIAL AND METHODS

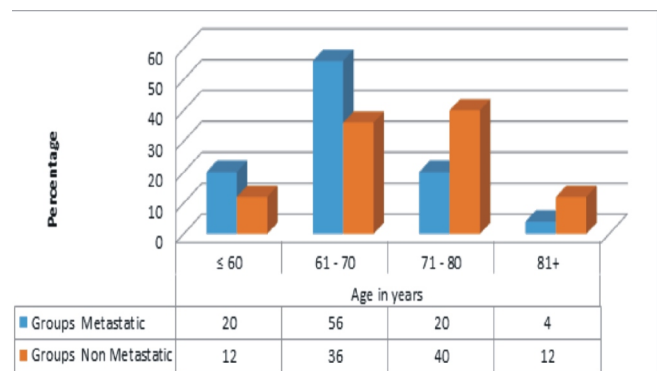
This study was cross sectional Analytical, which was conducted in Department of Urology Sheikh Zayed hospital Lahore during 2018. After getting the permission from the research board (ASRB), and informed consents of all registered patients diagnosed as Ca-prostate (metastatic and non-metastatic) on the basis of serum PSA levels, prostatic biopsy and evidence of metastasis on the basis of bone scan and CT scan were included in the study while those with considerably high serum IL-6 levels and having acute infections with fever (38.3C) or having known case of chronic inflammatory conditions were excluded. A total of 50 patients fulfilling the inclusion criteria were included in the study and divided into two groups; the metastatic and non-metastatic groups.

After informed consent, 5ml blood samples was collected from indoor-patients. Blood was centrifuged to separate serum and kept frozen at -20C. For serum IL6 estimation, standard commercial ELISA-based kits (manufactured by

Thermo scientific, USA) were used. The data was recorded in a structure checklist and analyzed on SPSS version 20 where mean and standard deviation were calculated for age and IL-6 levels, while categorical data were expressed in terms of frequency and percentages. Comparison of IL-6 levels between groups was performed by using independent sample T-test where P value of = 0.05 was be considered significant.

## RESULTS

A total of 50 patients (25 metastatic and 25 non-metastatic state) with mean age of 69±7.5 years. The age categories further indicated that 5 (20%) of patients with Metastatic and 3 (12%) with Non-Metastatic were belonging from age of = 60 years of age. Similarly, 14 (56%) of Metastatic group and 9 (36%) Of Non-Metastatic groups were from 61 70 years of age. among age ranged 71 80 year, majority were 10 (40%) were Non Metastatic patients while Metastatic were 5(20%). Similarly, 3 (12%) of Non Metastatic had 81+ years age and only 1 (4%) was from Metastatic group.



The average PSA level for metastatic and non-metastatic was 364.42±860.7 and 30.71±23.48 with median levels of 100 and 23.3 respectively. The difference compared among the groups was statistically significant with p-value <0.001. When Gleason score was compared between the groups it was observed that the average Gleason score for the metastatic and non- metastatic group was 7.16±0.85 and 6.28±0.54 with median levels of 7 and 6 respectively (Table 8). The difference compared between the groups was statistically significant with p-value <0.001 the mean average serum IL-6 levels for the metastatic and non-metastatic group was 25.73±80.48 and 1.56±1.38 with median levels of 2.59 and 0.98 respectively. The difference compared between the groups was statistically significant with p-value 0.014.

**Table 1. Comparison of PSA, Gleason score and interleukin-6 between Metastatic and Non-Metastatic prostatic cancer patients.**

Variables	Groups				P-Value
	Metastatic	Min-Max	Non-Metastatic	Min-Max	
	Mean + SD		Mean + SD		
Age	67+7	50-82	71+8	54-86	0.094
PSA	364.42+ 86.7	3.80-3953	30.71 +23.48	9.2-99	<0.001
Gleason score	7.16+0.85	6.0-9.0	6.28+0.54	5.0-7.0	<0.001
interleukin-6	25.73+80.48	0.0-400	1.56+1.38	0.0-3.51	0.014

**Table 2. Correlation between Interleukin-6 with age, PSA and Gleason score**

Correlation		Age	PSA	Gleason Score
Interleukin-6	R	-0.028	0.285*	0.222
	P	0.849	0.045	0.121
PSA	R			0.479**
	P			< 0.001

r= Correlation Coefficient, P= P-value

Association of Interleukin-6 was observed with age, PSA level and Gleason score. It was noted that the correlation with age was insignificant with  $\rho = -0.028$  and p-value 0.849, with PSA was significant with  $\rho = 0.285$  and p-value 0.045, whereas with Gleason score it was insignificant with  $\rho = 0.222$  and p-value 0.121. There was only weak positive association recorded between IL-6 and PSA. The correlation between PSA and Gleason Score was 0.479 with p-value <0.001 so significant with positive moderate relation.

## DISCUSSION

This study was carried out in order to measure and compare the serum IL-6 levels in both metastatic and non-metastatic prostate cancer patients in Pakistani population. Due to the increasing incidence of prostate cancer in western population as well as in eastern population, it has become a great challenge to evaluate the exact causative agent or biomarker for disease progression other than androgens, because prostate sensitive cancer becomes androgen insensitive prostate cancer after 12-18 months and even after taking androgen ablation therapy. It is suggested through recent research that presence of different inflammatory markers and cytokines at the site of tumor formation will eventually lead to the tumor cell survival, its further proliferation, invasion and metastasis. This factor also focus on the importance of inflammatory markers including IL-6, in the etiology, progress of PC. An increase in the levels of IL-6 in males with local PC and advanced stage of the disease makes IL-6 a very

suitable biomarker for PC development as well as its progression.<sup>18</sup>

The present study is among the first few studies which was conducted to find the IL-6 as biomarker for identification between metastatic and non-metastatic prostate cancer. The major finding of the present study showed that there is a significant association of high serum IL-6 levels with metastatic prostate cancer with a p value of 0.01. This is in correlation with different studies conducted by Adler et al<sup>20</sup>, which suggested that serum levels of IL-6 and transforming growth factor-b1 are increased in patients with metastatic prostate cancer, and that these elevated levels predominantly correlates with tumor burden as is evaluated by serum PSA or by clinically evident metastases. Our study also interprets that out of the total 9 patients which have distant metastatic disease (M1), 7 had elevated serum IL-6 levels. However, mean serum IL-6 was similar in patients with cancer confine to organ (pT2) and also in those with nodal metastases (N1). Eight out of the 12 patients with N1 disease had lowered serum IL-6 when compared to the pT2 group.<sup>20</sup> Similarly, the result of present study is also in consistence with study conducted with similar aim, demonstrates that there is an increases in serum IL-6 levels in male group with hormone-refractory prostate cancer compared to normal participants.<sup>21</sup> However, recently a study investigated the Paracrine Role for IL6 in Prostate Cancer Patients. The result indicate that IL-6 shown association with acute inflammation and in

metastatic disease, tumor cells was negative in lesion showing that IL-6 expression was restricted within vasculatures of bone and it was not expressed in soft tissue cells. This is suggestive that in PC patients, paracrine glands production is considered to be associated with the progression of disease rather than autocrine IL6 production<sup>22</sup>. There are many other studies in which a comparison between metastatic and localized PC was done, and significant differences in IL-6 levels among both the groups were reported. These studies suggested a significant increase in IL-6 levels among the patients with metastasis<sup>23 24</sup>. These findings reveals that IL-6 may work both as a positive as well as a negative growth factor in the respective target cells. It is considered to be one of the predominant growth-stimulatory factors for human myelomas. On the other side IL-6 also acts as an inhibitor of early stage melanomas and also in the case of mammary and lung carcinomas. In the present study we found a positive correlation of serum IL-6 with prostate specific antigen, and Gleason score ( $p < 0.05$ ). Similar fashion also reported by other studies<sup>25,26</sup>.

## CONCLUSION

Elevated IL-6 has been observed in patients having prostate carcinoma with bone metastasis. Moreover, increased serum IL-6 level has been also observed with raised PSA level and Gleason score.

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