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THE ASSOCIATION BETWEEN SERUM PROSTATE-SPECIFIC ANTIGEN, INTERLEUKIN-6 AND TUMOUR NECROSIS FACTOR-ALPHA IN PATIENTS WITH PRIMARY PROSTATE CANCER IN PORT HARCOURT, NIGERIA

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ABSTRACT

Prostate cancer is the most common malignancy in middle-aged and elderly male Nigerians. Serum prostate-specific antigen is its most acceptable biomarker. The aim of this study is to determine the association between serum PSA, interleukin-6 and TNF-α, which have been associated with poor prognosis of the tumour. **Materials and Methods:** Consecutive patients that presented to the Urology Clinic of University of Port Harcourt Teaching Hospital (UPTH), Nigeria were evaluated for prostate cancer. All patients with clinical features of prostatic disease, before prostate biopsy had pretreatment serum PSA, IL-6 and TNF-α measurements using the enzyme-linked immunosorbent assay. Karl Pearson's Correlation Test was used to determine the association between serum prostate-specific antigen, IL-6 and TNF-α. Results were organized into charts, tables and prose. **Results:** Forty-four (44) patients had histopathologically confirmed adenocarcinoma of the prostate. Fifty had benign prostatic hyperplasia. There was a moderate correlation between serum PSA and serum IL-6 ($P<0.001$ at 95% confidence level). The correlation coefficient was 0.63752 with coefficient of determination (R-squared value) of 0.4064 or 40.64%. Similarly, a moderate correlation of 0.66656832 was observed between serum PSA and serum TNFα ($P<0.001$ at 95% confidence level). The coefficient of determination was 0.444313 (44.43%). **Conclusion:** There was significant correlation between serum PSA and interleukin-6, and between serum PSA and TNF-α. In 40.64% and 44.43% respectively, the correlation between serum PSA and the proinflammatory cytokine was most likely due to prostate cancer and its associated inflammation. The clinical significance of these findings within this population needs further studies.

KEYWORDS: Association, Serum PSA and serum IL-6, Serum PSA and TNF-α, Primary prostate cancer, Port

INTRODUCTION

Adenocarcinoma of the prostate is the most common malignancy in men aged 50 years and older in Nigeria.[1] It is a grave health problem in Port Harcourt, the ethnic communities of the Niger Delta and the Eastern States of Nigeria.[2] The hospital incidence of the disease at the University of Port Harcourt Teaching Hospital is 114/100,000 patients.[3] In a recent study in Port Harcourt, the poorly differentiated variants of the disease (irrespective of age of patients) was found to have a prevalence among the city's prostate cancer population of 34% to 43%.[4]

Prostate-specific antigen '(human Kallikrein 3, hk3 protein or KLK 3 gene), the same as what was previously called gamma-semino-protein' by Hara et al,[5] is a glycoprotein with 7% carbohydrate, and has a molecular weight of 33 kilo Daltons.[6,7] It is a serine protease, a member of the family of glandular kallikreins with gene locus in chromosome 19, and is secreted by the secretory epithelial cells of the prostate gland.[6,7] The serum concentration of total PSA usually rises in prostatic hyperplasia, inflammation, following procedures on the gland and prostatic adenocarcinoma.[7] Despite its fallibilities, serum prostate-specific antigen has been used for decades as a biomarker for adenocarcinoma of the prostate. Serum PSA is used with digital rectal examination (DRE) for the screening and diagnosis of prostatic adenocarcinoma, and the determination of prostate cancer therapeutic responses.

Interleukin-6, (IL-6) and tumour necrosis factor-alpha (TNF-α) are produced by cells of the monocyte-macrophage lineage in response to tissue injury and

inflammation; the former is also produced by endothelial cells and enterocytes.[8] Both cytokines are secreted by adipocytes in such a manner that their serum concentrations have been found to have a correlation with the amount of adipose tissue in the body.[9] Both are inflammatory cytokines and have pleiotropic effects.[10] There is evidence in the literature that chronic inflammation has a role in prostate carcinogenesis and prostate cancer metastasis.[11]

Inflammatory mediators which have serum levels that correlate with serum PSA may therefore have important diagnostic, therapeutic and prognostic uses in clinical management of prostate cancer. This idea which was stated in previous independent studies, led to the study of the roles of non-steroidal anti-inflammatory drugs (NSAIDs) and pro-inflammatory mediators in the prevention and therapy of primary prostate cancer.[12,13] This study is aimed at determining the association between serum total prostate-specific antigen and serum levels of IL-6 and TNF-ά at diagnosis of primary adenocarcinoma of the prostate in Port Harcourt, Nigeria.

Despite its fallibilities, serum prostate-specific antigen (serum PSA) has been for decades accepted as the biomarker for adenocarcinoma of the prostate. The serum concentration of total PSA usually rises with tumour burden.[14] It has thus been used for screening and diagnosis of adenocarcinoma of the prostate, monitoring of patients' therapeutic responses and prognosis in treated patients, as well as monitoring prognosis in those undergoing active surveillance.[7]

Evidence that inflammation is an integral part of prostate carcinogenesis and metastasis is ample in the literature. However, there have been little or no studies on inflammation and inflammatory mediators in prostate cancer in Port Harcourt. This study is aimed at determining the association between serum total prostate-specific antigen and serum IL-6 levels on the one hand, and serum total PSA and TNF-ά on the other in patients diagnosed with primary adenocarcinoma of the prostate in Port Harcourt, Nigeria.

MATERIALS AND METHODS

The study was carried out on consecutive patients who presented to the Urology Clinic, University of Port Harcourt Teaching Hospital (UPTH) with features of prostatic disease. The study complied with the research protocol for the evaluation of prostatic disease in the hospital. Ethical approval for the study was obtained from the UPTH Research Ethics Committee. Each patient individually gave consent for both treatment in the hospital and participation in the study.

Each patient had a triple assessment comprising history-taking, general and systemic physical examination, and relevant investigations. Sociodemographic data, presenting complaints, and history of presenting complaints were obtained from each patient. Patients with lower urinary tract symptoms (L.U.T.S) were evaluated in detail. L.U.TS were hesitancy in micturition, weak stream of urine, intermittency, straining to void, feeling of incomplete voiding and terminal dribbling of urine (voiding symptoms), and storage symptoms-frequent micturition, urgency, nocturia, urge incontinence, and nocturnal enuresis. Other symptoms elicited included dysuria, haematuria, bone pains, waist pain, lethargy, paraparesis, paraplegia, anorexia, and weight loss. Other features of disease in each patient including present diagnoses ongoing treatments, and past medical, surgical, family and social histories were recorded. Results of general and systemic physical examinations were also recorded. Details suggestive of prostate cancer on digital rectal examination were hardness and nodularity of the prostate gland, asymmetrical enlargement, absence of the median sulcus, fullness of the lateral sulci, and laxity of the anal sphincter.

Patients with clinical features of prostate cancer further had the following: Diagnostic investigations which included serum PSA measurements, trans-rectal ultrasonography of the prostate (TRUS) and abdominopelvic ultrasonography. Transrectal prostate biopsy and histopathology were done for confirmation of prostate cancer diagnosis. Abdominopelvic CT-Scan with CT-Urography was done for selected patients that manifested with clinical features of retroperitoneal metastases, lymphedema and obstructive uropathy. Abdominopelvic magnetic resonance imaging (MRI) was done on patients billed for radical prostatectomy to rule out regional peri-prostatic malignant lymph node metastases. Similarly, MRI was done to define the involvement of the axial skeleton and spinal compression in those that presented with neurological deficits, (paraplegia, paraparesis, bone pains, pathological vertebral fractures and suspected spinal compression. Full blood count, serum electrolyte, urea and creatinine levels were measured. Fasting blood sugar measurements, urinalysis, urine microscopy, culture and antimicrobial susceptibility tests were done to detect urinary tract infections, other comorbidities, and to provide supportive treatments.

Determination of Serum PSA, IL-6 and TNF-ά Concentrations

The patients were given appointments for prostate biopsies every Thursday for histopathological confirmation of adenocarcinoma of the prostate. Before prostate biopsy 5ml of blood was collected from each patient, placed in a test tube and spun in a bucket centrifuge. The supernatant plasma was pipetted into a plain bottle, properly labelled and stored in the refrigerator at -80^0c for 3-6 months of the study. These samples were used for the determination of serum concentrations of PSA, IL-6 and TNF-ά for each patient. Concentrations of serum IL-6, TNF-ά and serum PSA were determined using enzyme-linked immunosorbent assay (ELISA) techniques. ELISA kits acquired from the

Aviva System Biology Corporation, San Diego, CALIFORNIA, USA were used for determination of serum IL-6 and serum TNF-α. Serum total prostate-specific antigen concentrations were quantified with ELISA kits from Monobind Inc, 100 North Pointe Drive, Lake Forest, California 92630, USA. The procedures were done at University of Port Harcourt Research Laboratory, Chemical Pathology Laboratory Complex, University of Port Harcourt Teaching Hospital. All quantities of reagents, temperatures, timing of reactions and observation of end points of reactions were done in compliance with procedures outlined in the ELISA kits from the manufacturers. Results obtained from tests on each patient were recorded and presented as charts and tables using the Microsoft Excel, and in the form of prose.

The non-probability sampling (quota sampling in this case) was used because of the set-out criterion of including only patients with histopathologically confirmed diagnosis of primary adenocarcinoma of the prostate in this study.

RESULTS

Ninety-eight patients were enlisted for the study out of one thousand three hundred and four (1304) consultations. Forty-four (44) patient had histopathologically confirmed diagnosis of primary prostate cancer. All were adenocarcinomas. Fifty patients had benign prostatic hyperplasia and were excluded.

Results of serum PSA, IL-6 and tumour necrosis factor alpha (TNF-α) concentrations of the 44 patients were measured. Results were presented in Tables 1 and 3. The relationship between the independent variables (serum PSA and serum Interleukin-6 on the one hand, and serum PSA and serum tumour necrosis factor-α on the other), are presented as scatter plots (figures 1 and 2 respectively).

Table 1: Serum Total Prostate-specific antigen (ng/ml) and Serum Interleukin-6 (pg/ml) in Patients with Primary Prostate Cancer at Diagnosis in Port Harcourt, Nigeria.

SERUM PSA ng/ml (x)	1.4	2.3	2.96	5.7	6.84	10.3	13.0	14.8	15.2	15.3	15.7	16.0
SERUM IL-6 (y)	2.8	3.2	4.0	4.3	4.3	4.3	4.3	4.6	4.6	4.6	4.6	4.6
SERUM PSA ng/ml (x)	18.3	19.2	24.9	26.5	27.8	31.1	44.4	49.5	50.9	55.7	59.5	60.3
SERUM IL-6 (y)	4.8	4.8	5.0	5.0	5.1	5.1	5.1	5.2	5.2	5.2	5.4	5.4
SERUM PSA ng/ml (x)	60.5	62.5	75.8	82.0	83.3	89.0	95.1	96.5	98.4	99.0	100.0	100.2
SERUM IL-6 (y)	5.5	5.7	5.8	6.1	6.1	6.5	6.9	7.0	7.0	7.4	7.7	7.9
SERUM PSA ng/ml (x)	159.0	175.0	179.2	350.0	370.0	810.8	900	960				
SERUM IL-6 (y)	8.7	9.1	10.0	11.4	14.5	16.5	35.0	63.3				

PSA, prostate-specific antigen; IL-6 = Interleukin-6.

Determination of the association between prostate-specific Antigen and Interleukin-6

The formula used for the calculation of Karl Pearson's correlation coefficient (r) was $r = \{\sum(xy) - (\sum x \sum y)/n\}/\sqrt{\{(\sum x^2 - (\sum x)^2/n)((\sum y)^2-(\sum y)^2/n)\}}$ [15, 16] ……. **Equation 1**

where x is the serum prostate-specific antigen (x_1 to x_{44}); and y = serum Interleukin-6 (y_1 to y_{44}. There are 44 pairs of variables x and y (Table1).

Table 2: Calculation of Karl Pearson's coefficient of correlation: There are 44 pairs of x and y (Table 1).

SERUM PSA (x) ng/ml, and SERUM IL-6 (y) pg/ml	∑ x	∑ y	∑xy	$\sum x^2$	$\sum y^2$	n
	4483.91	366.3	78269.37	1,457373.37	7171.72	44

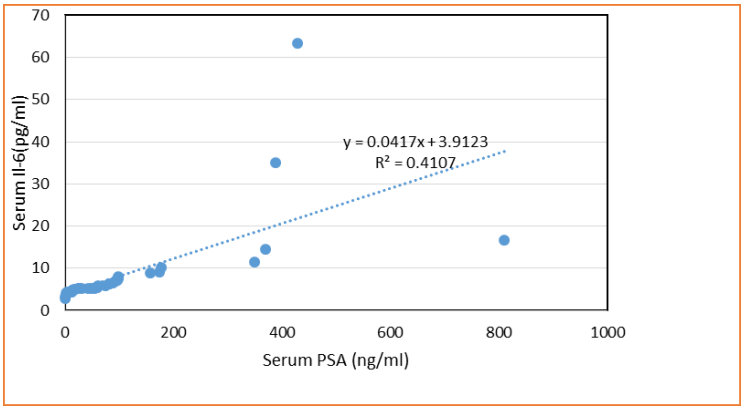

Figure 1: Scatter gram showing the relationship between serum prostate-specific antigen and Interleukin-6 in patients with primary adenocarcinoma of the prostate in Port Harcourt. PSA, prostate-specific antigen; Il-6, interleukin-6; R^2, R-Squared value, Coefficient of determination.

By substitution in Equation 1, Karl Pearson's coefficient of correlation (r) = 0.637519696 approximately 0.63752. This value of r corresponds to moderate correlation between serum PSA and serum interleukin-6 in patients with prostate cancer at diagnosis. There is a moderate correlation between serum PSA and serum interleukin-6 in patient with prostate cancer at diagnosis.

The standard error of correlation is given in large samples by $\sqrt{1}/(\square-1)$. The observed correlation coefficient (r) divided by the standard error of correlation i.e. =

Observed correlation coefficient (r)/ Standard error of correlation = $r\sqrt{(\square-1)}$ or $0.637519696\ \sqrt{(44-1)}$ as $\square = 44$ in this study; By substitution $r\sqrt{(\square-1)} = 4.181$. This value should be 2 instead of the calculated value, 4.181.

Coefficient of Determination

The coefficient of determination is the square of the correlation coefficient r (which is r^2). By substitution $r^2 = (0.637519696)^2 = 0.40643131362$ approximately 0.4064 or 40.64%.

Table 3: Serum PSA and corresponding values of serum tumour necrosis factor alpha in 44 patients at diagnosis of primary adenocarcinoma of the prostate in Port Harcourt.

SERUM PSA (x)	1.4	2.3	2.96	5.71	6.84	10.3	13.0	14.8	15.2	15.3	15.7	16.0
SERUM TNF-ɑ (y)	11.2	12.3	13.5	14.0	14.6	15.1	15.6	15.7	15.7	15.7	16.3	16.3
SERUM PSA (x)	18.3	19.2	24.9	26.5	27.8	31.1	44.4	49.5	50.9	55.7	59.5	60.3
SERUM TNF-ɑ (y)	16.8	17.3	17.9	18.5	18.5	19.1	19.6	20.2	20.2	20.4	20.7	20.7
SERUM PSA (x)	60.5	62.5	75.8	82.0	83.3	89.0	95.1	96.5	98.4	99.0	100.0	100.2
SERUM TNF-ɑ (y)	21.9	21.9	22.4	22.8	22.9	23.5	23.5	24.7	25.3	25.3	25.3	25.9
SERUM PSA (x)	159.0	175.0	179.2	350.0	370.0	810.8	390.0	430.0				
SERUM TNF-ɑ (y)	27.1	28.9	30.3	38.5	45.1	45.8	50.2	153.6				

Serum PSA, serum prostate-specific antigen; Serum TNF-ɑ.

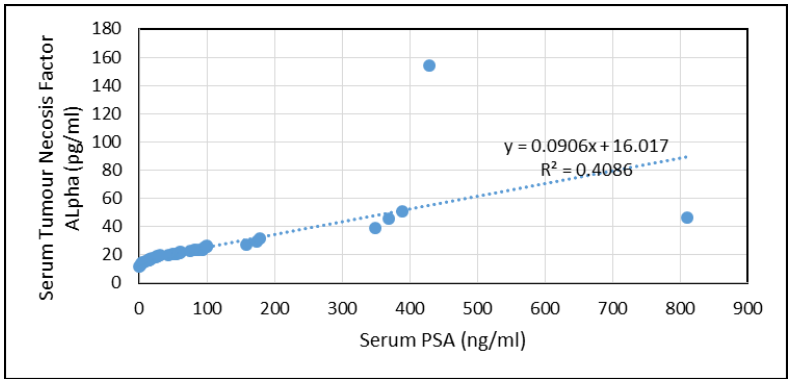

Figure 2: Scattergram showing the relationship between serum prostate-specific antigen and Tumour necrosis factor alpha in patients with primary adenocarcinoma of the prostate in Port Harcourt. PSA, prostate-specific antigen; Il-6, interleukin-6; R^2, R-Squared value, Coefficient of determination.

Table 4: Calculation of Pearson's Correlation Coefficient, Serum PSA and Serum TNF-ɑ.

SERUM PSA (x) ng/ml; SERUM TNFɑ (y_1(pg/ml)	$\sum x$	$\sum y_1$	$\sum xy_1$	$\sum x^2$	$\sum y_1^2$	**n**
	4483.91	1110.8	203765.154	1457373.37	46495.5	44

x, is serum prostate-specific antigen (serum PSA); y1, serum tumour necrosis factor alpha (TNFɑ). The values were calculated manually using data in Table 3.

Karl Pearson's correlation coefficient 'r' is given by the following formula

$r = \{(\sum(xy1) - (\sum x \sum y1)/\square\}/\sqrt{\{(\sum x^2 - (\sum x)^2/\square)((\sum y_1)^2 - (\sum y_1)^2/n)\}}$ [15, 16]

……. Equation 2

By substitution in Equation 2, the correlation coefficient is 0.666566832. If the correlation coefficient of a large population is zero, in large samples the correlation coefficients are normally distributed about the zero-population correlation coefficient.[15,16] The standard error of correlation is given as $\sqrt{1}/(n-1)$, where n is the sample size, which in this case is 44. The observed correlation divided by the standard error of correlation is $r\sqrt{(n-1)}$, 0r $0.66656832x\sqrt{(n-1)}$ i.e. $0.66656832x\sqrt{(44-1)}$, which is 4.371. The coefficient of determination is r^2 or $0.66656832^2 = 0.444313325$, approximately 44.43%.

The serum PSA levels were grouped with the number of patients in each group (Table 5).

Table 5: The profile of values of serum total PSA in the 44 patients with adenocarcinoma of the prostate in Port Harcourt, Nigeria.

RANGE OF SERUM PSA (ng/ml)	FREQUENCY (Number of patients	Percentage (%)
0-4	3	6.8
5-10	3	6.8
11-20	8	18.2
21-100	22	50.0
101-200	3	6.8
201-820	5	11.4
TOTAL	44	100.0

The range of serum total PSA was 1.4-810.8ng/ml. The mean serum total PSA was101.9ng/ml.

DISCUSSION

Certain features of prostate cancer facilitate its lethality. These include its therapeutic resistance and metastasis to the axial skeleton, with musculoskeletal and bone marrow dysfunction. Skeletal invasion is characterized, depending on tumour burden, by anaemia and musculoskeletal dysfunction, which includes bone pains, para paresis, paraplegia and bone marrow dysfunction. These features in addition to chronic renal disease due to obstructive nephropathy are frequently observed in advanced, metastatic and castration-resistant prostate cancer in UPTH, Port Harcourt, Nigeria.

Reports in the literature indicate that chronic inflammation and inflammatory mediators, interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α), are not only involved in prostate carcinogenesis, but promote its metastasis and worsen its prognosis.[17]

Conditions that may be associated with chronic inflammation of the prostate gland include prostatic trauma, urinary tract infection, poorly treated gonococcal urethritis /prostatitis[18], tuberculous prostatitis[19], trichomonas vaginalis infestation of the prostate and the urinary tract,[20] syphilitic prostatitis [21] and other forms of chronic bacterial and non-specific prostatitis. Viruses that have been implicated in prostatitis include human papilloma virus (HPV), cytomegaloviruses, human herpes virus type 2 and human herpes virus type 8.[22,23, 24] Others include iatrogenic and accidental pelvic with prostatic trauma, reflux of urine into the prostatic ducts with chemical inflammation,[2] consumption of red meat and animal fat[26], effects of dietary and environmental oestrogens[27,28], an autoimmune response to prostatic antigens[29], or a combination of these and other diseases. The hall mark of prostatic inflammation is the formation of proliferative inflammatory atrophy (PIA). PIA are lesions at the peripheral zone of the prostate resulting from inflammation, which have been described as intermediate lesions in the progression to low-grade prostatic intraepithelial neoplasia (LPIN), high-grade PIN (HGPIN) to prostatic neoplasia.[11]

Results of this our study indicate that, within the study population in Port Harcourt, there was a moderate positive correlation between serum total prostate-specific antigen and serum levels of interleukin-6, and between serum total PSA and tumour necrosis factor alpha in patients with primary adenocarcinoma of the prostate. These measurements were done simultaneously, and before treatment, or before procedures on the prostate. Similarly, serum levels of total prostate-specific antigen moderately correlated with pretreatment serum tumour necrosis factor-alpha. The manually calculated R^2 (coefficient of determination) of 0.4064313136 (r= correlation coefficient, 0.637519696) agrees with the Microsoft Excel automatically generated R-squared value of 0.4107 for the coefficient of determination. The coefficient of determination of 0.40643 or 40.64% observed in this study suggests that 40.64% of correlation between serum total PSA and the inflammatory cytokines might have been due to prostate cancer and associated inflammation, and that in 59.36% of cases correlation between serum PSA and these cytokines might have been due to other factors not considered in this study.

The correlation between serum total PSA, IL-6, and TNF-α observed in this study agrees with observations elsewhere.[30] The involvement of these IL-6 and TNF-α in prostate cancer metastasis, and its therapeutic resistance with worsening prognosis have been well reported.[30,31] However, there seems to be a knowledge gap in the reliable and universal clinical application of these findings.

Limitation of this study: One of the limitations of this study is the absence of universally accepted normal reference ranges of IL-6 and TNF-α for healthy adult individuals in the populations. This observation may be fairly global. Similar observations were made by Said EA et al[32], in a meta-analysis of pooled entries of reference values of serum IL-6 in normal blood donors. In the same study, the normal reference range of IL-6 was initially given as 0-43.5pg/ml. After analysis of 3166 reference values of serum IL-6 in 57 studies, the authors found the average IL-6 value to be 5.186 pg./ml. Said EA et al further made a salient finding of an age-related annual increase of 0.05 pg./ml of serum IL-6 level. A standard normal reference range of serum cytokines would form the baseline for evaluation changes due to diseases.

CONCLUSION

There was significant correlation between serum PSA and interleukin-6, and between serum PSA and TNF-α. In 40.64% and 44.43% respectively, the correlation between serum PSA and the proinflammatory cytokine was most likely due to prostate cancer and its associated inflammation. The clinical significance of these findings within this population needs further studies.

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REFERENCES

1. Ogunbiyi JO, Shittu OB. Increased incidence of prostate cancer in Nigerians. J Natl Med Assoc, 1999; 91: 159-164.
2. Monday K Sapira, Ndubuisi Eke, Alexander ME Nwofor. Ethnicity and prostate cancer in S outhern Nigeria: a preliminary report. Niger J Surg, 2015; 21: 96-101.
3. N Eke, MK Sapira. Prostate cancer in Port Harcourt, Nigeria: Features and outcome. The Nigerian Journal of Surgical Research; March-June, 2002; 4(1-2): 34-44.
4. Monday Komene Sapira. The risk of spread of primary prostrate cancer in patients with the disease in Port Harcourt, Niger Delta Region of Southern Nigeria. European Journal of Pharmaceutical and Medical Research. Sept., 2023; 10(10): 101-105.
5. Hara M, Inrre T, Fukuyama T. Some physico-chemical characteristics of grammasemino-protein, antigenic component specific for human seminal plasma. Jap J Legal Med., 1971; 25(25): 322-325. [PubMed, Google Scholar]
6. KW Watt, Paul-Jane Lee, Thabiso Mtimkulu, Wai-Pan Chan, Rueyming Loor. Human prostate-specific antigen: structural and functional similarity with serine proteases. Proceedings of the National Academy of Sciences, 1986; 83(10): 3166-3170.
7. Berman DM, Rodriguez R, Veltri RW. Development, Molecular Biology, and Physiology of the Prostate. In; Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA, Editors, Campbell-Walsh Urology 10th Edition; Philadelphia; ELSVIERS, 2012; 2533-2569.
8. Fink PM, The Inflammatory Response. In: Courtney M Townsend, R Daniel Beauchamp, B Mark Evers, Kenneth L Mattox, Editors, Sabiston Textbook of Surgery: The Biological Basis of Modern Surgical Practice, 19TH Edition; Philadelphia, ELSVIERS SAUNDERS, 2012; 40-65.
9. Popko K, Gorska E, Stelmaszcyk-Emmel A, Plywaczewski R, Stoklosa A, Gorecka D, Pyrzak B, Demkow U. Proinflammatory cytokines IL-6 and TNF-α and the development of inflammation in obese subjects. Eur J Med Res., Nov 4, 2010; 15(2): 120-122. Doi 10..1186/2047-783x-15-s2-120.
10. Toshio Tanaka, Masashi Narazaki and Tadamitsu Kishimoto. IL-6 in inflammation, immunity, and Disease Cold Spring Harb Perspect Biol, Oct., 2014; 6(10): a016295. Doi: 10. 1101/cshperspect. a016295
11. Angelo M De Marzo, Elizabeth A Platz, Siobhan Sutcliffe Jianfeng Xu, Henrik Gronberg, Charles G Drake, Yasumoto Nakai, William B Isaacs, William G Nelson. Inflammation in prostate carcinogenesis. Nat Rev Cancer. Apr., 2007; 7(4): 256-269. Doi 10.1038/nrc2090
12. Doat S, Cenee S, Tretarre B, Rebillard X, Lamy PJ, Bringer JP, Iborra F, Murez T, Sanchez M, Menegaux F. Non-steroidal anti-inflammatory drugs (NSAIDS) and prostate cancer risk: Results from the EPICAP study. Cancer Med., 2017; 6: 2461-2470.
13. De Marzo AM, Marchi VL, Epstein JI, Nelson WG. Proliferative inflammatory atrophy of the prostate: Implications for prostatic carcinogenesis.AM J Pathol., 1999; 155: 1985-1992.
14. Nguyen DP, Li J, Tewari AK. Inflammation and prostate cancer; the role of interleukin-6 (IL-6). BJU Int. Jun., 2014; 113(6): 986-92. Doi: 10,111/bju.12452
15. Mahajan BK. Correlation and Regression in Mahajan BK editor; Methods in Biostatistics for Medical Students and Research Workers Sixth Edition. New Delhi. JAYPEE BROTHERS Medical Publishers (P) LTD., 1997; 186-203.
16. Kothari CR, Gaurav Garg, Linear Regression; In Kothari CR, Gaurav Garg. Editors. Research Methodology Methods and Techniques, 3rd Edition; New Delhi, New Age International Publishers, 2014; P312-347.
17. Maddison Archer, Navneet Dogra, Natasha Kyprianou. Inflammation as a driver of prostate cancer metastasis and therapeutic resistance. Cancers, 2020; 12(10): 2984.
18. Pelouze PS. Gonorrhea in the male and female: a book for practitioners. Philadelphia: W. B. Saunders Company, 1935.
19. Poletti F, et al. Isolation of Chlamydia trachomatis from the prostate cells in patients affected by nonacute abacterial prostatitis. J. Urol, 1985; 134: 691-693.
20. Gardner WA, Jr, Culberson DE, Bennett BD. Trichomonas vaginalis in the prostate gland. Arch. Pathol. Lab. Med., 1986; 110: 430-432.
21. Thomson L. Syphilis of the prostate. Am. J. Syphilis, 1920; 4: 323-341.
22. Strickler HD, Goedert JJ. Sexual behavior and evidence for an infectious cause of prostate cancer. Epidemiol Rev., 2001; 23: 144-151.
23. Zambrano A, Kalantari M, Simoneau A, Jensen JL, Villarreal LP. Detection of human polyomaviruses and papillomaviruses in prostatic tissue reveals the prostate as a habitat for multiple viral infections. Prostate, 2002; 53: 263-276.
24. Samanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS. High prevalence of human cytomegalovirus in

prostatic intraepithelial neoplasia and prostatic carcinoma. J. Urol., 2003; 170: 998-1002.
25. Kirby RS, Lowe D, Bultitude MI, Shuttleworth KE. Intra-prostatic urinary reflux: an aetiological factor in abacterial prostatitis. Br. J. Urol., 1982; 54: 729-731
26. Sugimura T, Wakabayashi K, Nakagama H, Nagao M. Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. Cancer Sci., 2004; 95: 290-299.
27. Coffey DS. Similarities of prostate and breast cancer. Evolution, diet, and estrogens. Urology, 2001; 57: 31-38.
28. Harkonen PL, Makela SI. Role of estrogens in development of prostate cancer. J. Steroid Biochem. Mol. Biol., 2004; 92: 397-305.
29. Ponniah S, Arah I, Alexander RB. PSA is a candidate self-antigen in autoimmune chronic prostatitis/chronic pelvic pain syndrome. Prostate., 2000; 44: 49-54.
30. Nakashima J, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H, Murai M. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. Clin. Cancer Res., 2000; 6: 2702-2706.
31. Sharma J, Gray KP, Harshman LC, Evan C, Nakabayashi M, Fichorova R, Rider JR, Mucci L, Kantoff PW, Sweeney CJ, Elevated IL-8, TNF-alpha. And MCP-1 in men with metastatic prostate cancer starting androgen-deprivation therapy (ADT) are associated with shorter time to castration-resistance and overall survival.
32. Said EA, Al-Reesi I, Al-Shizawi N, Jaju S, Al-Balushi MS, Koh CY, Al-Jabri AA, Jeyaseelan L, Defining IL-6 levels in health individuals: A meta-analysis. J Med Virol. Jun., 2012; 93(6): 3915-3924. doi: 10. 1002/jmv. 26654. Epub 2020 Nov 22.