INTERNATIONAL JOURNAL OF CURRENT ADVANCED RESEARCH
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 14; Issue 01; January 2025; Page No.0034-0038
DOI: http://dx.doi.org/10.24327/ijcar.2025.0038.0008

Subject Area : Urology/Pathology

STUDY OF PROSTATIC OSTEOPONTIN EXPRESSION IN SYMPTOMATIC BENIGN PROSTATIC HYPERPLASIA IN RELATION TO THE SEVERITY OF THE DISEASE

Dr. Santhoju Kalyani, Dr. Bala Anand Shilpa, Dr. Padma Sunethri, Dr. M. Devojee, Dr. Keerthy Preethy. K, Dr. D. Vanaja

ARTICLE INFO

Article History:

Received 17th December, 2024
Received in revised form 29th December 2024
Accepted 15th January, 2025
Published online 28th January, 2025

Key words:

Osteopontin, Lower urinary tract symptoms, Benign prostatic hyperplasia.

ABSTRACT

Osteopontin (OPN) is secreted non-collagenous, sialic acid rich, chemokine like, matricellular phosphoglycoprotein that facilitates cell matrix interactions and promotes tumour progression. OPN is expressed by most of the immune cells. It is a cytokine & chemoattractant, and activates T-cells as well as macrophages. Osteopontin has been implicated in the pathogenesis of multiple fibrotic diseases. OPN, being a secreted protein, thus has also been explored for its function and diagnostic or prognostic potential in several cancers. Recently, it has been recognised that prostatic collagen accumulation is associated with lower urinary tract symptoms (LUTS) severity and treatment resistance. Also, Osteopontin has been found to increasingly expressed in the prostatic stroma of these patients. OPN is expressed across diverse prostate cell types including immune, epithelial, stromal and endothelial cells. Primarily, epithelial cells secrete OPN and stromal cells respond by up-regulating pro-inflammatory cytokines, in turn inducing more OPN secretion by epithelial cells. Abundance of inflammatory infiltrates in the prostate also correlates with symptom score and prostate volume. In the present study we have evaluated the expression of Osteopontin in relation to symptomatic benign adenoleiomyomatous hyperplasia.

INTRODUCTION

Osteopontin (OPN) is secreted non-collagenous, sialic acid rich, chemokine like, matricellular phosphoglycoprotein that facilitates cell matrix interactions and promotes tumour progression.[1] It is a cytokine, chemoattractant, and activates T-cells as well as macrophages.[2,3,4] OPN is expressed in a variety of tissues and bodily fluid, and associated with multiple pathologies including tissue injury, infection, auto-immune disease and cancer.[1] It has been implicated in the pathogenesis of multiple fibrotic diseases including benign prostatic hyperplasia.[5,6,7] OPN is a 34KDa protein that is extensively modified post translationally, it presents as a 60KDa, phosphoprotein initially called as transformation associated gene and a major sialoprotein in the extracellular matrix of bone.[1] It is a member of small integrin binding ligand N-linked glycoproteins (SIBLINGS), a family of five integrin binding glycophosphoproteins. The SIBLING family also encompasses bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP), and matrix extracellular phosphoglycoprotein (MEPE)[1] (fig.1). Osteopontin has been found to increasingly expressed in the prostatic stroma of these Benign prostatic hyperplasia (BPH) patients.[9] It is expressed across diverse prostate cell types including immune cells, epithelial cells, stromal cells and endothelial cells.[10] Primarily, epithelial cells secrete OPN and stromal cells respond by up-regulating pro-inflammatory cytokines, in turn inducing more osteopontin secretion by epithelial cells[10] (fig.2). Abundance of inflammatory infiltrates in the prostate also correlates with symptom score and prostate volume.[11]

Recently, it has been recognised that prostatic collagen accumulation is associated with lower urinary tract symptoms (LUTS) severity and treatment resistance. Thus, benign prostatic hyperplasia (BPH) is one of the most common causes of LUTS.[8]

In the present study we have evaluated the expression of osteopontin in relation to symptomatic benign adenoleiomyomatous hyperplasia of prostate. A positive association of osteopontin expression and symptom severity in these patients implies that the use of osteopontin inhibitors would benefit the symptomatic patients of benign prostatic hyperplasia.

AIM & OBJECTIVES

Aim is to study the expression of prostatic Osteopontin in symptomatic benign prostatic hyperplasia in relation to the severity of the disease.

Objectives

To study the expression of Prostatic Osteopontin in symptomatic patients with Benign Prostatic Hyperplasia.

To compare the Osteopontin expression in relation to the severity

*Corresponding author: **Dr. Santhoju Kalyani**

of the symptoms in symptomatic patients with Benign Prostatic Hyperplasia.

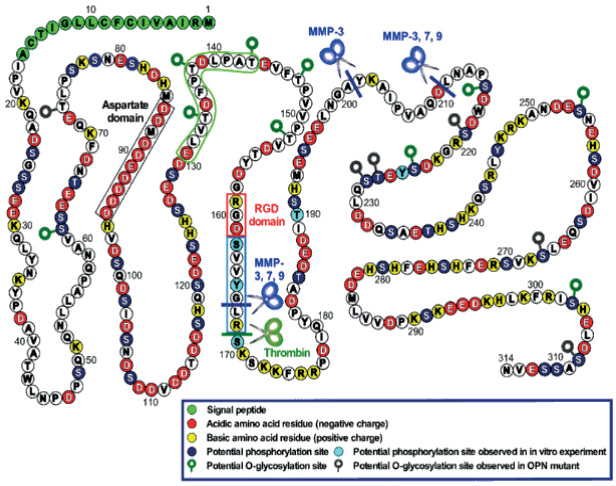

Figure 1 Primary structure and post-translational modifications of osteopontin.

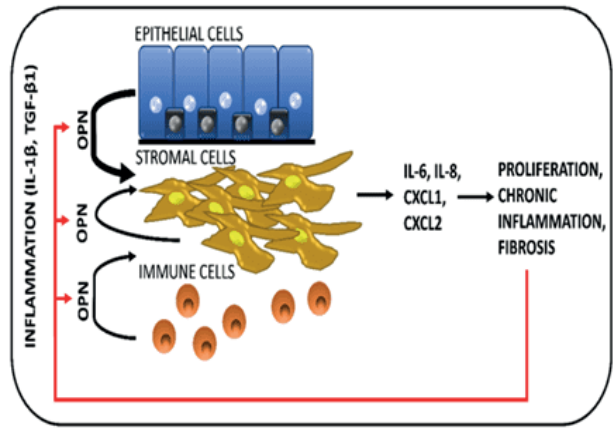

Figure 2 OPN exacerbates inflammation and fibrosis in the prostate.[10]

MATERIAL AND METHODS

The present study is an observational study conducted at the Department of Pathology, Gandhi Medical college/Hospital for a period of 24 months i.e., December 2020 to November 2022.

The following inclusion and exclusion criteria were considered for the study.

Inclusion criteria – All properly labelled and fixed prostatic samples which showed benign adenoleiomyomatous hyperplasia on routine H&E sections, Total prostatectomy specimens with benign adenoleiomyomatous hyperplasia have been included.

Exclusion criteria – All prostatic samples with malignancy have been excluded, Patients on chemotherapy or radiotherapy has been excluded.

A total of 100 cases which fulfilled inclusion criteria were included in the study group. The relevant clinical data pertaining to these cases including age and clinical symptoms was compiled.

Depending on the nature and severity of the symptoms, these patients were categorised into 2 groups:

Group 1 – Mild/moderate symptoms like hesitancy, weak flow, dribbling after urination, dysuria.

Group 2 – Severe symptoms like urinary tract infections (UTI), haematuria, inability to urinate.

The slides were stained with primary Ihc antibody, Osteopontin, monoclonal IgG antibody from Gene tex company.

RESULTS

Positive staining – Brown (due to DAB chromogen)

Osteopontin – Cytoplasmic positivity of glandular luminal epithelial cells was considered positive.

- The glandular luminal epithelial cells of BPH tissue showing cytoplasmic positivity were counted. Whereas, diffuse positivity in the stroma was ignored.
- The intensity of the staining was classified by the proportion of the cells stained.
- For each slide, 10 random microscopic fields were chosen and 100 cells counted in each field.[9]
- The percentage of the stained cells was decided by calculating the average of the 10 fields.[9]
- Tissues stained with less than 1% of the cells were classified as negative stain or unstained (-) and more than 1% of the cells were classified as positive stain and further categorized (Table 1).[9]
- Those with 1-30% of the cells stained were classified as weakly positive stain (+).[9]
- Tissues stained 30-70% were categorised as moderately positive stain (++) and more than 70% of the cells were classified as and strongly positive stain (+++) (Table 1).[9]

Table 1 Grading of Opn Staining Intensity[9]

S.no.	Grade	percentage of stained cells
1.	Negative (-)	<1% cells are positive
2.	Positive	>1% cells are positive
2a.	Weakly positive (+)	1-30% cells are positive
2b.	Moderately positive (++)	30-70% cells are positive
2c.	Strongly positive (+++)	>70% cells are positive

OBSERVATION AND RESULTS

A total of 100 cases were included in the study.

The majority of the cases in our study falls between the age group of 61-70 years (56%), followed by >70 years (25%) and then by 50-60 years (19%). No cases were identified below the age of 50 years. This suggests that the incidence of BPH is much higher in elderly individuals.

We have categorised the cases into two groups depending on the severity of symptoms.

Group 1 – Mild/moderate symptoms like hesitancy, weak flow, dribbling after urination, dysuria.

Group 2 – Severe symptoms like UTI, haematuria, inability to urinate.

The majority of the cases falls under group 2 (70%) out of which 38% patients had the symptom of UTI, 19% had haematuria and 13% have the inability to urinate.

Remaining 30% cases have mild or moderate symptoms thus categorised into group 1.

Out of 100 cases, 14 cases showed <1% cells stained positive for OPN, thus, considered as negative (-) and 86 cases have shown >1% cells stained positive for OPN, thus considered as positive (+). Out of 86 cases which are positive for OPN, 20 cases (23.2%) had group 1 symptoms whereas 66 cases (76.8%) had group 2 symptoms. Out of 20 positive cases in group 1, all 20 were showing weak positivity (+) with 1-30% of the cells staining positive for OPN. No cases have shown moderate (++) and strong (+++) positivity for OPN in this group. Out of 66 cases in group 2, 23 cases were weakly positive (+), 40 cases were moderately positive (++) and 3 cases were strongly positive (+++) for OPN. Thus, majority of the Group 2 cases showed moderate positivity. Overall, the cases of group 1 majorly shown weak positivity (+) whereas, majority cases of group 2 had shown moderate positivity (++) for OPN.

Table 2 Overview of distribution of cases based on staining intensity and symptom severity

Tissues	Number of cases	Stain intensity			
		-	+	++	+++
BPH with group-1 symptoms	30	10	20	0	0
BPH with group-2 symptoms	70	4	23	40	3

Thus, there is a significant correlation between OPN expression and symptom severity with a p value <0.0000001.In our study, the group 2 cases with severe symptoms had shown higher positivity rate (94%) compared to group 1 cases with mild/moderate symptoms (66.6%). This suggests that OPN positivity rate increases with symptom severity.

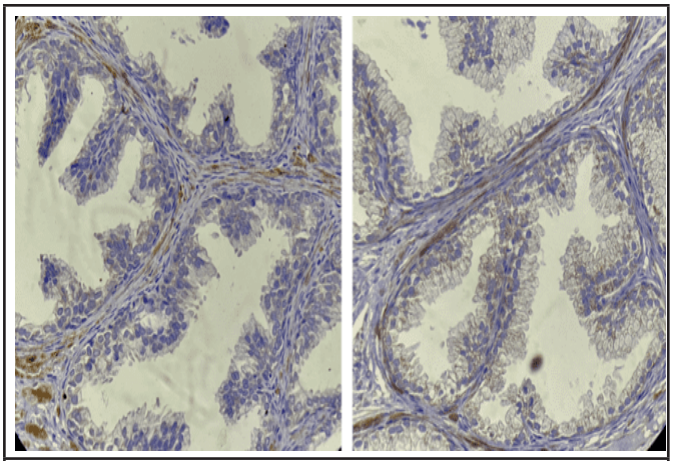

Figure 3a & b. IHC stain: Prostatic glandular epithelial cells weakly positive for OPN antibody

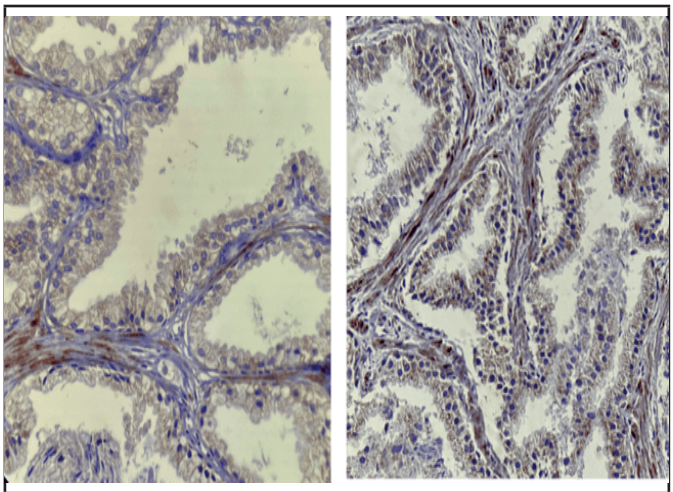

Figure 4a & b. IHC stain: Prostatic glandular epithelial cells moderately positive for OP N antibody

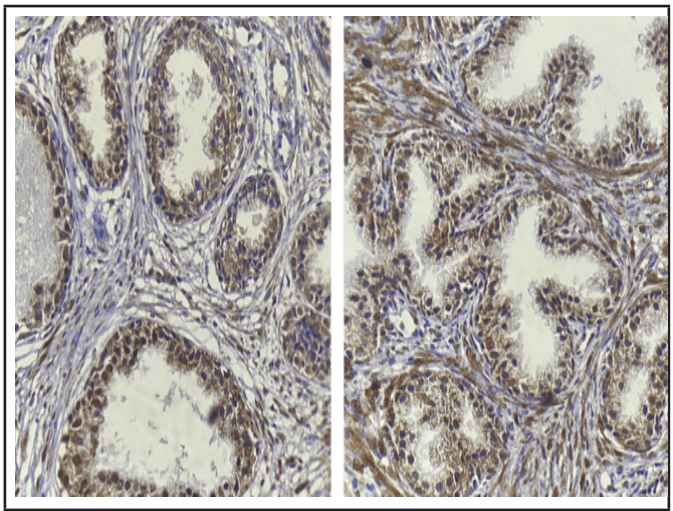

Figure 5a & b. IHC stain: Prostatic glandular epithelial cells strongly positive for OPN antibody

DISCUSSION

Benign prostatic hyperplasia (BPH) refers to non-malignant growth or hyperplasia of prostatic tissue. It is a common cause of lower urinary tract symptoms (LUTS) in men. Disease prevalence has been shown to increase with advancing age. The histological prevalence of BPH at autopsy is as high as 50-60% for males in their 60's, increasing to 80-90% over 70 years of age.[12]

Several definitions in literature when describing BPH includes bladder outlet obstruction (BOO), lower urinary tract symptoms (LUTS), benign prostatic enlargement (BPE). BPH describes the histological changes, BPE describes the increased size of the gland (usually secondary to BPH) and BOO describes the obstruction to flow. Those with BPE along with BOO are termed Benign Prostatic Obstruction[13]. LUTS includes urinary symptoms shared by disorders affecting the bladder and prostate (in reference to men).

- The development of BPH is characterized by stromal and epithelial cell proliferation in the prostate transition zone (surrounding the urethra), this leads to compression of the urethra and development of BOO which results in LUTS, urinary retention or infection due to incomplete bladder emptying. The aetiology of BPH is influenced by a variety of risk factors like old age, metabolic syndrome, obesity, hypertension and genetic factors. There is a direct hormonal effects on prostate mainly

dihydrotestosterone (DHT) derived from testosterone secreted by testis.[14]

The evaluation of BPH includes investigations like renal function tests, urinalysis, post-void residual, prostate specific antigen, ultrasound, urine flow studies, cystoscopy and IPSS. [15]

International prostate symptom score (IPSS):[16]

- Utilized to measure the severity of LUTS.
- It is a validated, reproducible scoring system to assess disease severity and response to therapy.
- It is not a reliable diagnostic tool for LUTS suggestive of BPH, but can be used to quantitatively measure LUTS after a diagnosis is made.
- It is made up of 7 questions related to voiding symptoms.
- Questions include:
- Incomplete emptying
- Frequency
- Intermittency
- Urgency
- Weak stream
- Straining
- Nocturia
- A score of 0-7 indicates mild symptoms, 8-19 indicates moderate symptoms and 20-35 indicates severe symptoms.[16]

Treatment for BPH includes, medical and surgical management. Medical therapy involves alpha blockers (tamsulosin-400mcg OD), 5 alpha-reductase inhibitors (finasteride-5mg OD), antimuscarinics. Surgical procedure involves transurethral resection/incision of prostate (TURP/TUIP), Holmium laser enucleation of the prostate (HoLEP), Urolift.[17]

In the present study, we have made an effort to categorise the patients who shall be benefit from medical management instead of going for invasive procedures. Thus, we have evaluated the expression of Osteopontin in relation to symptomatic benign adenoleiomyomatous hyperplasia. A positive association implies that the use of osteopontin inhibitors would benefit the symptomatic patients of BPH.

OPN is found in bone, breast, kidney, lung, nerve, pancreas, and skin tissues and is present in body fluids such as bile, blood, cerebrospinal fluid (CSF), milk and urine. It is also produced by epithelial cells, endothelial cells, fibroblasts, pericytes, hepatocytes, lens cells, tubular cells, immune cells (such as T cells, B cells, macrophages, NK cells, Kupffer cells), neural cells (such as neurons, glial cells, and schwann cells), osteoblasts, osteocytes, and vascular smooth muscle cells.

The OPNs produced by those cells are involved in various physiological and pathological processes including wound healing, biomineralization, bone remodelling, vascularization, diabetes, obesity, inflammation, fibrosis, urolithiasis, autoimmune diseases, tumorigenesis, and cancer invasion & metastasis.

Previous studies of OPN in prostatic disease primarily focused on malignancies and often used BPH prostate as a control. These studies reported weak staining/expression of OPN in BPH specimens compared to those with malignant diseases. However, very few studies have reported the upregulation of OPN in BPH versus normal prostate or healthy controls.[10]

In our study, there is an increased expression of OPN in patients with symptomatic BPH. Also studied that expression of OPN is higher in patients of BPH with severe symptoms i.e., Group 2 cases compared to the BPH with mild/moderate symptoms i.e., Group 1 cases. This implies that OPN might play an important role in BPH progression.

Our study correlates with the study done by Petra Popovics et al., where they have evaluated the expression of OPN by western blot in prostate stromal and epithelial cell lines of incidental-BPH (I-BPH) and surgical-BPH (S-BPH). They concluded that the expression of OPN is higher in S-BPH i.e., symptomatic patients who had to undergo surgery. They have reported that, abundance of inflammatory infiltrate in the prostate also correlates with symptom score and prostate volume. They also commented that OPN stimulates the expression of IL-6 and IL-8, which potentially leads to increased secretion of these cytokines similarly to the action of IFNγ and IL-17. Overexpression of IL-8 in prostatic epithelial cells leads to hyperplasia and the development of peri-glandular reactive stroma with increased pro-collagen 1 and tenascin levels consistent with fibrosis.[10]

Other study done by Shiva S. Forootan, the intensity of immunohistochemical staining amongst the normal, BPH and malignant carcinoma tissues has been compared so as to decide whether OPN is overexpressed in prostate carcinomas. The staining intensities of the tissue samples of low (Gleason score 2-4), moderate (Gleason score 5-7) and highly (Gleason score 8-10) malignant carcinomas have also been compared to assess whether the high level of OPN expression is related to the degree of malignancy. In this study, out of 36 BPH cases, 32 were weakly stained and 4 were unstained whereas majority of the malignant cases were strongly stained.[9] In our study, majority of the cases have shown moderately positive with significant correlation between OPN expression and symptom severity of BPH ($p = <0.0000001$).

OPN as a therapeutic target has been explored in various tumors including cancers of breast, lung, head and neck, stomach, colon and liver. The strategies often utilize OPN antibody to block its binding to receptors so as to inhibit the downstream signal transduction related to tumor growth and invasion, and deliver the small interfering RNA (siRNA) targeting OPN to tumor cells to decrease directly the expression of OPN and to reduce the effects triggered by elevated OPN.[18]

It is produced by various cells including tumour cells, endothelial cells, immune cells and fibroblasts. It is also expressed in many normal cells and plays an important role in physiological processes such as cell adhesion, migration, proliferation, survival, differentiation and immune modulation.[18]

Accordingly, the anti-OPN drugs can target the OPN molecule not only in the tumor tissues but also in normal tissues, which may cause severe side effects because of the inhibitory effects on the physiological activities of OPN. It is a biomarker which is associated with the response to cancer chemotherapy. [19]

Cetuximab, a monoclonal antibody targets EGFR thus increases IL-33 level and decreases OPN in the peripheral blood. Other neutralization monoclonal antibodies 100D3 and 100D6 are shown to block the binding of OPN to T cells and significantly increase the cytotoxic effects of tumor-specific CTLs and suppress tumor growth. Brefelamide, a novel inhibitor of Osteopontin, has been identified to reduce the invasion of human lung adenocarcinoma[20].

Drugs targeting the OPN are in research stage for their role in cancers and more studies are required to assess the importance of OPN in benign conditions like benign prostatic hyperplasia. Further clinical

studies are required to establish the use of these drugs in BPH.

CONCLUSION

BPH is one of the commonest diseases encountered in elderly individuals and most of them are treated with surgery. Literature on pathogenesis of BPH shows that the development of the disease is multifactorial and several inflammatory markers play a pivotal role. OPN is one such marker. In our study we have found that OPN is associated with the severity of the disease. Hence these patients would benefit with the usage of drugs targeting this molecule and thereby surpassing the invasive surgical procedure. Our study shall be a pilot study for further studies to be conducted in larger population groups and can also be a base to conduct clinical trials on the drugs targeting osteopontin in patients with BPH.

References

1. Lalita A. Shevde, Rajeev S. Samant. Role of osteopontin in the pathophysiology of cancer. Matrix Biology 37 (2014) 131–141.
2. Wang KX, Denhardt DT. Osteopontin: role in immune regulation and stress responses. Cytokine Growth Factor Rev. 2008;19(5-6):333-345.
3. Weber GF, Zawaideh S, Hikita S, Kumar VA, Cantor H, Ashkar S. Phosphorylation-dependent interaction of osteopontin with its receptors regulates macrophage migration and activation. J Leukoc Biol. 2002;72(4):752-761.
4. Ashkar S, Weber GF, Panoutsakopoulou V, et al. Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. Science. 2000;287(5454):860-864.
5. Leung TM, Wang X, Kitamura N, Fiel MI, Nieto N. Osteopontin delays resolution of liver fibrosis. Lab Invest. 2013;93(10):1082-1089.
6. Mori R, Shaw TJ, Martin P. Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring. J Exp Med. 2008;205(1):43-51.
7. Pardo A, Gibson K, Cisneros J, et al. Up-regulation and profibrotic role of osteopontin in human idiopathic pulmonary fibrosis. PLoS Med. 2005;2(9): e251.
8. Macoska JA, Uchtmann KS, Leverson GE, McVary KT, Ricke WA. Prostate Transition Zone Fibrosis is Associated with Clinical Progression in the MTOPS Study. J Urol. 2019;202(6):1240-1247.
9. Shiva S. Forootan. Prognostic significance of osteopontin expression in human prostate cancer. Int. J. Cancer: 118, 2255–2261 (2006) 2005 Wiley-Liss, Inc.
10. Petra Popovics et al. Prostatic osteopontin expression is associated with symptomatic benign prostatic hyperplasia. Pubmed.gov. Prostate. 2020 Jul;80(10): 731-741.
11. Robert G, Descazeaud A, Nicolaiew N, et al. Inflammation in benign prostatic hyperplasia: a 282 patients' immunohistochemical analysis. Prostate. 2009;69(16):1774-1780.
12. Roehrborn CG. Benign prostatic hyperplasia: an overview. Rev Urol. 2005;7 Suppl 9: S3-S14.
13. Abrams P. New words for old: lower urinary tract symptoms for "prostatism". BMJ. 1994 Apr 09;308(6934):929-30.
14. Parsons JK, Bergstrom J, Silberstein J, Barrett-Connor E. Prevalence and characteristics of lower urinary tract symptoms in men aged > or = 80 years. Urology. 2008;72(2):318-321.
15. Roehrborn CG. Pathology of benign prostatic hyperplasia. Int J Impot Res. 2008 Dec;20 Suppl 3: S11-8.
16. Barry MJ, Fowler FJ, O'leary MP, Bruskewitz RC, Holtgrewe HL, Mebust WK, Cockett AT., Measurement Committee of the American Urological Association. The American Urological Association Symptom Index for Benign Prostatic Hyperplasia. J Urol. 2017 Feb;197(2S): S189-S197. Gray's anatomy. The anatomical basis of clinical practice, 41st edition. Chapter 75, Page no. 1266-1268.
17. De la Rosette JJ, Alivizatos G, Madersbacher S, Perachino M, Thomas D, Desgrandchamps F, de Wildt M., European Association of Urology. EAU Guidelines on benign prostatic hyperplasia (BPH). Eur Urol. 2001 Sep;40(3):256-63; discussion 264.
18. Dong Xing Cao et al., Osteopontin as potential biomarker and therapeutic target in gastric and liver cancers. World J Gastrenterol 2012 August 14; 18(30): 3923-3930.
19. Yoshinobu Kariya, Yukiko Kariya. Osteopontin in cancer: mechanisms and therapeutic targets. Int. J. Trans. Med. 2022, 2, 419-447.
20. Jing Zhang et al., Identification of brefelamide as a novel inhibitor of osteopontin that suppress invasion of A549 lung cancer cells. Oncology reports 36: 2357-2364, 2016.

How to cite this article:

Santhoju Kalyani., Bala Anand Shilpa,. Padma Sunethri,. M. Devojee,.Keerthy Preethy. K and D.Vanaja .(2025). Study of prostatic osteopontin expression in symptomatic benign prostatic hyperplasia in relation to the severity of the disease, I*nternational Journal of Current Advanced Research*, 14(01), pp.0034-0038.

****