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COMPARATIVE ANALYSIS OF P40 AND 34BETAE12. IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF PROSTATE LESIONS: INSIGHTS INTO DIAGNOSTIC UTILITY

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ABSTRACT

Introduction: Prostate carcinoma remains one of the commonly diagnosed cancers and a leading cause of morbidity and mortality worldwide. The diagnostic challenge in distinguishing benign and malignant prostate lesions remains significant, especially in small biopsies. Immunohistochemistry(IHC) serves as a valuable adjunct tool in the

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diagnosis and management of prostate malignancies. This study aims to evaluate the utility of P40 expression in the diagnosis of prostate lesions and to compare with the immunohistochemical expression of 34betaE12 in benign, premalignant and malignant lesions of the prostate. **Materials & methods**: This investigation was done at the Sree Balaji Medical College and Hospital, Department of Pathology, Chennai, India. Total 41 males with prostate specimens prostatic specimens (biopsies and resections) satisfying inclusion and exclusion criteria were included in this cross-sectional research study.Initial sections weres tained with Hematoxylin and eosin stain followed by IHC staining with two markers, P40 and 34BetaE12. Data were analysed using the mean and standard deviation for quantitative variables, as well as frequency and percentage for categorical variables, for descriptive purposes. Statistical analysis was made with IBM SPSS 16.0 software and P value of <0.05 was considered significant. **Results**: Of the 41 cases examined, the most prevalent pathology was a benign lesion (51.2%), followed by 41.5% malignant and 7.3% had premalignant lesions. All patients with benign lesions and pre-malignant lesions were positive and all malignant lesions were negative for P40 staining. There was statistically significant increase in P40 and 35betaE12 staining among patients with benign and pre-malignant lesions. **Conclusion**: Our findings suggest that immunohistochemical markers 34betaE12 and p40 have been found to be of value in differentiating benign and malignant lesions of the prostate thereby playing an important role in management of patient and therapeutic outcome.

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KEYWORDS: Prostate carcinoma, Immunohistochemistry, P40, 34betaE12 & Basal cells.

ANÁLISIS COMPARATIVO DE P40 Y 34BETAE12. INMUNOHISTOQUÍMICA EN EL DIAGNÓSTICO DE LESIONES PROSTÁTICAS: PERSPECTIVAS SOBRE SU UTILIDAD DIAGNÓSTICA

RESUMEN

Introducción: El carcinoma de próstata sigue siendo uno de los cánceres más frecuentemente diagnosticados y una de las principales causas de morbilidad y mortalidad a nivel mundial. El desafío diagnóstico para distinguir entre lesiones prostáticas benignas y malignas sigue siendo significativo, especialmente en biopsias pequeñas. La inmunohistoquímica (IHQ) constituye una valiosa herramienta complementaria en el diagnóstico y tratamiento de las neoplasias malignas de próstata. Este estudio busca evaluar la utilidad de la expresión de P40 en el diagnóstico de lesiones prostáticas y compararla con la expresión inmunohistoquímica de 34ßE12 en lesiones prostáticas benignas, premalignas y malignas. **Materiales y métodos**: Esta investigación se realizó en el Departamento de Patología del Colegio Médico y Hospital Sree Balaji, Chennai, India. Se incluyeron en este estudio transversal 41 varones con muestras de próstata (biopsias y resecciones) que cumplían los criterios de inclusión y exclusión. Las secciones iniciales se tiñeron con hematoxilina y eosina, seguida de tinción inmunohistoquímica (IHQ) con dos marcadores: P40 y 34BetaE12. Los datos se analizaron utilizando la media y la desviación estándar para

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las variables cuantitativas, así como la frecuencia y el porcentaje para las variables categóricas, con fines descriptivos. El análisis estadístico se realizó con el programa informático IBM SPSS 16.0 y se consideró significativo un valor de $p < 0,05$. **Resultados**: De los 41 casos examinados, la patología más prevalente fue una lesión benigna (51,2%), seguida de una maligna (41,5%) y una premaligna (7,3%). Todos los pacientes con lesiones benignas y premalignas dieron positivo en la tinción de P40, y todas las lesiones malignas dieron negativo en la tinción de P40. Se observó un aumento estadísticamente significativo en la tinción de P40 y 35ßE12 entre los pacientes con lesiones benignas y premalignas. **Conclusión:** Nuestros hallazgos sugieren que los marcadores inmunohistoquímicos 34ßE12 y p40 han demostrado ser valiosos para diferenciar lesiones benignas y malignas de la próstata, desempeñando así un papel importante en el manejo del paciente y el resultado terapéutico.

PALABRAS CLAVE: Carcinoma de próstata; inmunohistoquímica; P40; 34betaE12 y células basales.

INTRODUCTION

In the histological diagnosis of prostate cancer based on architectural and cytological markers, the loss of basal cells is a hallmark of malignancy[1]. Immunohistochemical evaluation of the

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basal cells is a common supplementary strategy to confirm or rule out cancer when the growth pattern is hidden, as in core needle biopsies with few questionable glands[2]. Several basal cell markers, such as Keratin 903 (34betaE12), P-Cadherin, CK 5/6, p63, bcl-2, CD109, and D2-40.3, aid in the diagnosis of malignancy, allowing for the differentiation of benign and cancerous lesions[3].

Chromosome 3q27–29 has a p53 homologue, the p63 gene. The N-terminal transactivation domain of full-length TAp63 is transcriptionally active, while the N-terminal transactivation region of its isoform DNp63 is transcriptionally inactive (TA)[4,5]. To identify the p63 protein, most labs use the monoclonal antibody 4A4, which binds to a region of the protein shared by both isoforms. P40 has just been commercially accessible that recognises only the DN domain of the Np63 isoform, which is unique to that isoform[6].

It was found that aberrant labelling of cancer cells was far less common with p40 than p63. The fact that p40 is just the isoform Np63 of p63 makes it a reasonable candidate to be evaluated as a marker in a variety of diagnostic contexts. And also Squamous cell carcinomas can be detected with the same sensitivity as p63, but with a far higher specificity using the p40 marker, according to recent research[7,8].

The extremely infrequent occurrence of p63-positive prostate carcinomas can be a diagnostic stumbling block. An improved

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basal cell marker may be useful in these cases.34BetaE12 is a high molecular weight keratin specific for prostate basal cells and P40's value in diagnosing prostate illness is still up for debate. This is why the researchers investigated the use of p40 immunohistochemical staining in the identification of prostate lesions in comparison to 34BetaE12 staining.

The present study aims to evaluate the immunohistochemical expression of P40 and 34BetaE12 in prostatic lesions and also to compare the expression of these markers in various benign, premalignant and malignant lesions of the prostate.

MATERIALS & METHODS:

The study is a prospective cross sectional type conducted in Department of Pathology, Sree Balaji Medical college and Hospital with a sample size of 41. The study was conducted during the period from February 2020 to November 2021

Inclusion criteria: All the prostate samples (Biopsies & Resections) sent to the histopathology are included in the study irrespective of the age of the patients.

Exclusion criteria: Inflammatory lesions and Mesenchymal tumors of prostate are excluded from the study.

All Prostatic biopsies received were examined grossly and Dehydration, cleaning, and embedding procedures were followed in order to get a representative sections from the specimen. Sections were stained with Hematoxylin and eosin

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staining procedure. For Immunohistochemistry, 3 µm thickness sections were taken and transferred on an electropositive slide (Pathnsitu). The clinical history of the patient including the age, and previous biopsies done were obtained.

INTERPRETATION OF IMMUNOHISTOCHEMISTRY

P40 Expression

It was interpreted as either positive or negative. When the nucleus of the basal cell layer stained brown with negative stroma and luminal staining, it was considered positive.

Negative staining was evaluated only if it failed to identify any malignant cells in the focus and the material exhibited outstanding positive internal and external control staining.

34BETAE12 EXPRESSION:

An evaluation was made, and the results were either positive or negative. When the cytoplasm of the basal cell layer stained brown, it was interpreted as positive result.

A staining was declared a negative one only if it failed to identify any cancer cells in the sample's target area.

Statistical methods:

Data were analysed using the mean and standard deviation for quantitative variables, as well as frequency and

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percentage for categorical variables, for descriptive purposes. Bar graphs, pie diagrams, and box plots were also used to show the data.

In each explanatory variable category, all quantitative variables were visually inspected using histograms and normality Q-Q plots for normal distribution. Statistical analysis was made with IBM SPSS 16.0 software and P value of <0.05 was considered significant.

RESULTS:

In present study, comprising of 41 cases of prostatic lesions histolopathological analysis and the involvement of basal cell markers are studied during the period from February 2020 to November 2021.

The majority of patients in this study belong to age group of 51 to 55 years [Table 1].

Table 1: Distribution of age of the patients among the study population

Age (years)	n	%
≤ 50	2	4.9
51 – 55	13	31.7
56 – 60	8	19.5
61 – 65	8	19.5
66 – 70	3	7.3

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≥ 71	7	17.1
Total	41	100.0
Mean ± SD	61.02 ± 8.54 years	
Range	47 – 76 years	

In present study, majority of specimens (80.5%) were TURP specimens and 19.5% of specimens were trucut biopsy specimens.

Of the 41 cases examined, the most prevalent pathology was a benign lesion; accounting for 51.2% of the cases, followed by 41.5%malignant and 7.3% had premalignant lesions, respectively [Chart 1].

Chart 1: Pie chart showing distribution of patients according to type of lesions:

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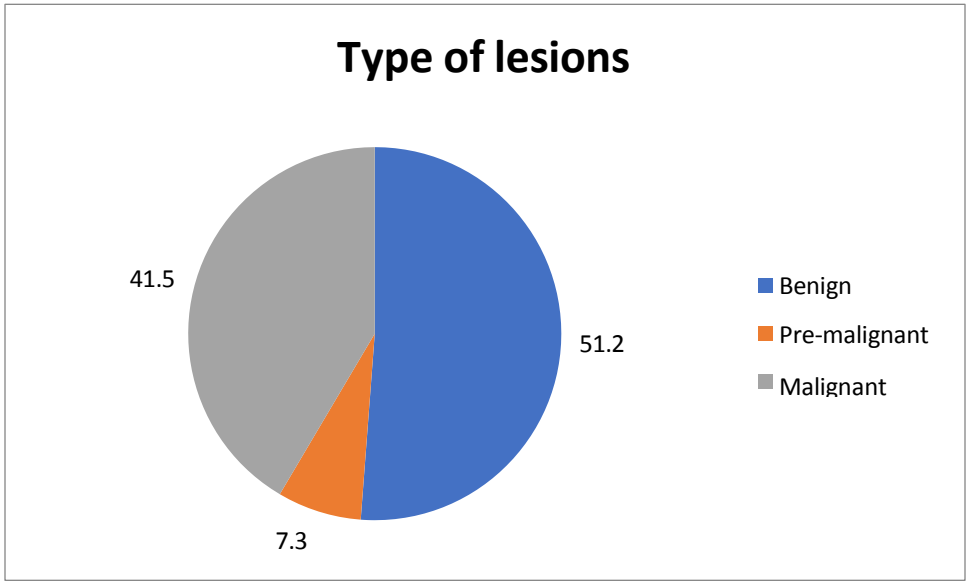

Majority of patients (48.8%) were diagnosed as benign prostatic hyperplasia followed by 41.5% of patients were diagnosed as prostatic adenocarcinoma, 7.3% of patients were diagnosed as prostatic intraepithelial neoplasia and one patient was diagnosed as clear cell cribriform hyperplasia by histopatholog y [Table 2] [Fig: 1,4,7,10&13].

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Table 2: Distribution of patients according to histopathology diagnosis (n=41):

HP diagnosis	**n**	**%**
Adenocarcinoma	17	41.5
Benign prostatic hyperplasia	20	48.8
Clear cell cribriform hyperplasia	1	2.4
Prostatic intraepithelial neoplasia	3	7.3

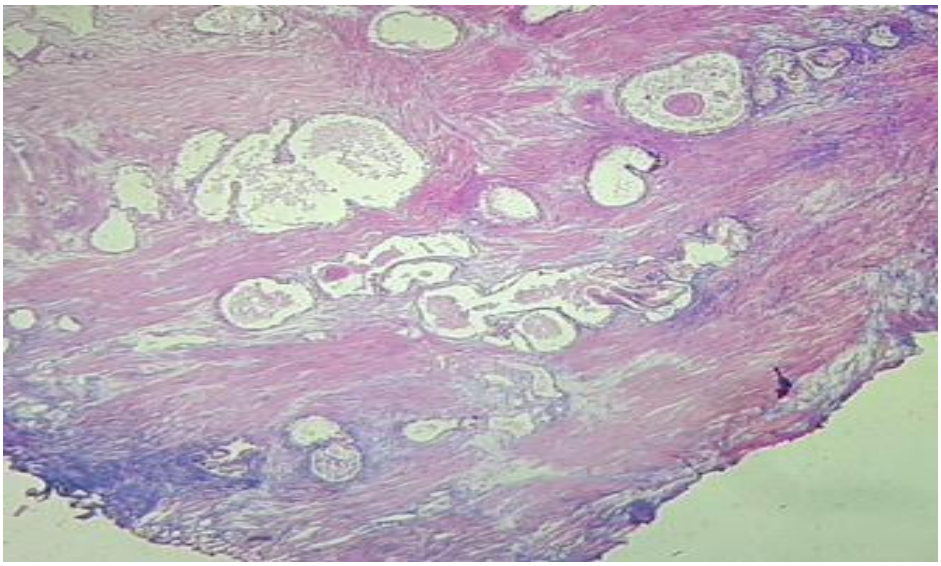

Figure- 1: BPH-Showing nodular lesions composed of variably sized glandular structures (H&E, 200x)

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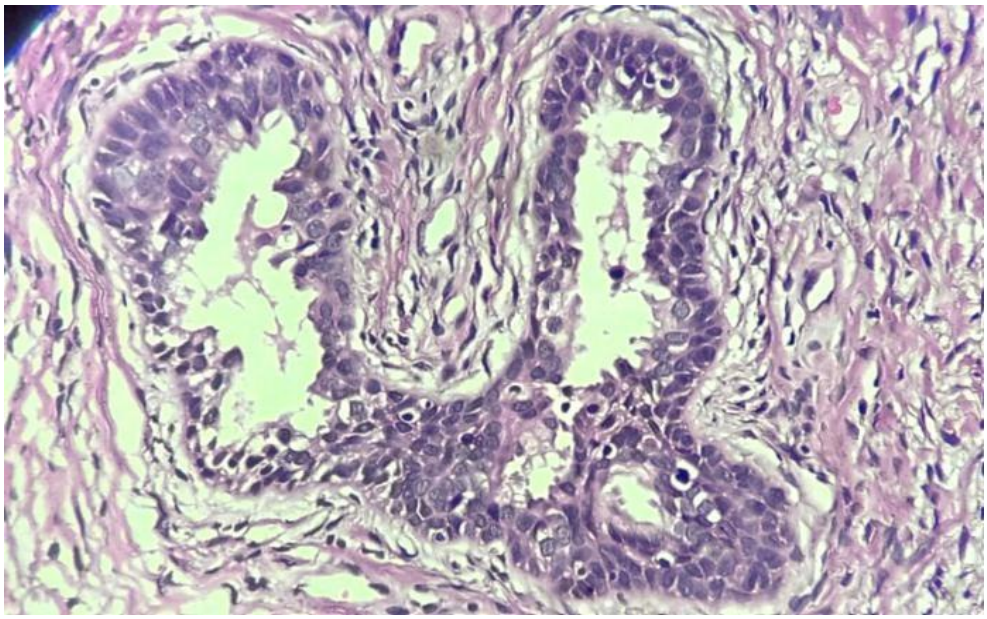

Figure- 4: H&E - High grade prostatic intraepithelial Neoplasia Showing prostatic glands exhibiting cytological atypia and prominent nucleoli (400x)

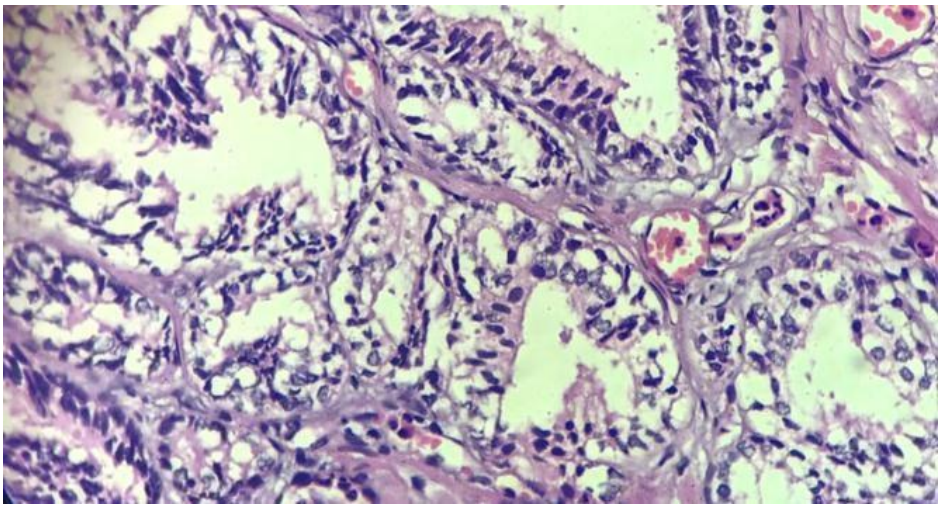

Figure-7: H&E Showing clear cellcribriform hyperplasia with epithelial cells having distinctive clear cytoplasm and small nuclei (400x)

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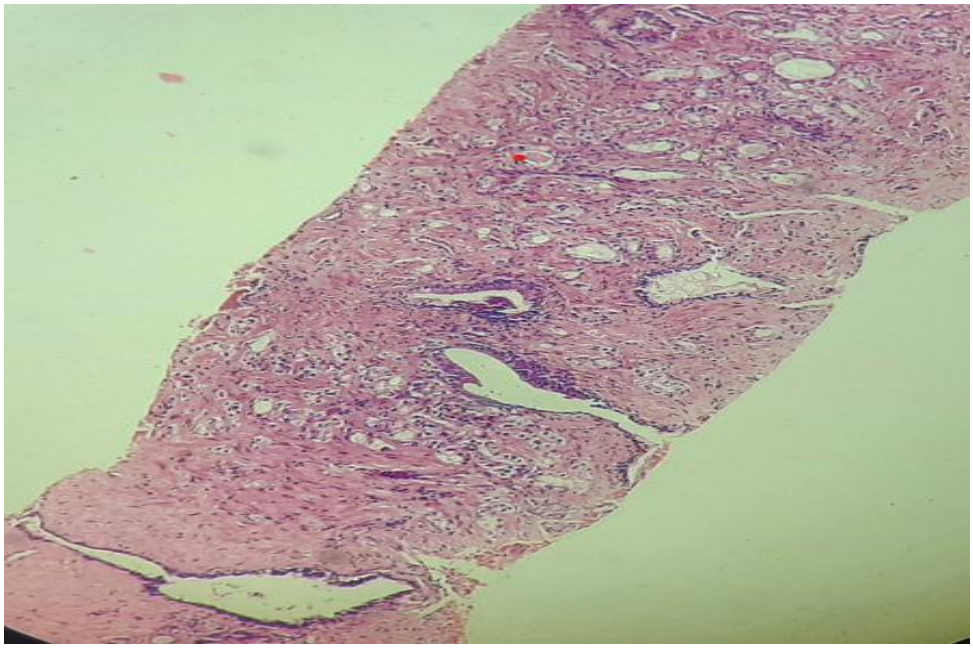

Figure -10: H&E Prostatic adenocarcinoma-Trucut biopsy (50x)

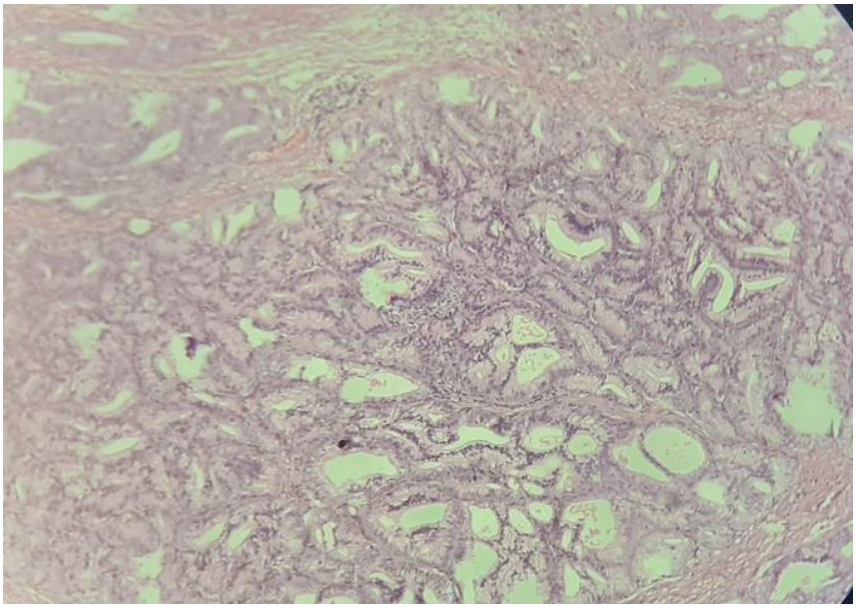

Figure- 13: H&E showing prostatic adenocarcinoma (200x)

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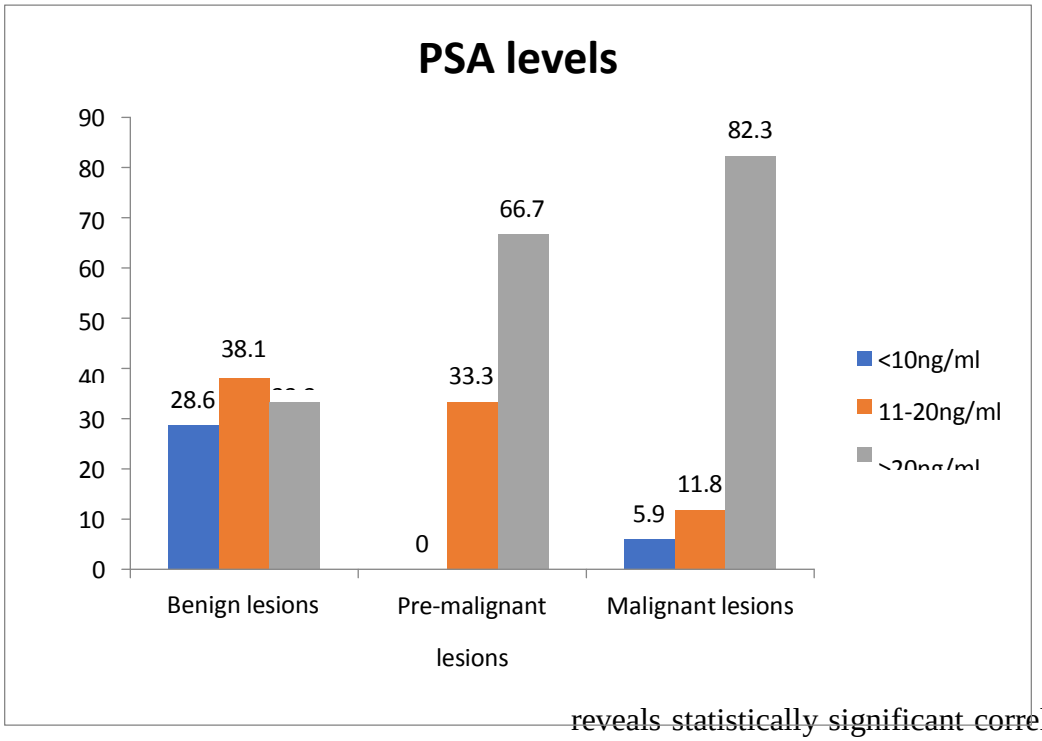
Serum PSA level were studied and found that Patients with malignant lesions had a significantly elevated PSA level [Chart 2].

Chart 2: Cluster bar chart showing distribution of patients according to PSA values:



P40 staining:

58.5% of patients were positive for P40 staining whereas 41.5% of patients were negative for P40 staining. Current study reveals statistically significant correlation between histopathology diagnosis and P40 staining (P value 0.000). All the patients diagnosed with benign prostatic

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hyperplasia, clear cell hyperplasia and prostatic intra-epithelial neoplasia were positive for P40 staining whereas all the patients diagnosed as adenocarcinoma of prostate were negative for P40 staining [Table 3] [Fig: 2,5,8,11 &14].

Table 3: Correlation between histopathology diagnosis with P40 among the study population (n=41):

Histopathology diagnosis	P40			
	Positive		Negative	
	N	%	N	%
Adenocarcinoma	0	0.0	17	100.0
Benign prostatic hyperplasia	20	83.3	0	0.0
Clear cell hyperplasia	1	4.2	0	0.0
Prostatic intraepithelial neoplasia	3	12.5	0	0.0
Total	24	100.0	17	100.0
Chi square	41.10			
P value	0.000			

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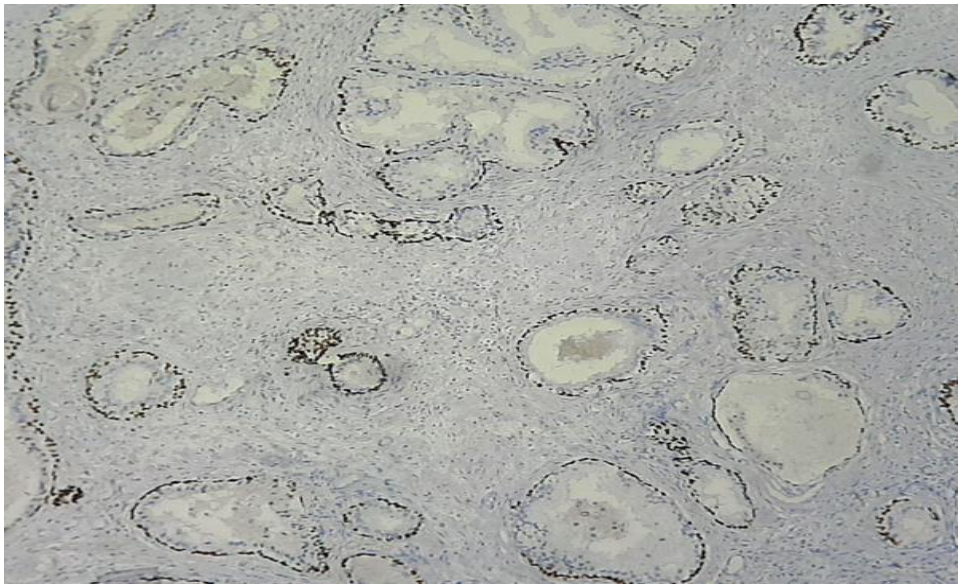

Figure-2: IHC Staining of BPH-Showing nuclear positivity for P40 (200x**)**

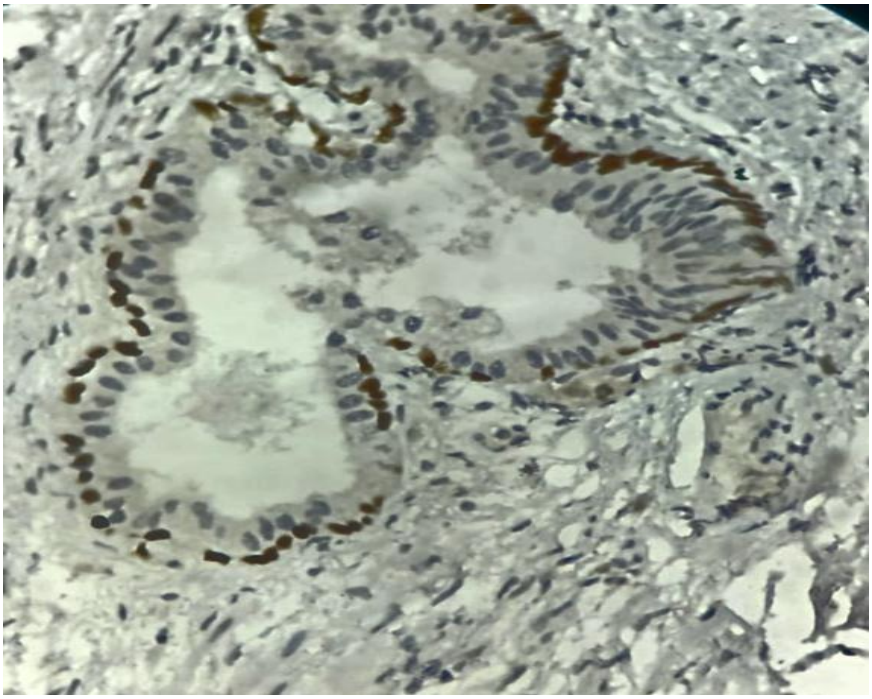

Figure-5: Prostatic intraepithelial neoplasia showing nuclear positivity for P40 (400x)

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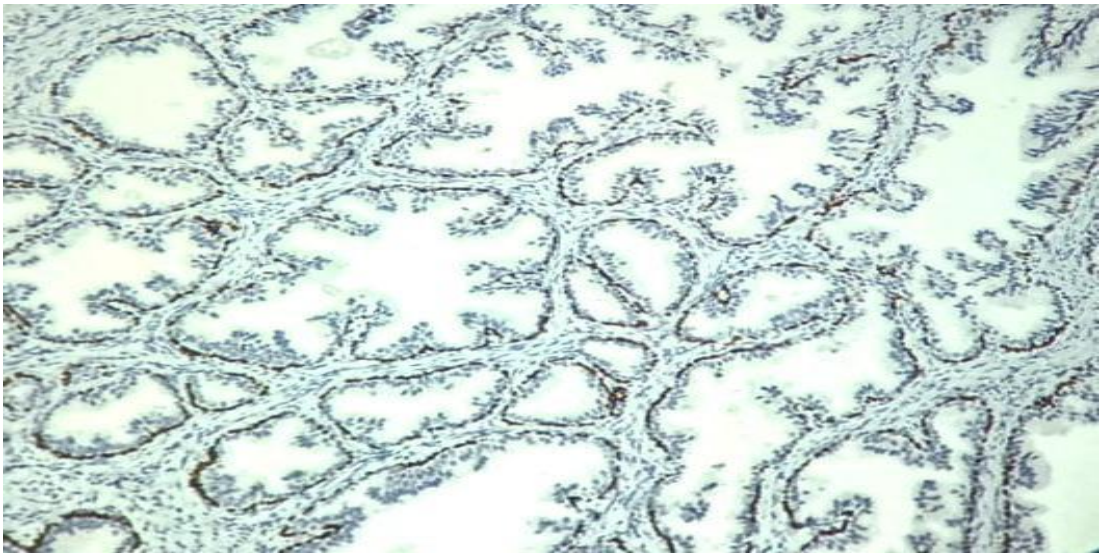

Figure-8: CCCH Showing Nuclear positivity for p40 (200x)

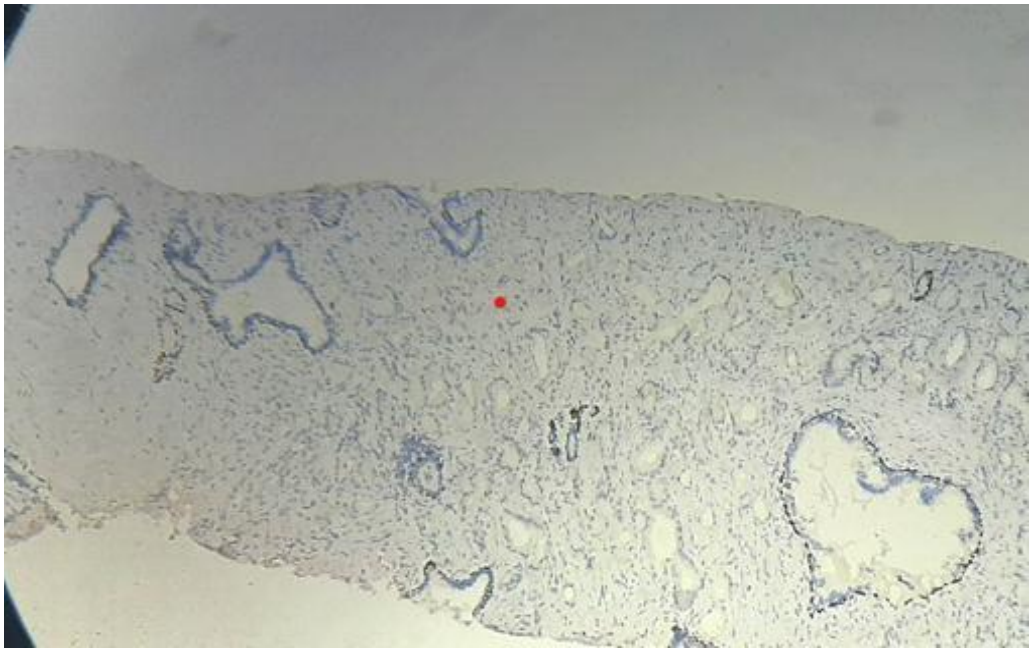

Figure-11: Adenocarcinoma-Negative for P40 (200x)

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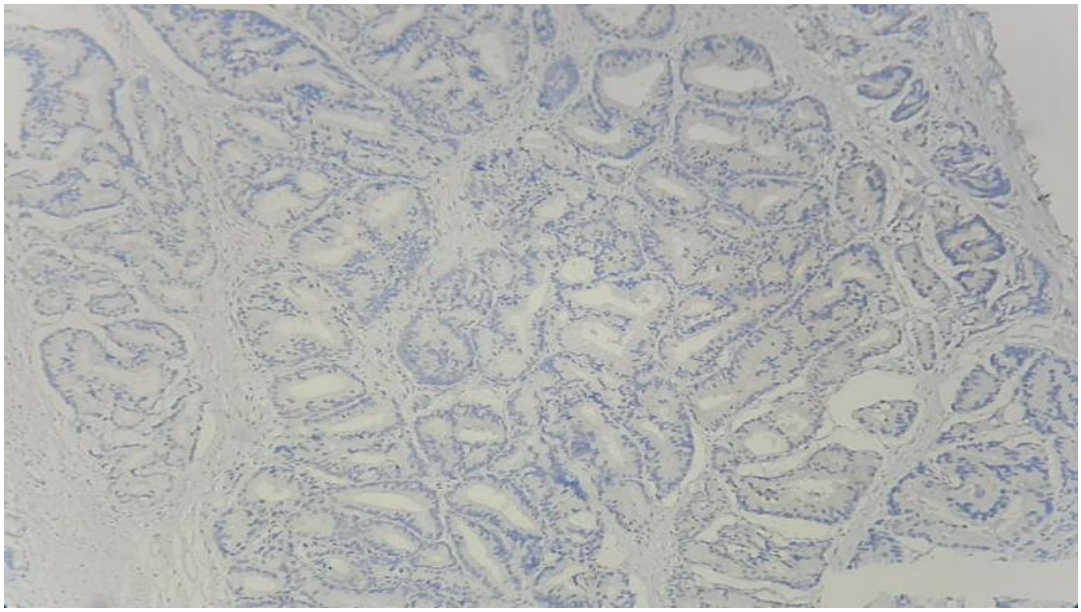

Figure- 14: Adenocarcinoma Negative for P40 (200x)

34Beta E12 staining:

In present study, 34beta E12 staining also show similar findings where all patients with benign lesions and pre-malignant lesions were positive for 34BETA E12 staining whereas all the patients with malignant lesions were negative for 34BETA E12 staining (Fig: 3,6,9,12&15). Patients with benign and pre-malignant lesions had higher levels of 34BETA E12 staining, which was statistically significant (P value 0.000) [Chart 3].

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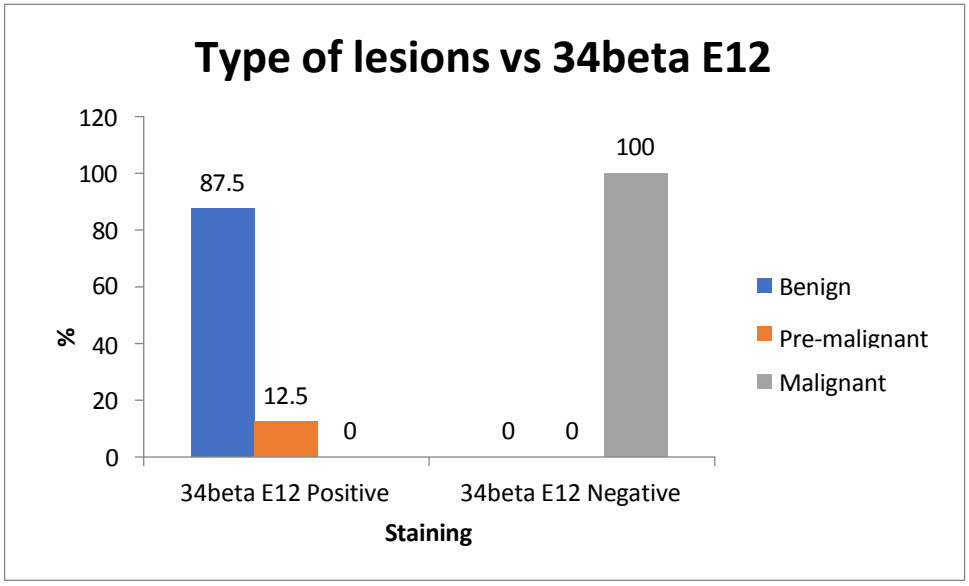
Chart 3: Cluster bar chart showing correlation type of lesion with 34beta E12 among the study population:



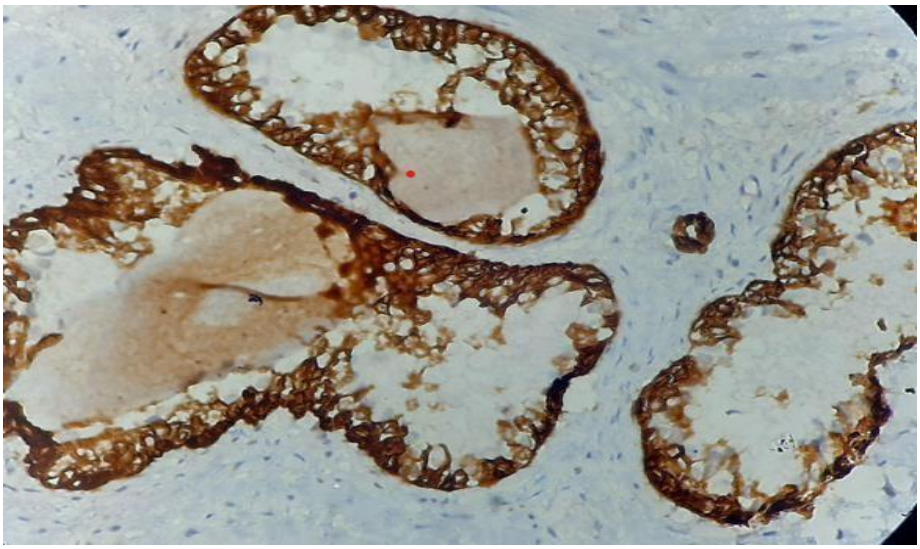


Figure- 3: BPH- Showing cytoplasmic positivity for 34betaE12 (400x)

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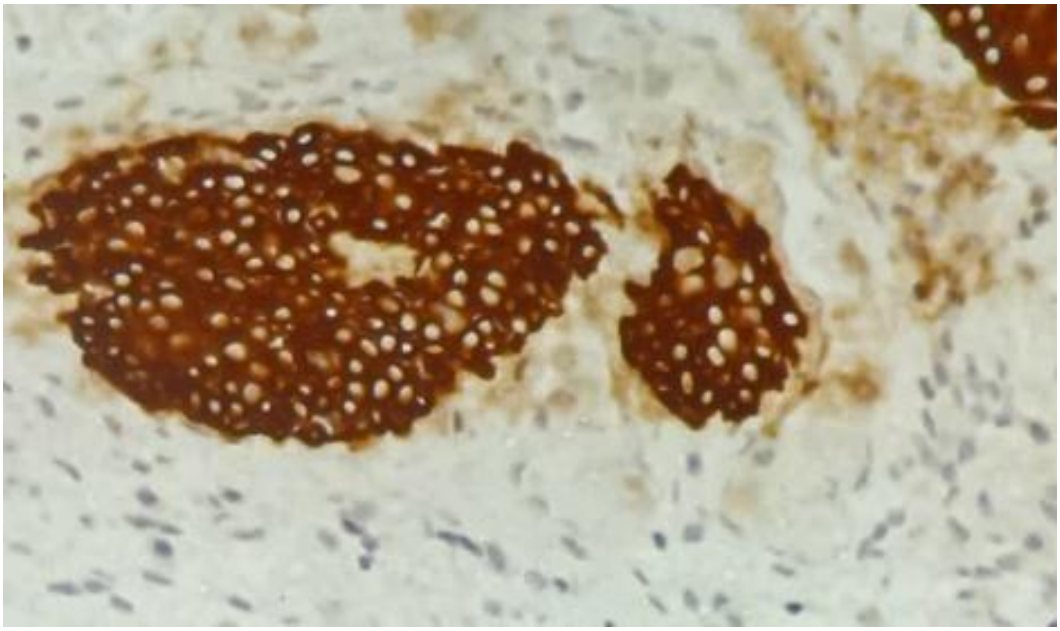

Figure-6: PIN Showing cytoplasmic positivity for 34betaE12 (400x)

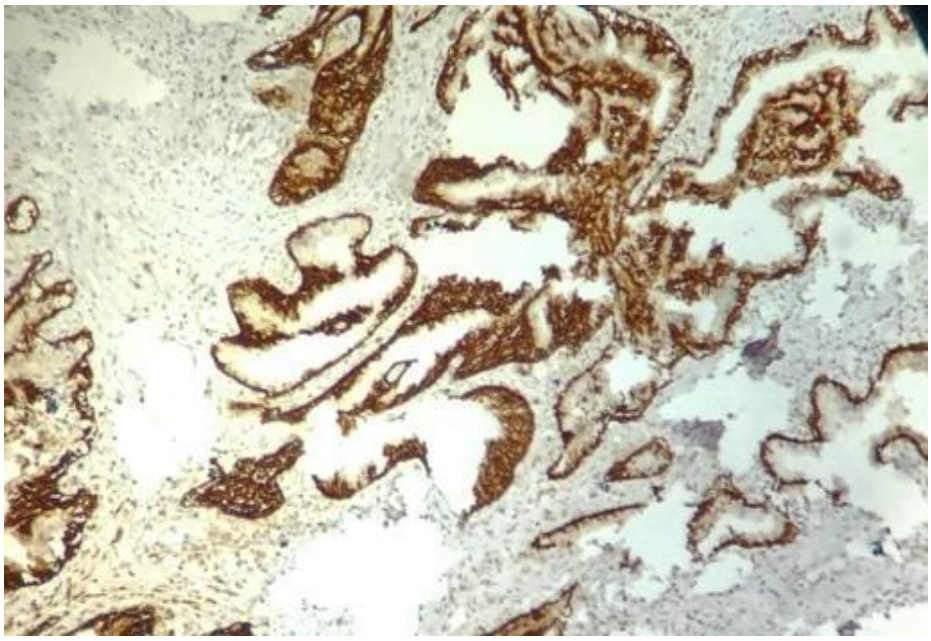

Figure-9: CCCH Showing cytoplasmic positivity for 34BETAE12(200x)

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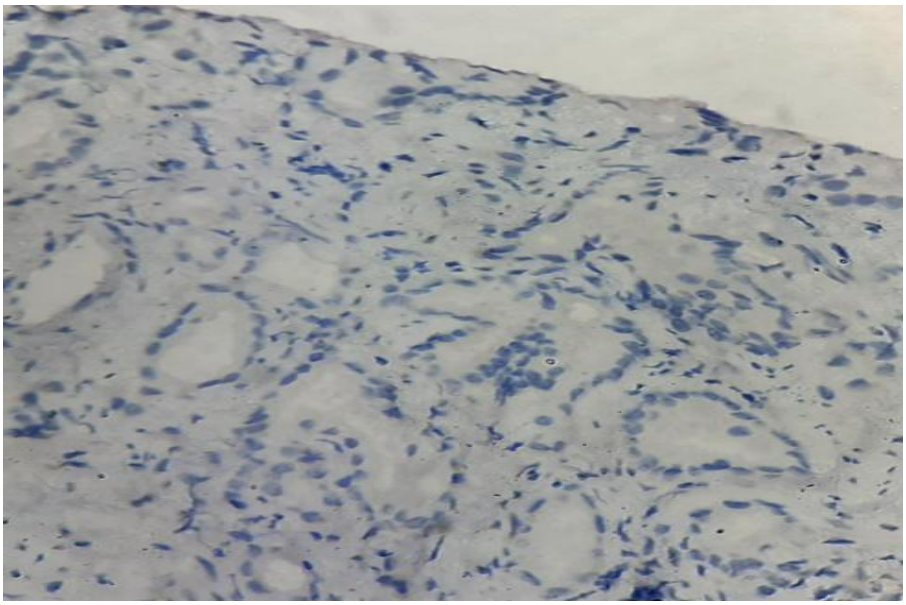

Figure-12: Adenocarcinoma-Negative for 34BETAE12 (400x)

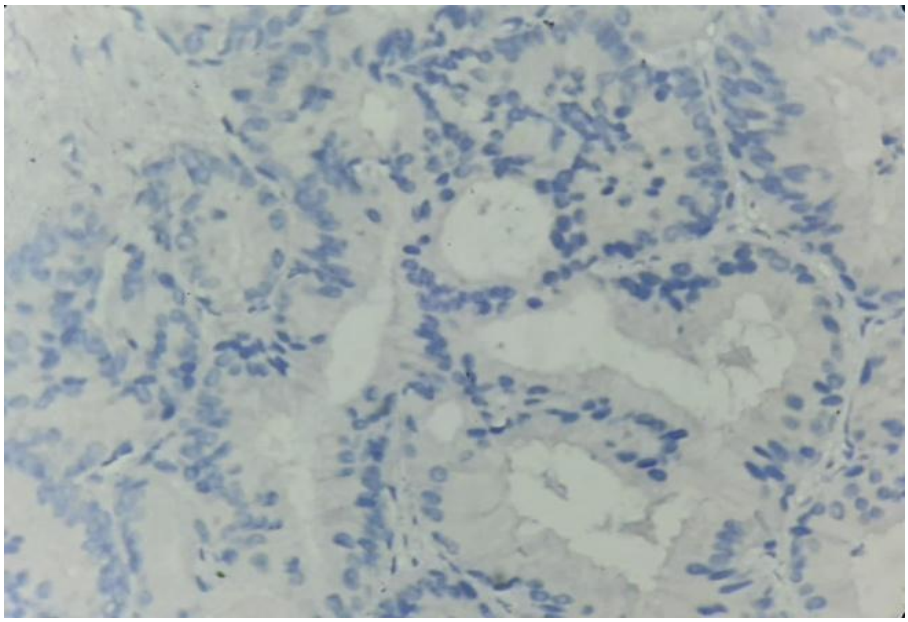

Figure- 15: Adenocarcinoma Negative for 34BETAE12(400x)

DISCUSSION

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The most common pathologies of the prostate among the geriatric males were benign prostatic hyperplasia and adenocarcinomas. Cell differentiation and proliferation led to both diseases. Benign prostatic hyperplasia is the most prevalent cause of lower urinary tract Symptoms in elderly men. Prostate cancer is the second most frequent cancer in males globally.

The prostate gland's androgen dependency has been well acknowledged. Nevertheless, the estrogenic nature of the prostate and the human prostate carcinogenesis have recently been described in the scientific literature. Nuclear hormone receptors have been linked to prostate development and differentiation, including androgen receptors (AR), progesterone receptors, and oestrogen receptors (ER).

There are PCa cell lines that are cytotoxic to the synthetic oestrogen diethylstilbestrol (DES), indicating that the ERs play an important role in the development of prostate cancer. Gene and non-genomic activities, as well as membrane signalling, lead to post-translational modifications of many proteins.

The Essential features for the histopathological diagnosis of prostate carcinoma include, Abnormal glandular architecture, Nuclear atypia and Loss of basal cells. Prostate adenocarcinoma often disrupts the normal glandular architecture of the prostate. Cancer cells may form irregular, fused, or cribriform glands. Unlike benign glands, the cancerous glands are typically smaller, more crowded, and lack the usual basal

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cell layer.The Gleason grading system is used to assess the architecture and differentiation of prostate cancer cells. It assigns a score based on the pattern of tumor cell growth.The scores of primary and secondary patterns are added together to form a Gleason score (range 2–10), with higher scores indicating more aggressive tumors.

Immunohistochemistry plays an important role in the diagnosis and management of prostae carcinoma, especially in the challenging and ambiguous cases. A frequent supplemental technique to confirm or exclude cancer is is immunohistochemical assessment of the basal cells,e specially when the development pattern is obscured, such as in core needle biopsies with few questionable glands and benign mimickers of prostate carcinoma like Atrophy, Basal cell hyperplasia etc.

For the most part, the nuclear immunoreactivity of p40 and p63 is identical in 88% of instances in benign tissues. 60% of cancer patients had cytoplasmic p40 staining, while 0.6 percent of cases have abnormal nuclear staining (compared with 1.4 percent aberrant staining with p63).

The mean age of the patients in current study was 61.02 ± 8.54 years with minimum age of 47 years and maximum age of 76 years and the Clinical signs such as urgency, increased frequency, and dribbling and a hard prostate were found in nearly half of the participants (48.8%).The studies conducted by George

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et al[10], Barakzai *et al*[11] and Hasan *et al*[12] on prostate pathology also reveals similar findings [Table 4].

Table-4 Comparision of age distribution in different studies

S.No	Study	Mean age
1	Present study	61.02 years
2	George et al[10]	66.81 years
3	Barakzai et al[11]	66.9 years
4	Hasan et al[12]	63.0 years

In a research by Physicians Health Study and the Health Professionals Follow Up Study, those over 80 with prostate lesions were shown to be less common[13].

Histopathological diagnosis:

In present study, majority of patients (48.8%) were diagnosed as benign prostatic hyperplasia followed by 41.5% of patients were diagnosed as prostatic adenocarcinoma, 7.3% of patients were diagnosed as Prostatic Intraepithelial neoplasia and one patient was diagnosed as clear cell hyperplasia by histopathology. These findings were

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compared with the similar studies as follows [Table 5].

Table-5 Comparision of benign,premalignant and malignant lesions in various studies

S no	Study	Benign	Pre-malignant	Malignant
1	Present study	48.8%	9.7%	41.5%
2	Hasan et al[12]	77.8%	-	22.2%

.PSA levels:

These findings were based on a study of 21 healthy men with benign lesions in which 38.1 percent of patients had PSA values between 11 to 20, as well as 33.3 percent have more than 20ng/ml , and 28.6 percent had levels below 10 ng/ml. Patients with pre-malignant lesions had PSA values of between 11 and 20ng/ml in 33.3% of the patients, whereas 66.7% of patients had PSA levels of more than 20ng/ml. As much as 82.3 percent of the

17 patients who had malignant lesions had PSA levels more than 20ng/ml, whereas 11.8% had values of 11 to 20ng/ml and 5.9% had PSA levels of less than 10 ng/ml.Patients with malignant lesions had a significantly elevated PSA level (P value 0.041).

Findings from a previous research by Grindstad T *et al*[14] reveal no significant

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association between PSA levels and kind of lesion. No significant correlation was found between PSA levels and the kind of lesions in a research conducted by Daniels G *et al*[15].

We studied the expression of two basal cell markers like p40 and 34betaE12 in 41 cases. Basal cells will be invariably lacking in prostatic carcinoma were not always present on benign prostatic epithelium on H&E stained slides. With advent of immunohistochemistry those cases in which basal cells were not identified in H&E sections were diagnosed as benign lesions based on basal cell positivity for P40.In our study we analyzed theexpression of P40 and 34betaE12 in benign,premalignant and malignant lesions.

P40 staining:

In present study, 58.5% of patients were positive for P40 staining whereas 41.5% of patients were negative for P40 staining. All the patients diagnosed with benign prostatic hyperplasia, clear cell hyperplasia and prostatic intra-epithelial neoplasia were positive for P40 staining whereas all the patients diagnosed as adenocarcinoma of prostate were negative for P40 staining. There was statistically significant correlation between histopathology diagnosis and P40 staining (P value 0.000).

All patients with benign lesions and pre-malignant lesions were positive for P40 staining whereas all the patients with malignant lesions were negative for P40 staining. Patients with benign and

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premalignant lesions had significantly higher levels of P40 positivity (P value 0.000).

34Beta E12:

In present study, 58.5% of patients were positive for 34beta E12 staining whereas 41.5% of patients were negative for 34beta E12 staining.

In present study, all the patients diagnosed with benign prostatic hyperplasia, clear cell hyperplasia and prostatic intra-epithelial neoplasia were positive for 34beta E12 staining whereas all the patients diagnosed as adenocarcinoma of prostate were negative for 34betaE12 staining. There was statistically significant correlation between histopathology diagnosis and 34beta E12 staining (P value 0.000).

In present study, all patients with benign lesions and pre-malignant lesions were positive for 34BETA E12 staining whereas all the patients with malignant lesions were negative for 34BETA E12 staining. Patients with benign and pre-malignant lesions had higher levels of 34BETA E12 staining, which was statistically significant (P value 0.000) [Table 6].

Table-6: Comparision of 34betaE12 in benign,premalignant and malignant lesions

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S no	Study	34Beta E12		
		Malignant	Pre-malignant	Benign
1	Present study	0%	100%	100%
2	Hasan et al[12]	4%	-	92.9%

In present study, there was no statistical significant correlation between P40 and 34beta E12 staining among the study population (P value 1.00).

In a study done by Ashwini et al9 showed that the sensitivity of both p40 and 34ßE12 is 95.92%, specificity being 100%, positive predictive value being 100% and the negative predictive value being 94.12% suggesting a reasonably good comparison with each other. Study concluded that the use of p40 in the diagnoses of suspicious prostate glands and compares favorably and has close correlation between staining with 34ßE12 in basal cells.

Prostate gland basal cell display was shown to be closely correlated to P40 and 34Beta E12 in a research conducted by Brustmann et al16, which may give more information on the dignity of prostate glandular proliferations.

CONCLUSION

Prostate lesions are responsible for noteworthy deaths and suffering among the elderly males globally. It is unusual to

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misdiagnose small focus of prostatic malignancies or over diagnosis of prostatic benign lesions which were imitating malignancies. Immunohistochemistry plays an important role in the diagnosis of prostate tumors by differentiating benign and mailgnant lesions, especially basal cell markers.

All the benign lesions and pre-malignant lesions showed positive staining for P40 and 34Beta E12 stains whereas all the malignant lesions were negative for P40 and 34Beta E12 staining in this study.

In present study, it was concluded that there was statistically significant increase in number of positivity for P40 staining and 34BetaE12 staining in benign and pre-malignant lesions when compared with malignant lesions. Thus these IHC markers can be used for differentiating benign, premalignant and malignant lesions thereby playing an important role in management of patient and therapeutic outcome.

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