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Trefoil factor 3 (TFF3) As A Prognostic and Pathogenic Factor in Prostatic Adenocarcinoma in Iraqi Patients

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Abstract

Background: The prostate gland has a high rate of benign and malignant disease, including "benign prostatic hyperplasia (BPH)" and prostatic adenocarcinoma. BPH occurs in as many as 75% of males by the eighth decade of life. "Prostate cancer" is the second most common cancer in the world, and in Iraq, it is the third most common cancer after lung and colorectal cancers. "Trefoil factor 3 (TFF3)" is a member of the trefoil factor family, which has been associated with epithelial repair and tumorigenesis. TFF3 promotes a cancerous state by increasing cell migration, invasion, and the formation of the tumor microenvironment, and by inhibiting apoptosis, likely through the activation of a phosphoinositide 3-kinase-protein kinase B (PI3K/AKT) signaling pathway.

Aim: This study aimed to evaluate TFF3 expression in prostatic adenocarcinoma versus BPH and to assess its correlation with pathological features, including age, preoperative PSA level, Gleason grade, perineural, and lymphovascular invasion.

Method: An examination of sixty formalin-fixed paraffin-embedded prostatic tissue blocks was performed: 30 "benign prostatic hyperplasia (BPH)" and 30 prostatic adenocarcinomas. Hematoxylin and eosin (H&E) staining and immunohistochemical staining with anti-TFF3 monoclonal antibody were performed on each block. TFF3 expression was qualitatively evaluated under light microscopy and correlated with computer-derived clinicopathologic characteristics.

Results: TFF3 expression was demonstrated in 93.3% of cases of prostatic adenocarcinoma and was expressed in only 20% of BPH cases. A highly statistically significant correlation was observed between TFF3 expression and Gleason grade ($p = 0.00004$) and with preoperative PSA ($p = 0.000012$). There was no statistically significant correlation with age, perineural, or lymphovascular invasion. TFF3 has a sensitivity of 93% and a specificity of 80% as a diagnostic marker.

Conclusion: TFF3 is significantly overexpressed in prostatic adenocarcinoma compared to BPH, particularly in high-grade tumors, supporting its potential as a diagnostic biomarker.

1. Introduction

Prostatic adenocarcinoma is known to be one of the most common cancers in men, and it is the fifth most common cause of cancer-related death. In 2020, prostatic adenocarcinoma accounted for approximately 1.41 million new cases and 375,304 deaths worldwide **[1]**. In the United States, “Prostate cancer” is the second most common cancer in men **[2]**In Iraq, prostate adenocarcinoma is the third most common cancer after lung and colorectal cancer. The Iraqi Cancer Registry reported for 2020: 1,117 new cases and 416 deaths from the disease in Iraq. **[3]**.

“Prostate cancer” incidence increases significantly with advancing age, with greater than 75% diagnosed in individuals 65 years and older **[4]**. A multifactorial etiology is recognised in the pathogenesis of “Prostate cancer”, including environmental, hormonal, and, most importantly, genetic risk factors. Men with a history of “Prostate cancer” in their family, especially multiple first-degree relatives, have a 5–10 times greater risk. **[5]**. Genetic instability has been associated with the initiation and progression of “Prostate cancer”, highlighting the importance of molecular markers for risk assessment and treatment outcomes **[6]**.

Although PSA-based screening is routinely performed, approximately 15% of “Prostate cancer” cases may present with normal PSA levels **[7]**. Histologically, prostatic adenocarcinoma typically exhibits acinar structures with varied architectural patterns, necessitating an assessment of both morphologic and immunohistochemical parameters for accurate diagnosis. Tumor grade, particularly as defined by the Gleason scoring system, is one of the strongest prognostic indicators of biological behavior **[8]**. The WHO classification from 2016 and the consensus from the ISUP presented a new grading scheme focusing on grade groups that more accurately reflect clinical outcomes **[9]**.

In contrast, “benign prostatic hyperplasia (BPH)” is a non-cancerous enlargement of the prostate that is associated with age; it involves both stromal and epithelial components of the prostate. BPH causes symptoms in the lower urinary tract, produces hyperplastic nodules on histology, and the processes that lead to BPH are largely unknown. The role of interactions between the stroma and epithelium remains particularly unclear **[10, 11]**.

Recently, the Trefoil Factor Family (TFF) of peptides, particularly TFF3, has gained attention in cancer biology. The TFF family contains three peptides—TFF1, TFF2, and TFF3—which are known as small secretory proteins mainly expressed in mucosal epithelia, especially in the gastrointestinal tract **[12]**. TFF3 has become an important factor in mucosal healing, epithelial restitution, and neoplastic transformation. TFF3 is a 59 amino acid peptide that can exist in a monomer or dimer form, with the dimer being more biologically active **[13]**.

TFF3 is a well-established factor in promoting the survival, migration, and invasion of cancer cells. This is mediated through multiple signaling pathways, including the PI3K/AKT and the MAPK/ERK pathway **[13, 14]**. TFF3 also has an anti-apoptotic effect, in part, by altering the balance of pro- and anti-apoptotic factors. For example, the downregulation of TFF3 has been linked to reduced expression of BCL-2 and increased expression of BAX, leading to the release of cytochrome c from the mitochondria, Smac/DIABLO, and subsequent activation of caspases 3 and 9. These molecular cascades illustrate their engagement in both tumor initiation and tumor progression **[15]**.

High levels of TFF3 expression have been shown to associate with a variety of cancers, including the breast, stomach, pancreas, colon, and prostate **[16, 17]**. For “Prostate cancer” specifically, studies have demonstrated a positive correlation between TFF3 expression, high-grade tumors, and aggressive features, suggesting it may be a valuable diagnostic and prognostic biomarker. TFF3 may serve as a surrogate marker for poor differentiation, high proliferative index, or invasive potential, making it an interesting drug target for both research activity and utility **[18]**.

TFF3 has significant expression in non-malignant disease processes, including type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), neurodegenerative diseases, and inflammatory bowel disease. All these studies further support the concept of TFF3 as a marker of pleiotropy in disease processes **[19, 20]**. The significant expression of TFF3 in multiple organs and response to metabolic and inflammatory signals further suggests its potential role as a general indicator of epithelial stress or regeneration **[21]**.

In prostate tissues, the increased expression of TFF3 in adenocarcinoma versus benign prostatic hyperplasia warrants consideration of using such differential expression for diagnostic purposes **[22]**. Several immunohistochemical investigations have provided evidence suggesting that TFF3 may be a useful marker to discriminate between benign and malignant lesions of the prostate **[16, 23]**. However, while preliminary data are promising, the diagnostic performance of TFF3, including its sensitivity and specificity, as well as its association with relevant clinical parameters such as PSA levels, "Gleason score", and perineural or lymphovascular invasion, has yet to be fully investigated.

Given this background, the present study was undertaken to assess the expression pattern of TFF3 in prostatic adenocarcinoma and benign prostatic hyperplasia. Additionally, the study aimed to investigate any correlation between TFF3 immunoreactivity and various clinicopathological characteristics, including age, preoperative PSA levels, histological grade, perineural invasion, and lymphovascular invasion. The goal of the study was to determine whether TFF3 can be a reliable diagnostic and possibly prognostic marker in the clinical evaluation of prostatic cancer in an Iraqi patient cohort.

3. Patients, Materials, and Methods

3.1 Study Design

This retrospective case-control study was conducted in the Histopathology Unit of Al-Hussein Medical City, Karbala, Iraq, from January 2022 to November 2022.

3.2 Study Population

A total of 60 formalin-fixed, paraffin-embedded (FFPE) prostatic tissue samples were enrolled in the study, comprising 30 cases of "benign prostatic hyperplasia (BPH)" and 30 cases of prostatic adenocarcinoma (PAC) of various histological grades. These cases were obtained from the archives of the Histopathology Laboratory at Al-Hussein Medical City and a private specialised pathology laboratory, covering the period from 2020 to 2022.

All cases had been previously diagnosed as BPH or prostatic adenocarcinoma. Re-evaluation of the hematoxylin and eosin (H&E)-stained sections by experienced pathologists was conducted to confirm the diagnosis, determine tumor grade according to the 2016 WHO classification, and assess for perineural and lymphovascular invasion. The prostatic specimens were derived from various procedures, including transurethral resection of the prostate (TURP), core needle biopsies, and open prostatectomies, as shown in Table 3.1.

Table 3.1: Type of Prostatic Biopsy and Number of Cases

Type of Biopsy	Number of Cases
Core Needle Biopsy	40
TURP	15
Open Prostatectomy	5
Total	**60**

Clinicopathological parameters, including patient age, serum PSA level, tumor grade, perineural invasion, and lymphovascular invasion, were extracted from pathology reports and hospital records from the Oncology Department at Al-Hussein Medical City.

3.3 Inclusion and Exclusion Criteria

3.3.1 Inclusion Criteria

1. Male patients aged between 50 and 90 years.
2. Availability of preoperative serum PSA levels.
3. Prostatic specimens obtained via TURP, core needle biopsy, or open prostatectomy with confirmed histopathological diagnosis of PAC or BPH.
4. Cases with cytoplasmic immunohistochemical positivity for TFF3.

3.3.2 Exclusion Criteria

1. Specimens with technical artifacts or inadequate fixation/staining.
2. Insufficient tissue material for proper histopathological analysis.
3. Cases with nonspecific or ambiguous immunohistochemical staining.

3.4 Materials

3.4.1 Equipment

- Memmert oven and water bath (Type WNE 22).
- Rotary microtome.
- Positively charged slides (DAKO).
- Glass staining jars, timer, slide box, and cover slips.
- Olympus light microscope with camera.
- Samsung microwave oven.
- PAP pen.
- Laboratory refrigerator.

3.4.2 Chemicals and Reagents

- Ethanol in graded concentrations (50%, 70%, 90%, absolute).
- Xylene.
- Distilled water.
- H&E stain.
- Buffer solutions.
- Mounting medium (DPX).

3.4.3 Immunohistochemistry Reagents

- **Detection System:** DAKO Monoclonal Mouse PolyExcel, UK (sufficient for 100 slides)

- **Primary Antibody:**

    - Name: Anti-TFF3.
    - Manufacturer: Elabscience, India.
    - Clonality: Polyclonal.
    - Host: Rabbit.
    - Isotype: IgG.
    - Reactivity: Human.
    - Localization: Cytoplasmic.
    - Storage: –20°C.
    - Dilution: 1:50.

- **Controls:**
    - Positive: Normal colonic mucosa and colonic carcinoma.
    - Negative: Sections processed without the primary antibody.

3.5 Tissue Processing and Staining

After being routinely dehydrated with graded ethanol, cleaned with xylene, and preserved in 10% neutral-buffered formalin, all biopsies were embedded in paraffin. For histological analysis, sections that were 5 µm thick were cut and stained with hematoxylin and eosin..

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3.6 Immunohistochemical Technique

3.6.1 Sectioning and Slide Preparation

FFPE blocks were cut into 4 µm-thick sections, which were then put on positively charged slides. After 45 minutes of incubation at 60°C, the slides were left to incubate overnight at room temperature..

3.6.2 Staining Protocol for TFF3

1. **Deparaffinization and Rehydration:** Slides were passed through xylene (2x for 5 min), then rehydrated through 95% and 70% ethanol (5 min each), followed by rinsing in distilled water.
2. **Antigen Retrieval:** The slides were immersed in Tris-EDTA buffer (pH 9.0) and subjected to heat-induced epitope retrieval in a microwave at 98°C for 35 minutes, followed by a 20-minute cooling period and washing in buffer.
3. **Blocking Endogenous Peroxidase:** Peroxidase block solution was applied for 5 minutes in a humidified chamber.
4. **Primary Antibody Incubation:** After 30 minutes of room temperature incubation with an anti-TFF3 antibody (1:50 dilution), the slides were buffer washed..
5. **Secondary Detection:** PolyExcel poly-HRP reagent was applied for 10 minutes, followed by buffer wash.
6. **Chromogen Development:** Substrate-chromogen solution was applied for 5 minutes in the dark.
7. **Counterstaining:** A 3-minute hematoxylin counterstain was applied, followed by rinsing with distilled water.
8. **Dehydration and Mounting:** Sections were cleaned in xylene, dehydrated using a series of graded ethanols, and then mounted using DPX. They were then covered with cover slips.
9. **Microscopy:** Stained slides were evaluated under a light microscope for cytoplasmic TFF3 immunoreactivity.

3.7 Scoring System

Cytoplasmic brown staining was considered a positive immunohistochemical reaction, as per manufacturer guidelines.

"Intensity Score:

- 0 = Negative
- 1 = Weak
- 2 = Moderate
- 3 = Strong

Proportion Score (Percentage of Positive Cells):

- 0 = 1–5%
- 1 = 6–19%
- 2 = 20–49%
- 3 = >50%

Immunoreactive Score (IRS)":

Calculated by multiplying intensity and proportion scores (range 0–9):

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- 0–3 = Negative (IRS 0)
- 4–5 = Low expression (+1)
- 6–9 = High expression (+2)

3.8 Image Capture
Representative immunohistochemical fields were identified and captured using an Olympus digital microscope camera. Selection was based on areas showing the most representative TFF3 expression per case.

3.9 Statistical Analysis
For all statistical studies, SPSS software version 28.0 was used. The data was summarized using descriptive statistics, such as mean, percentage, and frequency. To assess relationships between categorical variables, the chi-square test and Fisher's exact test were used. P-values less than 0.05 were deemed statistically significant..

4. Results
4.1 Age Distribution
The study included 60 cases divided equally between “benign prostatic hyperplasia (BPH)” and prostatic adenocarcinoma (PAC). The age of BPH patients ranged from 48 to 86 years, with a mean of 67.2 ± 40.38 years. In PAC cases, ages ranged from 50 to 89 years, with a mean of 69.3 ± 50.36 years. Among the PAC group, 11 patients (36.7%) were aged ≤65 years, whereas 19 patients (63.3%) were aged >65 years (Figure 4.1).

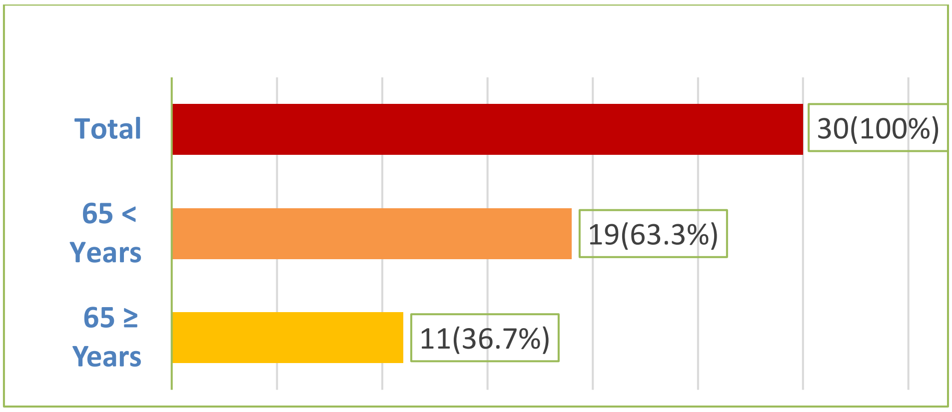

Figure (4.1) Frequency of the Age groups among patients with Prostatic adenocarcinoma (PAC)

4.2 “Serum Prostate-Specific Antigen (PSA)” Levels in PAC Patients
PSA levels were categorized into three groups. Most patients (n = 23; 76.7%) had PSA levels >20 ng/mL, followed by 5 patients (16.7%) within the 10.1–20 ng/mL range, and only 2 patients (6.6%) had PSA levels <10 ng/mL. The overall mean PSA level in PAC patients was 78.40 ± 54.38 ng/mL (Figure 4.2).

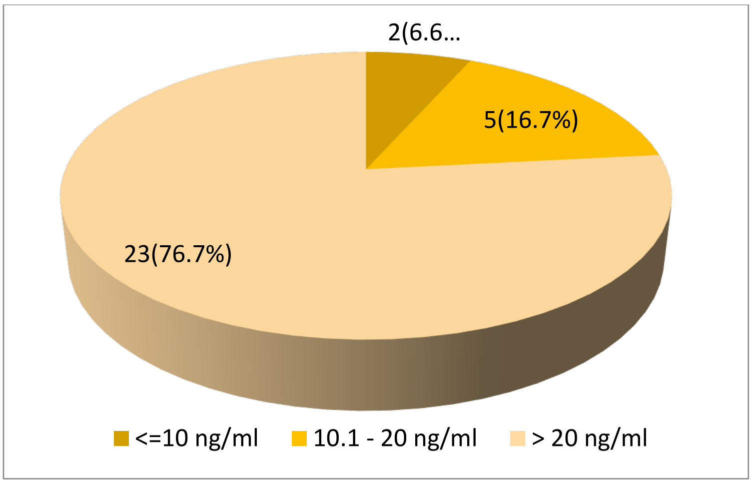

Figure (4.2) Prostatic specific antigen levels (PSA) groups among patients with prostatic adenocarcinoma

4.3 Distribution of Prostatic Adenocarcinoma Grade Groups

Analysis of tumor grade revealed that 13 cases (43.3%) were classified as Grade Group V, 7 cases (23.3%) as Grade IV, 3 cases (10%) each in Grade II and Grade III, and 4 cases (13.3%) as Grade I (Figure 4.3).

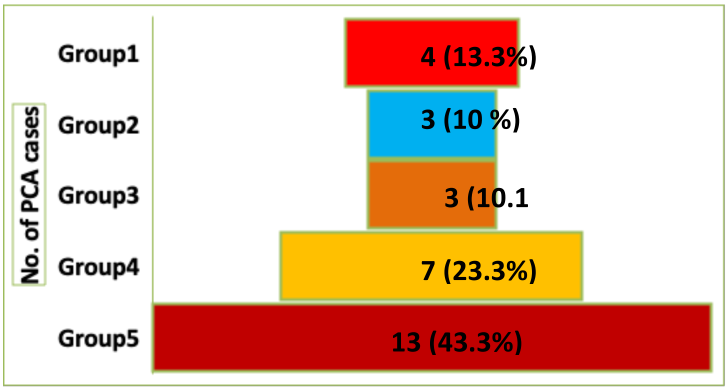

Figure (4.3) Frequency of the Grade group among patients with Prostatic adenocarcinoma (PA)

4.4 Perineural Invasion

Perineural invasion was identified in 19 of 30 PAC cases (63.3%), while 11 cases (36.7%) showed no perineural invasion (Figure 4.4).

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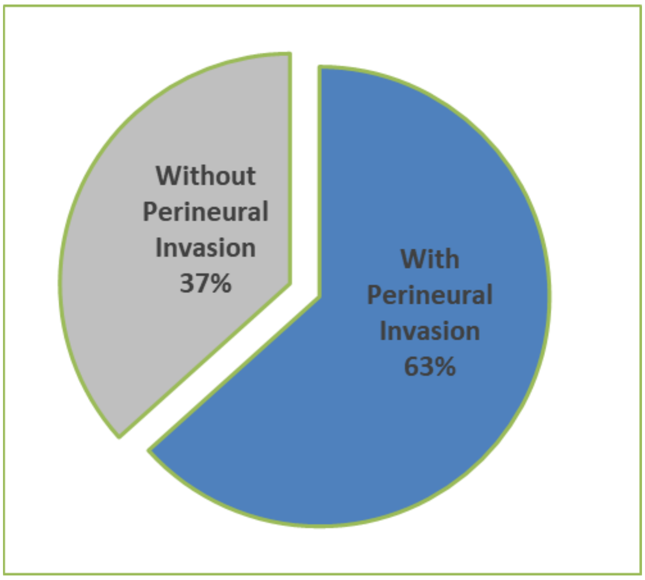

Figure (4.4) Perineural invasion distribution among patients with Prostatic adenocarcinoma (PA) included:19 (63%) cases with perineural invasion and 11(37%) without perineural invasion

4.5 Lymphovascular Invasion

Among the 30 PAC cases, lymphovascular invasion was observed in 7 cases (23.3%), while 23 cases (76.7%) did not exhibit lymphovascular involvement (Figure 4.5).

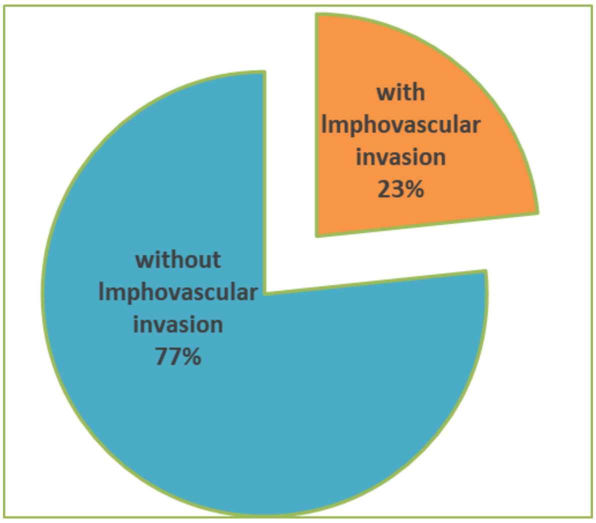

Figure (4.5) distribution of lymphovascular invasion among the patients with prostatic adenocarcinoma: included 23% with lymphovascular invasion and 77% without lymphovascular invasion.

4.6 Immunohistochemical Evaluation of TFF3 Expression

4.6.1 TFF3 Expression in BPH vs. PAC

Immunohistochemical analysis of TFF3 demonstrated cytoplasmic staining in 28 out of 30 PAC cases (93.3%) and in 6 of 30 BPH cases (20%). Among the TFF3-positive BPH cases, 4 cases (66.7%) had an immunoreactive score (IRS) of 6, while 2 cases (33.3%) scored 4. In contrast, 2 PAC cases (6.7%) were TFF3-negative. A statistically significant difference in TFF3 expression between BPH and PAC was observed ($p = 0.00006$) (Figure 4.6).

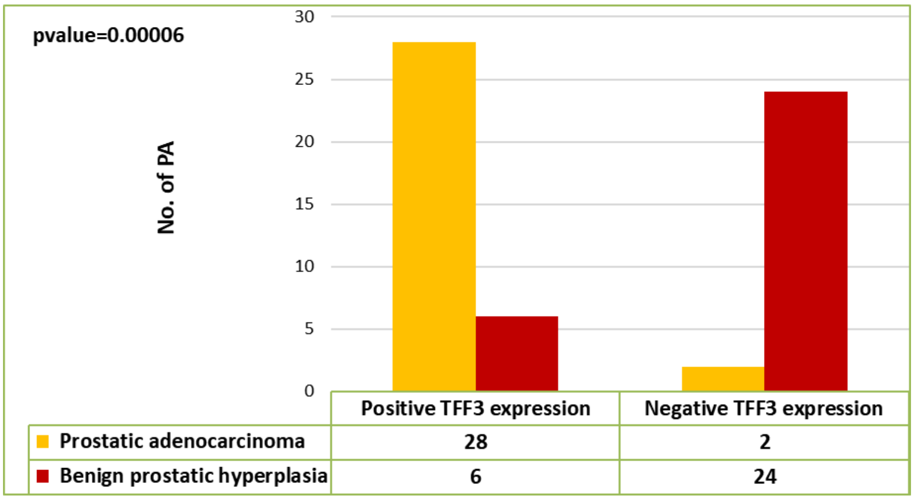

Figure (4.6): Positive and negative TFF-3 expression among Prostatic adenocarcinoma patients and benign prostatic hyperplasia.

4.6.2 Association Between TFF3 Score and Age in PAC

Within the PAC group, 11 patients (36.67%) were aged ≤65 years: 7 (63.6%) had high TFF3 expression (IRS 6–9), and 4 (36.4%) had low expression (IRS 4–5). Among the 19 patients aged >65 years, 15 (78.9%) exhibited high expression (IRS 6–9), 2 (10.5%) had low expression (IRS 4–5), and 2 (10.5%) were negative (IRS 1–3). No statistically significant association was found between age and TFF3 expression ($p > 0.05$).

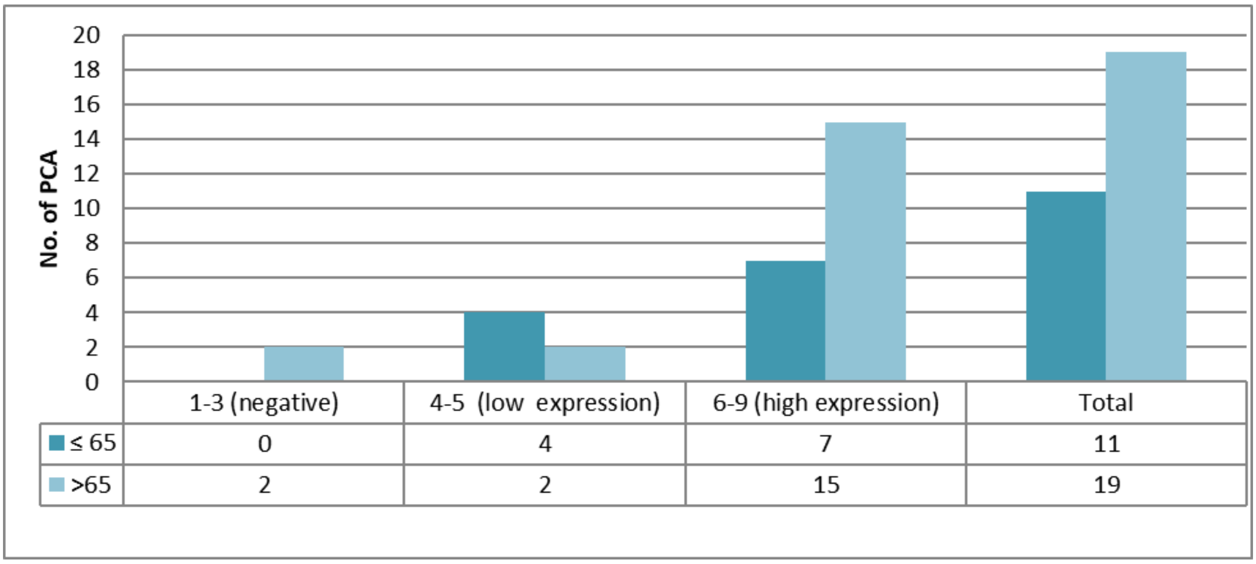

Figure (4.6) TFF3 scoring association to the Prostatic adenocarcinoma patients according to the Age groups

4.6.3 Association Between TFF3 Score and PSA Levels in PAC

A strong positive correlation was found between TFF3 expression and PSA levels. Among patients with a PSA level of <10 ng/mL (n=3), 2 cases (66.7%) exhibited negative expression (IRS 1–3), and 1 case (33.3%) showed low expression (IRS 4–5). All patients with PSA levels of 10.1–20 ng/mL (n=5) had low TFF3 expression (IRS 4–5). Notably, all 22 patients with PSA >20 ng/mL had high TFF3 expression (IRS 6–9). This association was statistically highly significant ($p = 0.000012$) (Figure 4.7).

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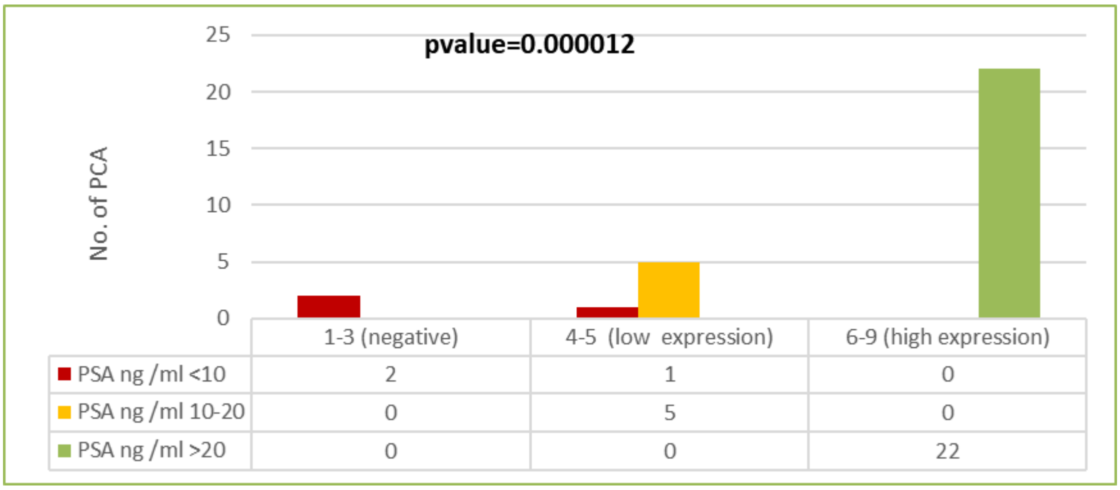

Figure (4.7): TFF_3 scoring association with the Prostatic adenocarcinoma patients according to PSA level

4.6.4 Association Between TFF3 Expression and Tumor Grade

TFF3 expression was strongly correlated with higher tumor grade. Among PAC cases in Grade Groups IV and V, the majority exhibited high TFF3 expression (IRS 6–9). Lower-grade tumors (Groups I–III) demonstrated lower TFF3 scores or negative expression. A statistically highly significant correlation was observed between TFF3 IRS and tumor grade ($p = 0.00004$), as shown in Table 4.1 and Figure 4.8.

Table 4.1: Association Between Grade Group and TFF3 Immunoreactivity in Prostatic Adenocarcinoma

Grade Group	Negative (IRS 1–3)	Low Expression (IRS 4–5)	High Expression (IRS 6–9)	P value
1	2 (50.0%)	2 (50.0%)	0 (0.0%)	**0.00004**
2	0 (0.0%)	3 (100.0%)	0 (0.0%)	
3	0 (0.0%)	0 (0.0%)	3 (100.0%)	
4	0 (0.0%)	0 (0.0%)	7 (100.0%)	
5	0 (0.0%)	1 (7.7%)	12 (92.3%)	

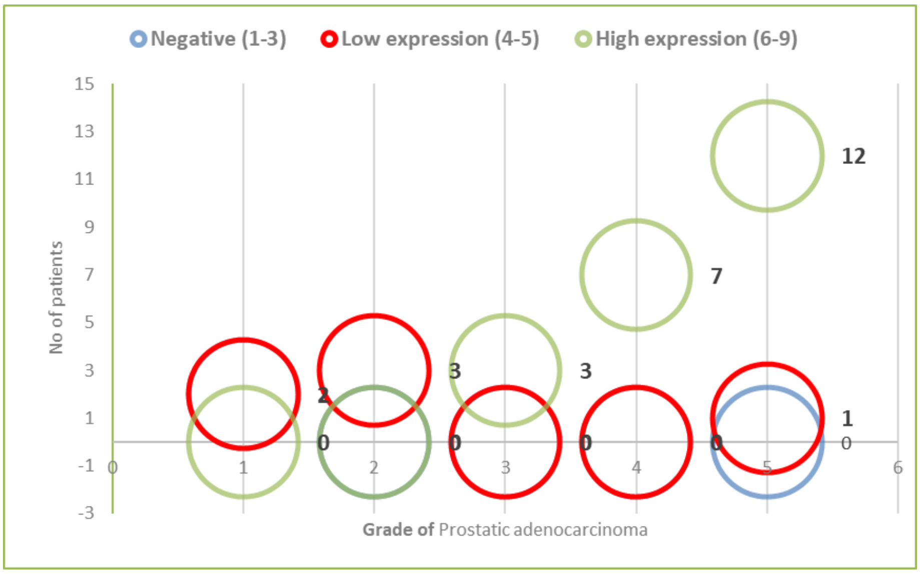

Figure (4.8) The Frequency of the TFF3 score with grade of Prostatic adenocarcinoma

4.6.5 Association Between TFF3 Expression and Perineural Invasion

Of the 19 PAC cases with perineural invasion, 16 (84.2%) had high TFF3 expression (IRS 6–9), and 3 (15.8%) had low expression (IRS 4–5). None of these cases were TFF3-negative. Among the 11 PAC cases without perineural invasion, 6 (54.5%) had high TFF3 expression, and 3 (27.3%) had low expression. Although there was a trend toward increased TFF3 expression in cases with perineural invasion, the association was not statistically significant (p = 0.07) (Table 4.2).

Table 4.2: Association Between TFF3 Score and Perineural Invasion in Prostatic Adenocarcinoma

Perineural Invasion	Negative (IRS 1–3)	Low Expression (IRS 4–5)	High Expression (IRS 6–9)	Total Cases	P value
Present (+)	0 (0.0%)	3 (15.8%)	16 (84.2%)	19 (100%)	0.07
Absent (–)	2 (18.2%)	3 (27.3%)	6 (54.5%)	11 (100%)	

4.6.6 Association Between TFF3 Expression and Lymphovascular Invasion

Of the 7 PAC cases with lymphovascular invasion, 1 (14.3%) had high TFF3 expression (IRS 6–9), and 2 (28.6%) showed low expression (IRS 4–5). Among the 23 cases without lymphovascular invasion, 21 (91.3%) demonstrated high TFF3 expression, 3 (13%) showed low expression, and 2 cases (8.7%) were negative. There was no statistically significant association between TFF3 expression and lymphovascular invasion (p = 0.10) (Table 4.3).

Table 4.3: Association Between TFF3 Score and Lymphovascular Invasion in Prostatic Adenocarcinoma

Lymphovascular Invasion	Negative (IRS 1–3)	Low Expression (IRS 4–5)	High Expression (IRS 6–9)	Total Cases	P value
Present (+)	0 (0.0%)	2 (66.7%)	1 (33.3%)	3 (100%)	0.10
Absent (–)	2 (7.4%)	4 (14.8%)	21 (77.8%)	27 (100%)	

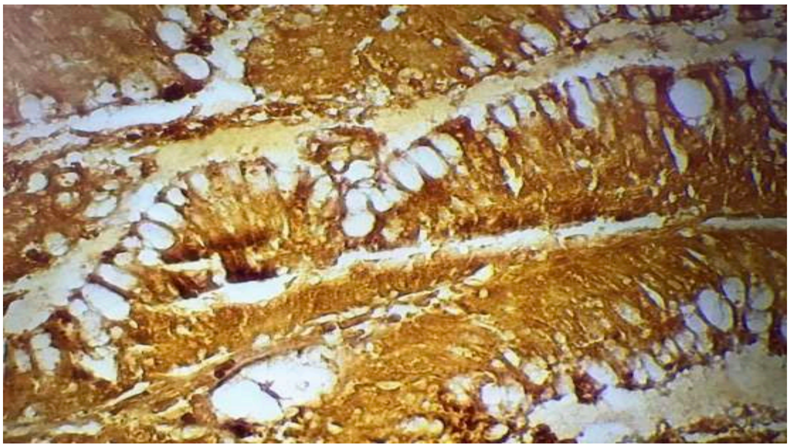

Figure (4.9): TFF3 immunoreactivity in normal colonic mucosa (control), note cytoplasmic expression (X40).

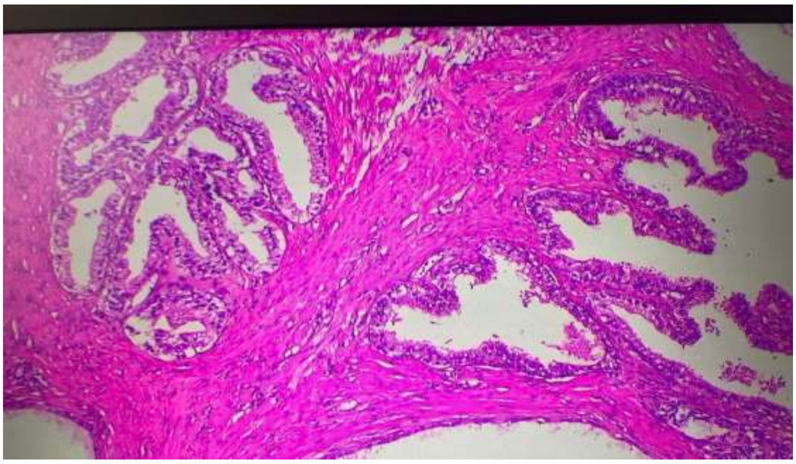

Figure (4.10): Prostate biopsy (TUR) shows benign prostatic hyperplasia, glandular epithelial proliferation plus stromal proliferation, H&E (X10).

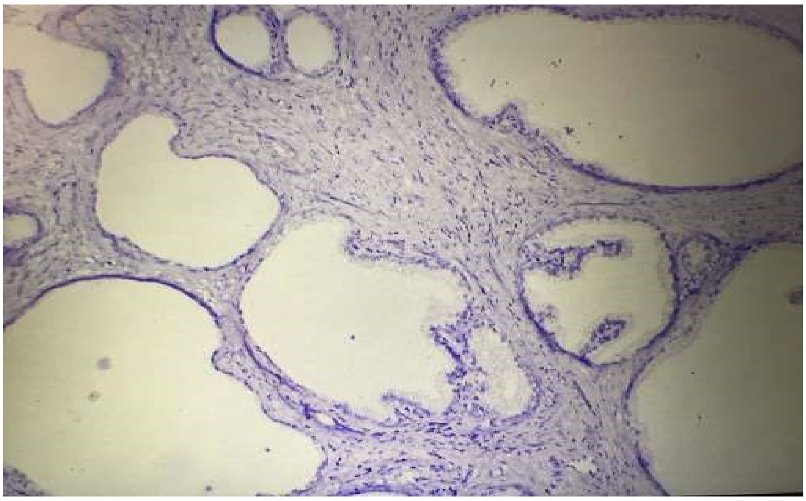

Figure (4.11): Prostate biopsy (TUR) shows TFF3 immunoreactivity (0 score) for BPH (X10).

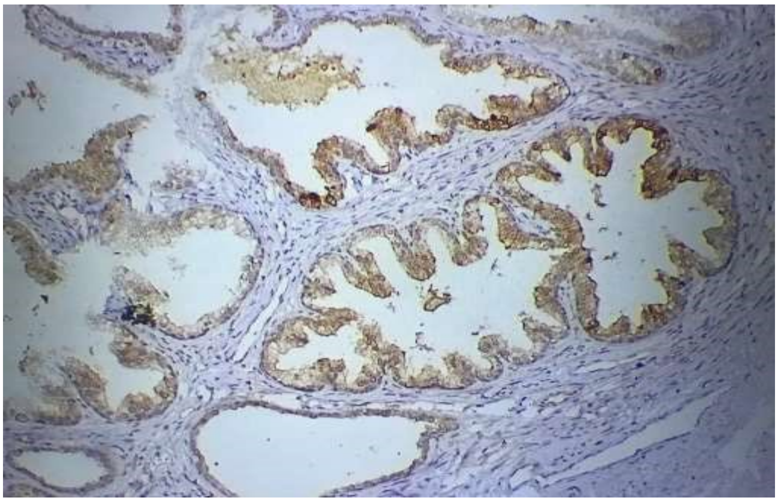

Figure (4.12): TFF3 immunohistochemistry staining in BPH (+1) score (X10).

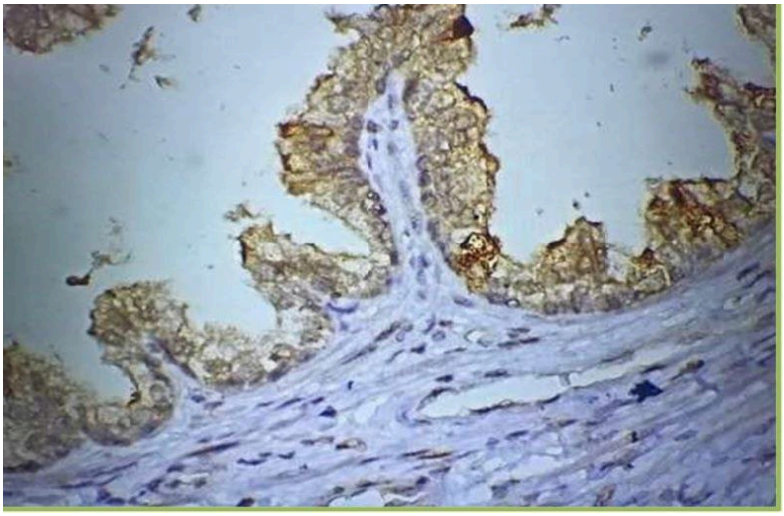

Figure (4.13): TFF3 immunoreactivity in BPH, note the faint staining of the epithelial cells' cytoplasm(+1) score (X40).

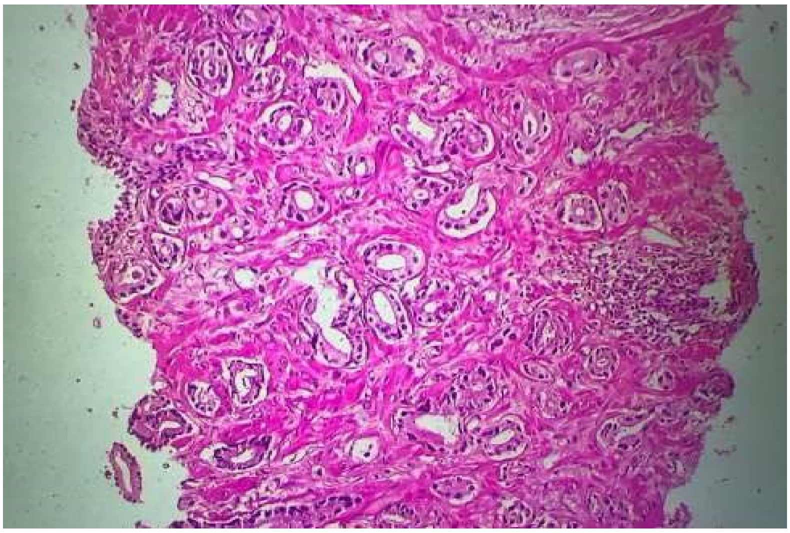

Figure (4.14): Prostate core needle biopsy shows prostatic carcinoma with small well-formed malignant glands, “Gleason score” 3+3=6, grade group 1, H&E (X10).

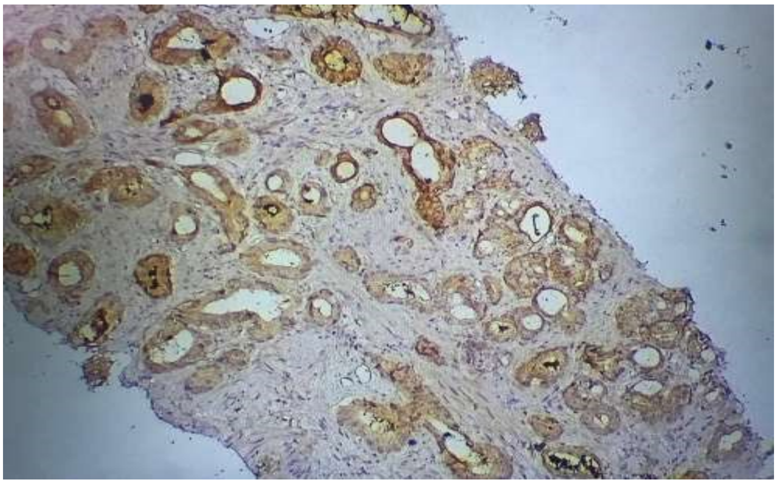

Figure (4.15): Prostate core needle biopsy shows TFF3 immunoreactivity (+1) score for PCa, “Gleason score” 3+3=6 (X10)

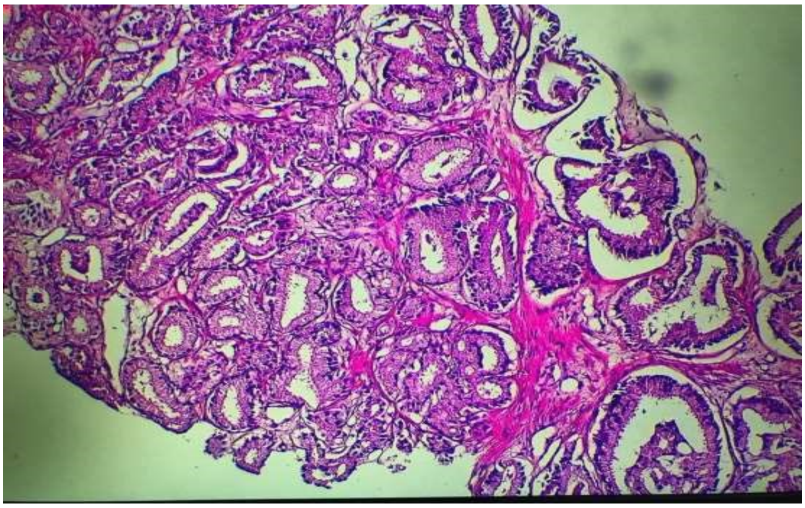

Figure (4.16): Prostate core needle biopsy shows fused malignant glands with cribriform growth pattern, G score 4+3=7, Grage group 3. H&E, (X10).

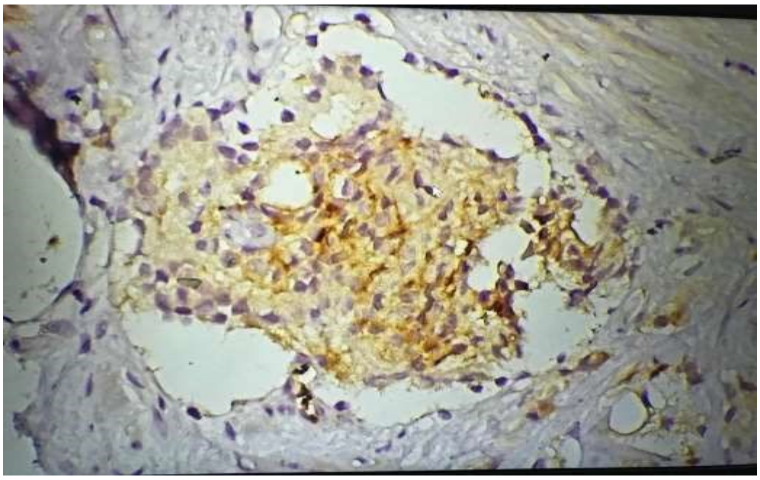

Figure (4.17): TFF3 immunohistochemistry staining in PAC (+1) score, G score 4+3=7, grade 3 (X40).

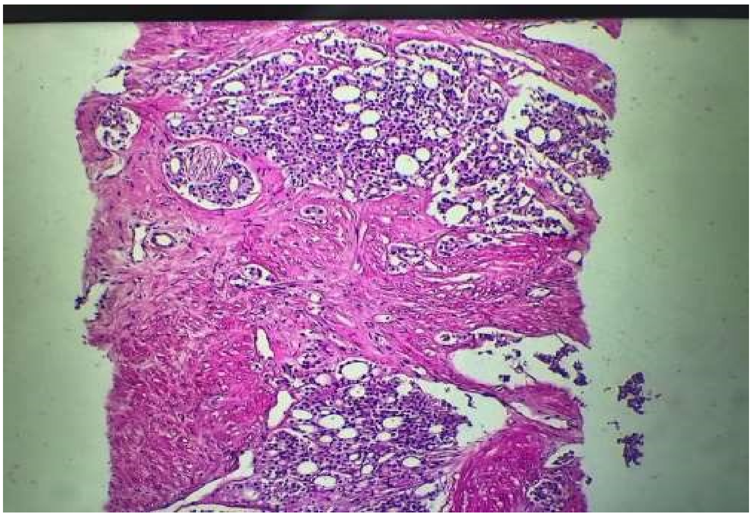

Figure (4.18): Prostate core needle biopsy shows predominantly cribiform growth pattern of PAC, G score 4+4=8, grade group 4 H&E(X10).

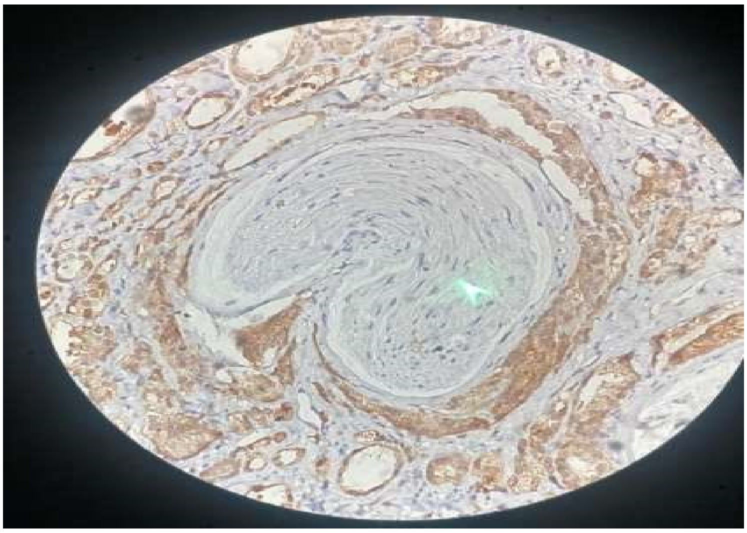

Figure (4.19): TFF3 immunoreactivity for PC (+2) score with perinural invasion (X40).

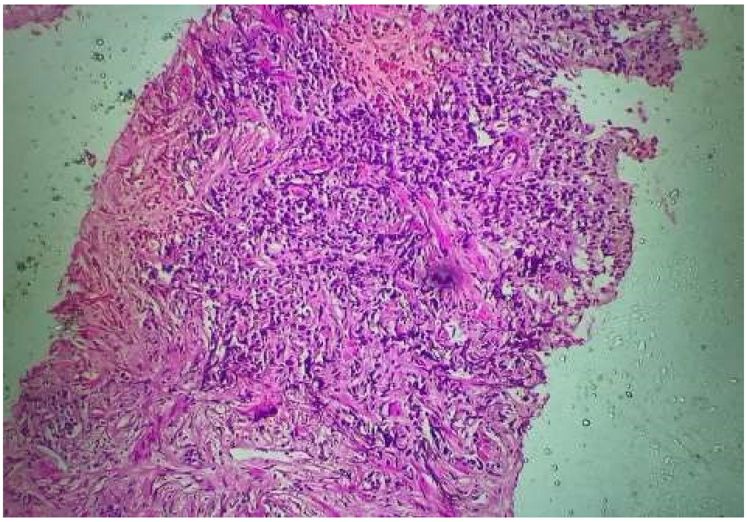

Figure (4.20): A section of prostatic adenocarcinoma, “Gleason score” 5+4=9, grade group 5 (sheet, single cell infiltration) X10.

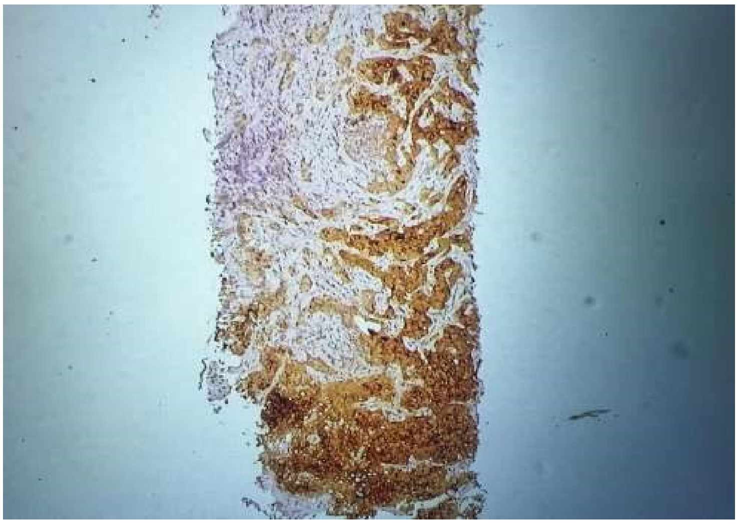

Figure (4.21): TFF3 immunoreactivity for PC (+2) score, high expression for prostatic carcinoma, Gleasseon score 5+4=9 (X4).

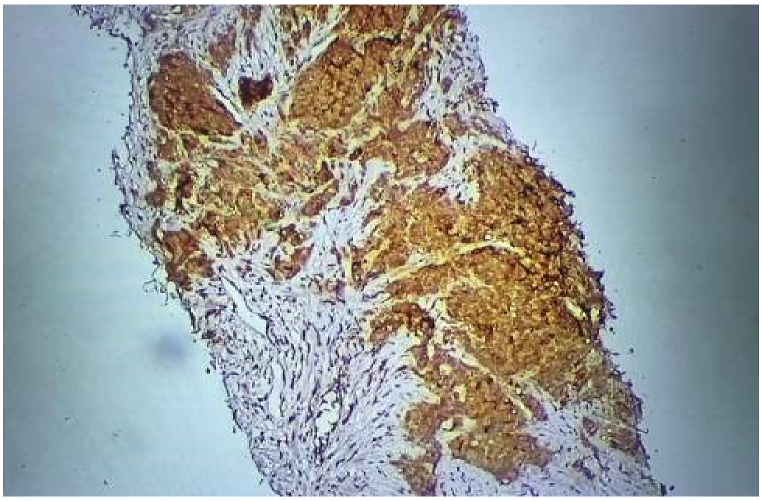

Figure (4.22): TFF3 immunoreactivity (+2) score, high expression for prostatic carcinoma gleasseon score 5+4=9 (X10)

4. Discussion

Prostatic cancer is the third most common cancer among Iraqis, as reported by the Iraqi Cancer Registry (2020). "Trefoil factor 3 (TFF3)" is a secreted peptide involved in tumor cell migration, invasion, and anti-apoptotic signaling. Altered TFF3 expression has been observed in multiple cancers, including prostatic adenocarcinoma [24].

4.1. Age Distribution

The mean age of patients with prostatic adenocarcinoma in this study was 69.3±50.36 years. This aligns with previous studies, where the mean ages were 66 years [25], 63 years [17], 64 years [26], and 59±6.35 years [16]. Our findings show a similar age distribution to previous research, with slight variations attributed to sample size differences.

4.2. Prostate-Specific Antigen (PSA) Levels

In our study, 76.7% of patients had PSA levels >20 ng/mL, whereas in [25], only 19.04% of patients had PSA levels >20 ng/mL. The differences may be due to sample size variations and missing PSA data in some cases.

4.3. Grade Group and "Gleason score"

The "Gleason score" remains a crucial prognostic indicator. Our study found that 43.3% of cases were in grade group 5, while the distribution varied in other studies: [26] reported 43.3% in grade group 5, while [25] found only 7.61% in the same category. Differences are likely due to the absence of screening programs and reliance on core needle biopsies.

4.4. Perineural and Lymphovascular Invasion

Perineural invasion was present in 63.3% of cases, comparable to [25], where 75.24% had perineural invasion. Similarly, lymphovascular invasion was observed in 10% of our cases, whereas it was present in 17.1% in [25] and 17.5% in [29]. Differences in sample collection methods may account for these variations.

4.5. Immunohistochemical Expression of TFF3

Our study found that 80% of "benign prostatic hyperplasia (BPH)" cases were negative for TFF3, whereas 93.3% of prostatic adenocarcinoma cases exhibited TFF3 expression ($p<0.001$). Similar results were reported in [26], where 18% of BPH cases expressed TFF3. [25] found TFF3 expression in 77.13% of prostatic adenocarcinoma cases, and [17] reported 54.6% expression, indicating some variability in detection methodologies.

4.6. Correlation with Age, PSA, and Grade Group

TFF3 expression and patient age did not significantly correlate ($p=0.22$), which is consistent with [25] and [29]. Nonetheless, in line with results from [30], there was a significant connection between preoperative PSA levels and TFF3 expression ($p=0.000012$). Additionally, there was a significant association ($p=0.0004$) between grade group and TFF3 expression, which is consistent with [25]..

4.7. Correlation with Perineural and Lymphovascular Invasion

No significant correlation was found between TFF3 expression and perineural invasion (p=0.07), which aligns with [25] and [29]. Similarly, no significant correlation was observed between TFF3 expression and lymphovascular invasion (p=0.1), consistent with [25] and [29].

4.8. Diagnostic Potential

The sensitivity and specificity of TFF3 were 93% and 80%, respectively, suggesting its potential use as a diagnostic marker. This study is the first of its kind in Iraq and the Middle East, though it has limitations, including a small sample size and lack of radical prostatectomy specimens for further pathological correlation. The COVID-19 pandemic also impacted sample collection.

These findings contribute to the growing evidence supporting TFF3 as a potential biomarker in prostatic adenocarcinoma while emphasizing the need for larger, multi-center studies to validate its clinical utility.

Conclusion

1. The epithelial cytoplasmic Immunohistochemical expression for TFF3 in cases of BPH and PA suggests the participation of Trefoil factor 3 in hyperplasia and carcinoma.
2. Higher Immunohistochemical expression of TFF3 was observed in prostatic adenocarcinoma than in benign prostatic hyperplasia, which means that TFF3 plays a role in tumorigenesis.
3. TFF3 is expressed mainly in high-grade prostatic adenocarcinoma.
4. TFF3 immunohistochemistry expression has high sensitivity and specificity in prostatic adenocarcinoma

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