CORRELATION OF TMPRSS2-ERG GENE FUSION STATUS WITH CLINICOPATHOLOGICAL CHARACTERISTICS IN PROSTATE CANCER OF IRAQI PATIENTS

Ammar K. A.[1] **M. F. Altaee[2]**
Assist. lecturer **Assist. Prof.**
[1]-Dentistry Department/ Al-Esraa University College\Iraq
[2]-Biotech. Dept./ Coll. Scie. / University of Baghdad/ Iraq.
ammar.kadhim@esraa.edu.iq maha.fakhry@sc.uobaghdad.edu.iq

ABSTRACT

The aim of this study was to establish the existence and interaction of *TMPRSS2 – ERG* gene fusion status with clinicopathological features of prostate cancer patients. This research consisted of 123 embedded formalin-fixed tissues obtained from the prostate tumor patients. The above gene fusion is detected through the technique of fluorescent in situ hybridization (FISH) by means of a triple color probe. Seven samples have not been scored due to technical difficulties and 46 patients have fusion (39.6%), while the remaining (70) have not been seen with fusion. Of the 46 fusion-positive, 17 (36%) were caused by ERG-translocation, of the other 29 (63%) were caused by the interstitial segment deletion between the two genes due to their sequential position. In order to find a substantial correlation between 116 patients (fusion took place in 46 and non-fusion in 70) with clinicopathological features, the following findings were calculated: Our findings recorded non-significant correlation at age, BMI and tumor size at which (P-value >0.05).While results showed significant association with: pathological T stage (P value = 0.011), Gleason score (P value = 0.002), perineural invasion (P value = 0.047) and PSA (P value = 0.033). The clinicopathological features which had a considerable correlation with fusion status are very important as they aid the doctors in prognosis and treatment of such tumor.

Key words: "FISH" technique, age, gender, formalin-fixed tissues

مجلة العلوم الزراعية العراقية -2022 :53(3):487-495 الزبيدي والطائي

العلاقة بين الاندماج الجيني لـ TMPRSS2 - ERG مع الصفات السريرية المرضية للمرضى العراقيين المصابين بسرطان البروستات

عمار كاظم الزبيدي[1] **مها فخري الطائي [2]**
مدرس مساعد **استاذ مساعد**
[1] قسم طب الاسنان / كية الاسراء الجامعة / العراق [2] قسم التقنيات الاحيائية / كلية العلوم / جامعة بغداد / العراق

المستخلص

كان الهدف الرئيسي من هذه الدراسة هو تمييز وجود الاندماج الجيني TMPRSS2 – ERG وارتباطه بالخصائص السريرية المرضية لمرضى سرطان البروستات. تضمنت هذه الدراسة 123 نسيجًا ثابتًا من الفورمالين تم جمعها من مرضى سرطان البروستاتا. تم الكشف عن اندماج الجينات أعلاه باستخدام تقنية التهجين الفلوري باستخدام مسبار ثلاثي الألوان. لم يتم تسجيل سبع عينات بسبب مشاكل فنية في حين وجد الاندماج في 46 مريضًا (39.6٪) بينما لم يظهر الاندماج في 70 مريضًا. من بين 46 إيجابية الاندماج ، كان سبب 17 (36 ٪) هو الانتقال في جين ERG بينما كان السبب في 29 (63 ٪) هو الحذف في المقطع الخلالي بين الجينين لأنهما موجودان بالتسلسل. تم حساب قيمة P للعثور على الارتباط المعنوي لـ 116 مريضًا (حدث الاندماج في 46 وغير الاندماج في 70) مع الخصائص السريرية المرضية التي أعطتنا النتيجة الموضحة كما يلي: لم تظهر النتائج اي علاقه معنويه بالنسبة للعمر و مؤشر كتلة الجسم وحجم الورم حيث كانت قيمة P أكبر من (0.05) في حين أظهرت نتائجنا ارتباطًا معنويًا بـ: درجة انتشار الورم (P – value = 0.01)، درجة غليسون (P – value = 0.002)ومع وجود او عدم وجود الخلايا السرطانية حول الالياف العصبية للبروستاتا (P – value = 0.047) و مستوى مستضد البروستات النوعي (P – value = 0.033). تعتبر السمات السريرية المرضية التي كانت لها علاقة قوية بحالة الاندماج مهمة جدًا لأنها تساعد الأطباء في تشخيص وعلاج مثل هذا الورم.

كلمات مفتاحيه: تقنيه التهجين الفلوري , العمر, الجنس, النسيج المثبت بالفورمالين.

Received:22/1/2021, Accepted:11/4/2021

INTRODUCTION

The prostate is a gland present in some organisms in the male reproductive system. It rests underneath the bladder in the pelvis and encircles the urethra (28). The inner structure of the prostate is characterized by both lobes and zones. It generates some of the semen fluid (10). Prostate cancer (PCa) continues to be a critical cause of morbidity and mortality as it is the second commonest male malignancy and the fifth - largest cause of death. It accounts for 3.8% of all cancer deaths throughout the male population (5, 15). Incidences regarding prostate cancer have risen dramatically in emerging and Asian countries, including Iraq. PCa mortality in Iraq became 0.25 percent's annual data released by the World Health Organization (WHO). In addition, the death rate is 7,02 out of 100,000 in Iraq (22). Prostate cancer has quite an incredibly complex genetic makeup spanning copy number, point mutations, structural rearrangements, and chromosome number changes (20). A large share of prostate carcinomas (PCA), though different incidences across various patient cohorts, become present in chromosome rearrangement involving E26 transformation-specific genes (ETS) (21). ERG (E26 oncogene chromosome homolog 21q22.3) is only detected in prostate cancer. ERG is proven to affect prostate cancer. In 50% of clinically established and metastatic PCa samples it was first confirmed that the oncogene ERG was abundantly expressed in transcript level (26). Many investigations have said that ERG fusion was the basis for this over-expression, and that the commonest HGPIN variant revealing TMRPSS2-ERG fusion has been blended with TMPRSS2-ERG PCa co-existing with androgen-driven gene (16, 25). TMPRSS2-ERG fusion with a reported range of 15%-80%, depending on the clinical cohort and the methods of detection, is a common rearrangement in prostate cancer. There's also a tiny proportion (<10 percent) of the fusions between ERG and 5' sensitised androgens, such as (SLC45A3) (18). New studies showed its potential role as a prognosis measure and marker of prostate cancer with the detection of ERG (13). In 2006, *Perner et al.* concluded that there is a major link between the existence of rearrangements with ERG followed by 5′-ERG deletion and the presence of metastatic diseases involving pelvic lymph node (19). Later in 2007, *Demichelis et al.* reported a substantial correlation between TMPRSS 2-ERG fusion and prostate death in a watchful waiting cohort of 111 patients and a connection between ERG alterations and major Gleason score (7). Many methods were used to recognize new biomarkers of prostate carcinogenic cancer in patients' tissues and blood to replace PSA routine usage at present. This study was aimed to determine the correlation between TMPRSS2-ERG gene fusion and certain clinical markers as a new prognostic marker for prostate cancer by examining a cohort of prostate cancer in a sample of Iraqi patients using fluorescence in situ Hybridization (FISH) technique.

MATERIALS AND METHODS

Study subjects: One hundred and forty-nine individuals were involved in this study for the period from February – 2019 to February – 2020. The sample type was FFPE (formalin – fixed embedded tissue) from the government as well as from private facilities and laboratories at Baghdad, Basra and Kufa/ Iraq. 123 samples of pre-diagnosed patients with prostate cancer, aged between 48 and 86 years.

Staining method: The key staining method that is used in histology is hematoxylin and eosin stain (abbreviated as: H and E stain). This protocol gave us an overview of the pattern and distribution of cells in the tissue that offers information of the structure of the sample. A standard procedure for restricting the tumor region of the tissue section has been used to glean the protocol and the following step was assumed as a precept (6).

Tissue microarrays (TMAs) construction: A high-performance method of evaluating prospective biomarkers and therapeutic goals in preserved pathologic samples are available. In several tumors on a single diaphragm, TMAs provide simple routes for rapid evaluation of molecular variation. Fluorescent in situ hybridization (FISH) have been used for in situ tissue analysis. Tissue microarrays are made by exposing the T-SueTM Array (15 cores for each 4mm) for fired oven at 75° C for 30 minutes, according to guidelines (T-SueTM Microarray Kit). Leisurely immerse

liquid paraffin till the surface of core rods was submerged. The embedding cassette was attached to the mould and paraffin was added and cooled for 60 minutes at approximately 2 - 8 °C. the cassette was removed slowly from the T-SueTM array mould. The tissue cores from the donor blocks based on the outcome of H and E stain were extracted with the punch needle T-SueTM. The tissue cores will then be moved to the receiver block. A slide of glass (facing) was placed onto block and the corresponding hole in blocks was incubated at 37 °C to 45°C overnight. The blocks were let to cool for 20 minutes with the slide in a dry ice tray. Then the slide was quickly removed from the block and became ready for microtome cutting (3).

FISH technique

TriChechTM DNA probe (ZytoVision) ZytoLight® SPEC ERG/TMPRSS2 DNA designed to detect deletions from the genes ERG and TMPRSS2 and other rearrangements (translocations) of both genes as shown in (figure 1). The slides were heated to 70°C (each with 15 cores) for 10 minutes, including (dewaxing, proteolysis) pretreatments were performed in accordance with the ZytoLight FISH-Tissue Implementation Package. Then a denaturing phase of 75°C was taken about 10 minutes. Finally, at 37°C overnight, slides were incubated to a hybridizing moisture trap. DAPI/Dura Tech-Solution for cell nuclei staining and suitable filter sets were adopted in fluorescent microscopy (9).

FISH interpretation

The appropriate filter sets have been used, the green (distal to the ERG), orange (close to the ERG) and blue (distal to the TMPRSS2) (9).

Standard case

Two orange/green fusion signals and two blue signals in normal cells or cells without rearranging their respective gene regions (9).

Aberration

A 21q22 locomotive with a deletion of 21q22.2 resulting in the TMPRSS2-ERG fusion is indicated by an orange signal with a blue signal and a green signal loss. A separate orange signal and a blue signal co-located with the separate green signal are indicated for an ERG translocation without the participation of TMPRSS2 (9).

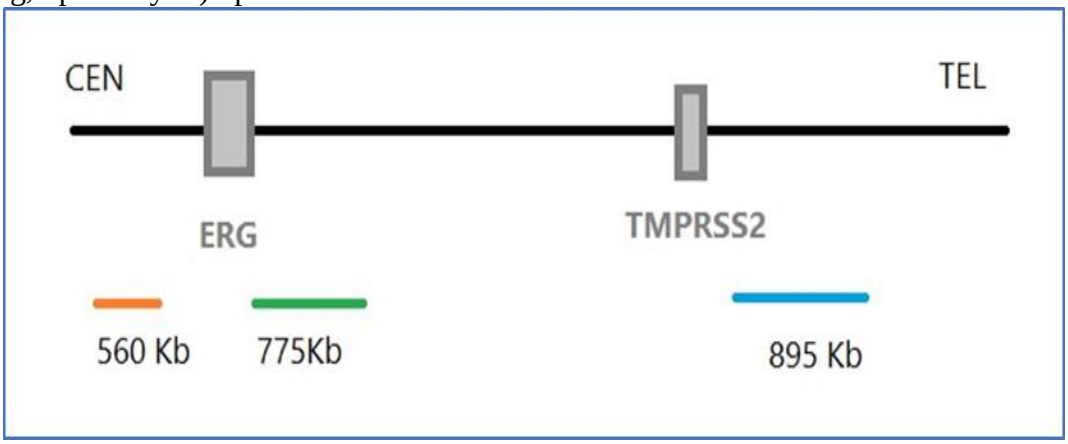


Figure 1. ERG\TMPRSS2 probe map

Statistical analysis

Statistically, the discovery of a combination of the positive and negative fusion TMPRSS2-ERG with age, bodily mass index, PSA stage, the Gleason score and tumor size) were analyzed with the SPSS software version 26. The results were evaluated for the checking of the chi square and Fisher's reliable two by two contingency tables were used and the results had statistically significant when P is < 0.05 (2).

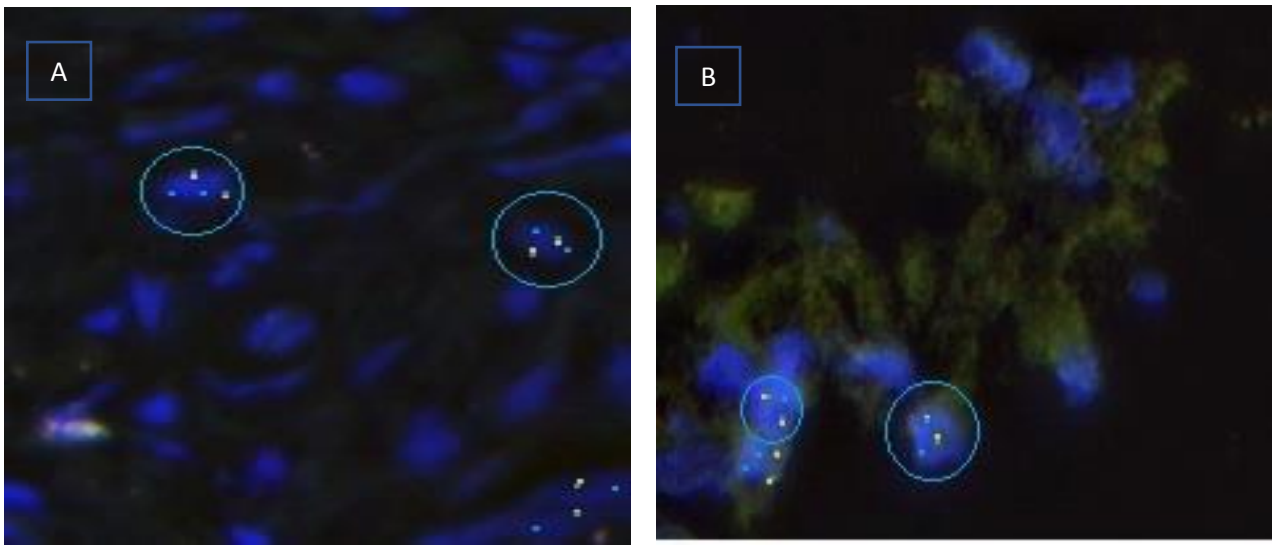
RESULTS AND DISCUSSION

This cohort of (116 prostate patients) with its clinicopathological features was described in Table 1. All the characteristics were taken at the early diagnosis, which included age (that has been divided into two groups (≥68 and <68) body mass index, tumor area, tumor Stage, Gleason score, perineural invasion and PSA level.

Table 1. Clinicopathological features for 116 prostate cancer patients

Parameter	Number (116)	Percentage
Age	≥68(61)	53%
	<68(55)	47%
Body mass index	18.5-24.9 (24)	21%
	25-29.9 (62)	53%
	≥30(30)	26%
Tumor area	≥ 40% (65)	56%
	< 40% (51)	44%
Stage	2(45)	39%
	3(43)	37%
	4(28)	24%
Gleason score	3+4 (32)	27%
	4+3 (14)	12%
	8 (41)	35%
	9,10 (29)	25%
Perineural invasion	seen (55)	54%
	not seen (46)	46%
PSA level	≤ 4 (7)	6%
	4-9.9 (64)	55%
	10-19.9 (27)	23%
	≥20 (18)	16%

Of 123 samples, 7 were not scored due to technical difficulties. The FISH results showed that the 46 were considered positives (TMPRSS2 - ERG gene fusion had occurred) and 70 were negative that fusion was not confirmed between the two genes. The Forty - six samples with fusion genes are categorized depending on fusion mechanisms; firstly, translocation of the ERG gene but not TMPRSS2 in 17 (36%) samples; secondly, deletion in the interstitial segment between the genes in 29 (63%) samples as illustrated in (Figure 2).



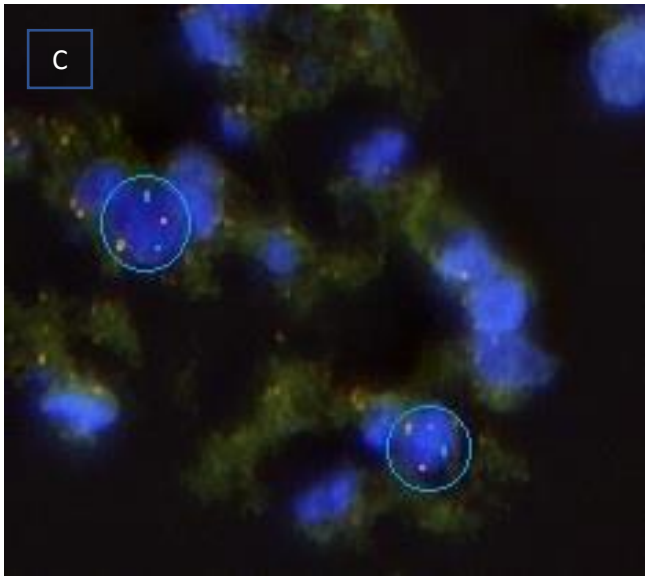


Figure 2. The result of fluorescent in situ hybridization technique:
A- no gene aberration in which two green\orange and two separated blue signals
B- Deletion of interstitial segment between both genes results in gene fusion (one blue close to one orange and one green is missing).
C- ERG translocation with 5 UTR of TMPRSS2 resulting in fusion gene (one blue close to green with separated orange).

Fusion status and clinicopathological characteristics: The range of patient's age for those with fusion genes (52 - 86 years) and without fusion (48 - 86 years) is classified into two groups: 61 were ≥68 years from which 27 (44%) positives and 34 (56%) negatives for fusion status and 55 were <68 years, 19 (35%) positives and 36 (56%) negatives. No significant correlation based on P - value was

Table 2. Association of age with fusion status

Age groups	Total	Positive (46)	Percentage	Negative (70)	Percentage
≥68	61	27	44%	34	56%
<68	55	19	35%	36	65%
P - value = 0.380; non-significant					

For BMI's relationship with T2E-caused prostate cancer, the three classes were evaluated; first was the standard range between (18.5 - 24.9 kg/m) 9 (38%) and 15 (62%), second is overweight which range between (25 - 29.9 kg \ m) 21 (34%) and 41 (66%), last one is obese in which (≥30 kg\m) (16,14) for positive and negative respectively. No relation among BMI and prostate cancer patients (P - value = 0.21) because the muscle mass is not adequately reduced compared to their fat mass for the elderly, many of them have conditions that can also improve their fluid mass.

Table 3. BMI values in comparison to positive and negative T2E

BMI	Total	Positive (46)	Percentage	Negative (70)	Percentage
18.5-24.9	24	9	38%	15	62%
25-29.9	62	21	34%	41	66%
≥30	30	16	53%	14	47%
chi-sq. = 1.569 [DF = 1] P = 0.210; non-significant					

The region of a tumor was determined from the total tumor cells of the sample to generate two categories: ≥ 40% n = 65 including 29 (45%) positive and 36 (55%) negative while the other <40% n = 51 including 17 (33%) positive and 34 (67%) negative and were not correlated with fusion status (P - value = 0.297).

Table 4. Association of tumor area with fusion status

Tumor area	Total	Positive (46)	Percentage	Negative (70)	Percentage
≥ 40%	65	29	45%	36	55%
< 40%	51	17	33%	34	67%
P - value = 0.297; non-significant (Fisher's exact test)					

For the classification of cancer pathological stages according to the Union for International Cancer Control (UICC), the TNM system is used in this study there were available three stages (T2, T3, T4). P-value (0.011) indicates that the results reported were significantly linked to cancer (Table 5).

Table 5. Association of pathological T stage with fusion status

Stage	Total	Positive (46)	Percentage	Negative (70)	Percentage
2	45	10	22%	35	78%
3	43	22	51%	21	49%
4	28	14	50%	14	50%
X^2 = 6.500, P - value = 0.011; significant					

Prostate cancer pathologists use numbers from 1 to 5 obviously depends on how often the cancerous tissue cells appear under the microscope. This is called the scheme of Gleason score. GS is very important to predict prostate cancer behavior and to assess appropriate treatment options. (3+3) or grade 1 is not regularly used because it could be well differentiated or low grades and is probably less aggressive; in other words they appear to spread and expand slowly, so that other four grades has been taken to analysis these results which revealed significant differences when P-value = 0.002 (table 6).

Table 6. Association of Gleason score with fusion status

Gleason score	Total	Positive (46)	Percentage	Negative (70)	Percentage
3+4	32	4	13%	28	87%
4+3	14	8	57%	6	43%
8	41	22	54%	19	46%
9,10	29	12	41%	17	59%
X2 = 15.046, P - value = 0.002 significant					

Perineural invasion triggers the surrounding or monitoring of cancer cells along a nerve fiber in the prostate. This means that there is a greater risk of cancer spreading beyond the prostate. The recorded data are only valid for 101 samples and also compared to both fusion statuses. The findings were important, as shown in (table 7) since P - value = 0.047.

Table 7. Association of perineural invasion with fusion status

PNI	Total	Positive (46)	Percentage	Negative (70)	Percentage
seen	55	29	53%	26	47%
Not seen	46	15	33%	31	67%
P - value = 0047; significant					

Blood level of a person's prostate-specific antigen (PSA) is also a good indication of whether therapy is successful or has been effective. It is also significant in the Gleason score and the Pathological T stage prediction. Most doctors found 4.0 ng/mL and lower PSA levels to be normal. In our data set, four separate PSA levels classes were considered compared with the positive and negative fusion genes. The findings were significant as P - value = 0.033, as demonstrated in table 8.

Table 8. Association of PSA level with fusion status

PSA level	Total	Positive (46)	Percentage	Negative (70)	Percentage
≤ 4	7	3	43%	4	57%
4-9.9	64	18	28%	46	72%
10-19.9	27	14	52%	13	48%
≥20	18	11	61%	7	39%
X^2 = 8.727 P - value = 0.033; significant					

TMPRSS2: ERG fusions generally occur early in prostate carcinogenesis with approximately 10%–20% of high-grade prostatic intraepithelial neoplasia (a histologic precursor to invasive prostate cancer) harboring this fusion. Although there are several methods to detect such gene rearrangement, in this study, it has been proven that the FISH technique is one of the best methods for diagnosing this type of genetic fusion. In addition to diagnosing the presence or absence of fusion, the researcher is able to know the mechanism of the fusion gene, as in this study where it was found translocation of the ERG gene but not TMPRSS2 in 17 (36%) samples and deletion in the interstitial segment between the genes in 29 (63%) of the total fusion samples. However, Hagen et al. also found that FISH

technique is reliable in detecting the fusion (12). In this study, the results showed no significant relation between fusion status with age, body mass index and tumor area. Our results were consistent with many previous studies, including Suh et al. (22), Mucci, Lorelei et al. (17), and Yoshimoto et al. (27) as most prostate cancer patients are in close age stages starting from the age of 60 and up. At these ages, the body mass index is affected by many conditions includes the decreasing in muscle mass and the possibility of the presence of fat in certain areas in addition to the effects of some diseases such as chronic diseases with taking some medications, which may have side effects with gain or loss weight. The reason for the lack of a significant association with the area of the tumor is because the biopsy taken may be for the purpose of the initial diagnosis, and sometimes the tumor has passed to the rest of the organs such as the bone, so there is no need for the excision until after the implantation and to ascertain the real cause and make the decision for an appropriate treatment for the case and in each, the biopsy does not represent the actual area of the tumor. Our findings showed an association of TMPRSS2-ERG rearrangement with clinical and pathologic features of and aggressive form of prostate cancer whereas 36 (78%) of total positive fusion were linked to stage three and four. Similarly, such association of fusion positive with pathological T stage was confirmed by Attard et al. (1) and Sung, Ji-Younet al. (21). The other indicator of strong association in which 34 (74%) of total fusion positive were (8,9 and 10) according to Gleason score referring to poorly differentiated cells with lack of gland formation which corresponding to the study of Büscheck et al. (4) and Suryavanshi et al (24). Moreover, PNI was seen in 29 (53%) and this characteristic if present, it considered definitively a diagnostic of malignancy. This observation has been concordant with Gopalan et al. (11) and Lee, Seung-Ryeol et al. (14). Finally, a number of benign (not cancerous) conditions can cause a man's PSA level to rise. including prostatitis (inflammation of the prostate) and benign prostatic hyperplasia (BPH) (enlargement of the prostate). Although there is no evidence that prostatitis or BPH leads to prostate cancer, many doctors now use features of the cancer (such as the pathological T stage, initial PSA level, and prostate biopsy results) to divide the risk into the following risk groups (very low, low, intermediate, high and very high). Our survey has a significant association with PSA level and agree with Eguchi et al. (8) and Suryavanshi et al. (24).

CONCLOSION

TMPRSS2-ERG gene fusion has been observed in 39.6% (46 out of 116) patients, which corresponds almost to the global ratio between 20 and 50 percent of prostate cancer caused by the above-mentioned fusion genes. The fusion patients were closely correlated with their highest PT stage, Major GS and PNI. While there are many methods to predict the fusion genes, FISH technique is in order to detect mechanisms of fusion, very accurate for the detection of such rearrangements.

REFERENCES

1. Attard, G., J. Clark, L. Ambroisine, G. Fisher, G. Kovacs, P. Flohr and C. Cooper. 2008. Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. Oncogene. 27(3): 253-263

2. Bhanushali, A., P. Rao, V. Raman, P. Kokate, A. Ambekar, S. Mandva, S. Bhatia, and B.R Das. 2018. Status of TMPRSS2–ERG fusion in prostate cancer patients from India: correlation with clinico-pathological details and TMPRSS2 Met160Val Polymorphism.Prostate International. 6(4): 145-150

3. Brown, L.A. and D. Huntsman, 2007. Fluorescent in situ hybridization on tissue microarrays: challenges and solutions. Journal of Molecular Histology. 38(2): 151-157

4. Büscheck, F., M. Sulimankhil, N Melling, D. Höflmayer, C. Hube-Magg, R. Simon, and C. Fraune. 2020. Loss of cytoplasmic survivin expression is an independent predictor of poor prognosis in radically operated prostate cancer patients. Cancer Medicine. 9(4): 1409-1418

5. Čamdžić, N., S. Kuskunović-Vlahovljak, M. Dorić, S. Radović, E.L Salčin, and M. Babić. 2020. Serum total prostate-specific antigen (tPSA): correlation with diagnosis and grading of prostate cancer in core needle biopsy. Medicinski Glasnik: Official Publication of the Medical Association of Zenica-doboj Canton, Bosnia and Herzegovina. 18(1).

6. Chan, J. K. 2014. The wonderful colors of the hematoxylin–eosin stain in diagnostic surgical pathology. International Journal of Surgical Pathology. 22(1): 12-32

7. Demichelis, F., K. Fall, S. Perner, O. Andrén, F. Schmidt, S. R Setlur and M. A. Rubin. 2007. TMPRSS2: ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene. 26(31): 4596-4599

8. Eguchi, F. C., E. F. Faria, C. S. Neto, A. Longatto-Filho, C. Zanardo-Oliveira, S. R. Taboga, and S. G. Campos. 2014. The role of TMPRSS2: ERG in molecular stratification of PCa and its association with tumor aggressiveness: a study in Brazilian Patients. Scientific Reports. 4(1): 1-6

9. Esgueva, R., S. Perner, C. J. LaFargue, V. Scheble, C. Stephan, M. Lein, and M. A. Rubin. 2010. Prevalence of TMPRSS2–ERG and SLC45A3–ERG gene fusions in a large prostatectomy cohort. Modern Pathology. 23(4): 539-546

10. Goddard, J. C., 2019. The history of the prostate, part one: say what you see. Trends in Urology & Men's Health. 10(1): 28-30

11. Gopalan, A., M. A. Leversha, J. M. Satagopan, Q. Zhou, H. A. Al-Ahmadie, S. W. Fine, J. A. Eastham, P. T. Scardino, H. I. Scher, S. K. Tickoo, and V. E. Reuter. 2009. TMPRSS2-ERG gene fusion is not associated with outcome in patients treated by prostatectomy. Cancer Research. 69(4): 1400-1406

12. Hagen, R. M., P. Adamo, S. Karamat, J. Oxley, J. J. Aning, D. Gillatt, and A. Rhodes. 2014. Quantitative analysis of ERG expression and its splice isoforms in formalin-fixed, paraffin-embedded prostate cancer samples: association with seminal vesicle invasion and biochemical recurrence. American Journal of Clinical Pathology. 142(4): 533-540

13. Kolar, Z., A. Burdova, T. Jamaspishvili, J. Bouchal, R. Kucerova, M. Bienova, M. Kral, and V. Student. 2014. Relation of ETS transcription factor family member ERG, androgen receptor and topoisomerase 2β expression to TMPRSS2-ERG fusion status in prostate cancer. Neoplasma, 61(1): 9-16

14. Lee, S.R., Y. D. Choi, and N. H. Cho. 2018. Association between pathologic factors and ERG expression in prostate cancer: Finding pivotal networking. Journal of Cancer Research and Clinical Oncology. 144(9): 1665-1683

15. Maha Fakhry Altaee, R. W. Younis and K. Zaid Kamona 2020. Activity of *Annona Squamosa* peels extracts against two pathogenic bacteria and two blood cancer cell lines. Iraqi Journal of Science. 51(6): 1496-1503

16. Mosquera, J. M., R. Mehra, M. M. Regan, S. Perner, E. M. Genega, G. Bueti, R. B. Shah, S. Gaston, S. A. Tomlins, J. T. Wei, and M. C. Kearney. 2009. Prevalence of TMPRSS2-ERG fusion prostate cancer among men undergoing prostate biopsy in the United States. Clinical Cancer Research. 15(14): 4706-4711

17. Mucci, L.; N. Luedeke; A. Rinckleb, S. Kolb; J. L. Wright; C. Maier; M. L. Neuhouser and J. L. Stanford 2008. Obesity and the TMPRSS2: ERG translocation in prostate cancer. AACR. Am. J. Epidemiol.181(9): 706–713

18. Park, K., S. A. Tomlins, K. M. Mudaliar, Y. L. Chiu, R. Esgueva, R. Mehra, K. Suleman, S. Varambally, J. C. Brenner, T. MacDonald and A. Srivastava. 2010. Antibody-based detection of ERG rearrangement-positive prostate cancer. Neoplasia. 12(7): 590-IN21

19. Perner, S., F. Demichelis, R. Beroukhim, F. H. Schmidt, J. M. Mosquera, S. Setlur and M. A. Rubin. 2006 TMPRSS2: ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. Cancer Research. 66(17): 8337-8341

20. Schoenborn, J. R., P. Nelson and M. Fang. 2013. Genomic profiling defines subtypes of prostate cancer with the potential for therapeutic stratification. Clinical Cancer Research, 19(15): 4058-4066

21. Sung, J.Y., H. G. Jeon, B. C. Jeong, S. I. Seo, S. S. Jeon, H. M. Lee, H. Y. Choi, S. Y. Kang, Y. L. Choi and G. Y. Kwon. 2016. Correlation of ERG immunohistochemistry with molecular detection of TMPRSS2-ERG gene fusion. Journal of Clinical Pathology. 69(7): 586-592

22. Shkaaer, M. T. and N. M. Utba. 2019. IL-18 Gene Polymorphisms Impacts on Its Serum Levels in Prostate Cancer Iraqi Patients. Iraqi Journal of Science. 60(6): 1188-1196

23. Suh, J. H., J. H. Park, C. Lee and K. C. Moon. 2017. Intrafocal heterogeneity of ERG protein expression and gene fusion pattern in prostate cancer. The Prostate. 77(14): 1438-1445

24. Suryavanshi, M., A. Mehta, J. Jaipuria, A. K. Sharma, S. Rawal and N. Seth. 2015. Weaker ERG expression in patients with ERG-positive prostate cancer is associated with advanced disease and weaker androgen receptor expression: An Indian outlook. in Urologic Oncology: Seminars and Original Investigations. 33(7): 331-e9

25. Tomlins, S. A., A. Bjartell, A. M. Chinnaiyan, G. Jenster, R. K. Nam, M. A. Rubin and J. A. Schalken. 2009. ETS gene fusions in prostate cancer: from discovery to daily clinical practice. European Urology. 56(2): 275-286

26. Vanaja, D. K., J. C. Cheville, S. J. Iturria and C. Y. Young. 2003. Transcriptional silencing of zinc finger protein 185 identified by expression profiling is associated with prostate cancer progression. Cancer Research. 63(14): 3877-3882

27. Yoshimoto, M., A. M. Joshua, I. W. Cunha, R. A. Coudry, F. P. Fonseca, O. Ludkovski and J. A. Squire. 2008. Absence of TMPRSS2: ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. Modern Pathology. 21(12): 1451-1460

28. Young, B., P. Woodford and G. O'Dow. 2013. Wheater's Functional Histology E-Book: A Text and Colour Atlas. Elsevier Health Sciences.