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Original Research

Neurotrophic tyrosine receptor kinase (NTRK) 1 and 2 expression in squamous cell carcinoma and squamous intraepithelial lesions of the cervix uteri

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Background: Selective tropomyosin receptor kinase (Trk) inhibitors are known to provide promising outcomes in selected tumor patients with neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions. In the present study, we aimed to determine whether there was a NTRK1 and NTRK2 expression in cervical squamous cell carcinoma (SCC) and precursor lesions. *Materials and methods*: A total of 92 formalin-fixed paraffin-embedded tissue samples of cervical SCC, squamous intraepithelial lesion (SIL) and non-neoplastic cervical tissue (NCT) were subjected to immunohistochemical (IHC) staining with NTRK1 and NTRK2 antibodies. These stainings were compared with p16/Ki-67 (IHC) and the low-risk/high-risk Human papillomavirus (HPV) *in situ* hybridization stainings that were previously administered. *Results*: In IHC analysis, NTRK1 expression was detected in 3.2% and 12.5% of SCC and cervical intraepithelial neoplasia (CIN)-2 samples, respectively. In addition, NTRK2 expression was detected in 6.5% and 6.3% of SCC and CIN-2 samples, respectively. However, NTRK1/NTRK2 expression was not detected in samples of NCT, CIN-1, and CIN-3. The p16 and Ki-67 expression and high-risk HPV positivity increased as the grade of the lesion increased. *Conclusions*: The results indicated that both NTRK1 and NTRK2 had no contributory effect on the grading and differentiation of cervical SCC and SIL. We consider that the IHC method is likely to be used for the screening of *NTRK* gene fusions in patients with cervical SCC. Trk inhibitors are likely to be a favorable therapeutic alternative for this low number of SCC cases with positive NTRK1/NTRK2 staining. Further studies are needed to confirm these ideas.

Keywords

Cervix uteri; NTRK1; NTRK2; Squamous cell carcinoma; Squamous intraepithelial lesion

1. Introduction

Cervical cancer (CC) is both the fourth leading cancer diagnosis and the fourth leading cause of death from cancer in women worldwide. In many countries, however, CC is the most common cancer and a leading cause of death from cancer among women [1]. According to the 2017 Health Statistics, CC is the ninth leading cancer among women in Turkey, representing 2.5% of all cancers [2]. Squamous cell carcinoma (SCC) accounts for about 70–80% of CCs and constitutes the majority [3–5]. Almost all CCs are known to be caused by oncogenic *Human papillomavirus* (HPV). Low-risk HPV (LR-HPV) types such as 6 and 11 are generally associated with genital condyloma and low-grade squamous intraepithelial lesion (LSIL) while high-risk HPV (HR-HPV) types such as 16 and 18 are typically associated with CC and high-grade squamous intraepithelial lesion (HSIL) [3, 5, 6]. *In situ* hybridization (ISH) is highly specific for detecting HPV and can also predict viral integration status. Immunohistochemical (IHC) studies with $p16^{ink4A}$ and Ki-67 are commonly used as an adjunct technique in the diagnosis and grading of patients with squamous intraepithelial lesions (SIL) [4–6].

The tropomyosin receptor kinase (Trk) family contains three transmembrane proteins consisting of TrkA, TrkB, and TrkC receptors. The neurotrophic tyrosine receptor kinase (*NTRK1*), *NTRK2*, and *NTRK3* genes encode TrkA, TrkB, and TrkC receptors, respectively. These genes are associated with neuronal development and cell survival. In some cancer types, *NTRK* gene fusions potentially lead to oncogenesis through structural activation or overexpression of Trk receptors [7, 8]. Glial tumors, melanomas, some sarcomas (soft tissue, uterine), and some carcinomas (thyroid, colon, stomach, lung, cervix uteri, breast, appendix, gallbladder, salivary gland, pancreas, bladder, head-and-neck) have a low frequency of *NTRK* gene fusions [7–17]. Due to their oncogenic activities, *NTRK* gene fusions have been studied as therapeutic targets, and favorable outcomes have been obtained by the administration of Trk inhibitors such as entrectinib and larotrectinib in some malignant tumors harboring *NTRK* gene fusions [7, 8, 10, 14, 16]. Accordingly, the detection of *NTRK* gene fusions is highly important. Molecular studies including fluorescence ISH and next-generation sequencing are good methods for detecting *NTRK* gene fusions. There are also studies using IHC methods that are less costly and more practical [8, 9, 11, 12, 14, 16–19].

The present study aimed to investigate NTRK1/NTRK2 expression using the IHC method in the diagnosis and grad-

ing of cases with SCC/SIL of the cervix uteri and to analyze the relationship between NTRK1/NTRK2 expression and LR/HR-HPV types and p16/Ki-67 expression. We also aimed to investigate the diagnostic and potential value of NTRK1/NTRK2 in SCC/SIL.

2. Materials and methods

2.1 Sample selection

The study included formalin-fixed paraffin-embedded tissue samples and slides of SCC/SIL of the cervix uteri and non-neoplastic cervical tissue (NCT) which had been profiled at Van Yuzuncu Yil University Medical School Pathology Department over the period 2001–2011. Samples that had no slides with HR-HPV, LR-HPV, p16, and Ki-67 staining and those that had inadequate tissue were excluded from the study. The diagnosis of each sample was confirmed by Hematoxylin-Eosin (H&E) staining and a total of 92 suitable samples were selected for IHC. These samples comprised: 31 (33.7%) with SCC; 18 (19.6%) with cervical intraepithelial neoplasia (CIN)-1; 16 (17.4%) with CIN-2, 14 (15.2%); CIN-3, and 13 (14.1%) with NCT. Clinical characteristics including patient age and tumor staging were retrieved from pathological reports and hospital records. The SCC samples were graded as well-, moderately- or poorly-differentiated and staged as early-stage (Stage I and IIA) or advanced-stage (Stage IIB, III and IV) [4–6]. The study was conducted in accordance with the Helsinki Declaration and was initiated after obtaining an approval from Van Yuzuncu Yil University Noninterventional Clinical Research Ethics Committee (Approval No: 06; Date: 20 June 2017).

2.2 Immunohistochemical staining

Tissue sections of 4 μm thickness were obtained from paraffin blocks and mounted on poly-l-lysine-coated slides. The sections were exposed to primary antibodies including NTRK1 (Phospho-TrkA [Tyr701] Polyclonal Antibody, Dilution: 1/75, Catalog number: PA5-40161, ThermoFisher Scientific, Rockford, IL, USA) and NTRK2 (Phospho-TrkB [Tyr705] Polyclonal Antibody, Dilution: 1/75, Catalog number: PA5-38077, ThermoFisher Scientific, Rockford, IL, USA). Subsequently, the sections were stained using Ventana BenchMark XT Automated IHC/ISH Staining System with a Ventana UltraView Universal DAB Detection Kit (REF 760-500, Ventana Medical Systems, Inc., Tucson, AZ, USA). Normal brain tissue was used as a positive control and the blood vessels of the same tissue were used as a negative control [9, 11, 13, 17]. Tissue slides that had previously been stained with Ki-67 IHC primary antibody (CONFIRM^{TM} Anti-Ki-67 {30-9} Rabbit Monoclonal Primary Antibody, Dilution: 1/250, Catalog number: 790-4286, Ventana Medical Systems, Inc., Tucson, AZ, USA) and p16 IHC primary antibody (CINtec® p16 Histology, Dilution: 1/150, REF 805-4713, Ventana Medical Systems, Inc., Tucson, AZ, USA) using the same system and kit were included in the study.

2.3 In situ hybridization

Tissue slides that had been previously stained using an HR-HPV probe set that detects genotypes 16, 18, 31, 33, 35, 45, 52, 56, 58, and 66 (INFORM HPV III Family 16 Probe set [B]; REF 800-4295, Ventana Medical Systems, Inc., Tucson, AZ, USA) and an LR-HPV probe set that detects genotypes 6 and 11 (INFORM HPV II Family 6 Probe set; REF 800-2220, Ventana Medical Systems, Inc., Tucson, AZ, USA) on a Ventana BenchMark XT device with a VENTANA ISH iVIEWBlue Plus Detection Kit (REF 760-097, Ventana Medical Systems, Inc., Tucson, AZ, USA) were used for HR-HPV and LR-HPV chromogenic ISH.

2.4 Scoring

NTRK1 and NTRK2 expressions were assessed based on cytoplasmic or cytoplasmic/membranous staining [9, 11, 13, 15, 17]. Staining percentage was scored as 0 = no staining, 1 = 1–50%, and 2 = 51–100%. Staining intensity was scored as 0 = no staining, 1 = weak, 2 = intermediate, and 3 = strong. The sum of these two scores was accepted as the total staining score for each sample. A total score of 0–2 was regarded as a negative staining and a total score of 3–5 was regarded as a positive staining [13, 20, 21]. The p16 expression was assessed based on nuclear and nuclear/cytoplasmic staining. Staining percentage was scored as 0 = 0–4%; 1 = 5–25%; 2 = 26–50%; and 3 = 51–100% and staining intensity was scored as 0 = no staining or weak; 1 = weak-intermediate, and 2 = intermediate-strong. The total score was calculated by multiplying the intensity score by the percentage score. A total score of 0–2 was regarded as a low expression and a total score of 3–6 was regarded as a high expression [22]. The Ki-67 expression, was assessed based on nuclear staining only, and staining percentage was scored as 1 = 0–25%; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100%. A score of 1–2 was regarded as low expression and a score of 3–4 was regarded as high expression [23]. In HR-HPV and LR-HPV chromogenic ISH, integrated and/or episomal type nuclear staining in squamous epithelial cells was accepted as positive staining while the absence of staining, diffuse brown staining, cytoplasmic staining of stromal cells, and background staining were accepted as negative staining [24].

2.5 Statistical analysis

Data were analyzed using MINITAB (version 14, Minitab LCC, State College, PA, USA). Continuous variables were expressed as mean, standard deviation (SD), and minimum and maximum values. Categorical variables were expressed as frequencies (n) and percentages (%). Differences between means were compared using Z-test. A kappa (κ) value was calculated to assess the agreement between staining methods. A p-value of <0.05 was considered significant.

3. Results

The study included samples from 92 adult cases with a mean age of 45.4 $\pm$ 12.2 (range, 28–75) years. Table 1 presents the distribution of cases with regard to ages.

Table 1. Age-based distribution of the cases included in the study.

	Total number n (%)	Age range (years)	Age (years) (median)	Age (years) (Mean ± SD)	*p* value
NCT	13 (14.1)	30–63	39	41.2 ± 11.1^a	0.01
CIN-1	18 (19.6)	28–70	42	42.7 ± 11.7^a	
CIN-2	16 (17.4)	30–60	37	40.3 ± 9.5^a	
CIN-3	14 (15.2)	32–64	38	43.1 ± 10.9^a	
SCC	31 (33.7)	30–75	52	52.4 ± 12.1^b	
Grade					
WD	10 (32.3)	32–71	53	50.9 ± 12.4	0.52
MD	12 (38.7)	37–75	54	55.5 ± 12.9	
PD	9 (29.0)	30–64	51	49.8 ± 11.0	
Stage					
Early	14 (45.2)	32–69	53	52.4 ± 12.1	0.99
Advanced	17 (54.8)	30–75	49	52.4 ± 12.3	
All	92 (100.0)	28–75		45.4 ± 12.2	45.4 ± 12.2

^{a,b}: Assignment of a different letter indicates the presence of a significant difference between the group means ($p < 0.05$).
NCT, non-neoplastic cervical tissue; CIN, cervical intraepithelial neoplasia; SCC, squamous cell carcinoma; WD, well-differentiated; MD, moderately-differentiated; PD, poorly-differentiated.

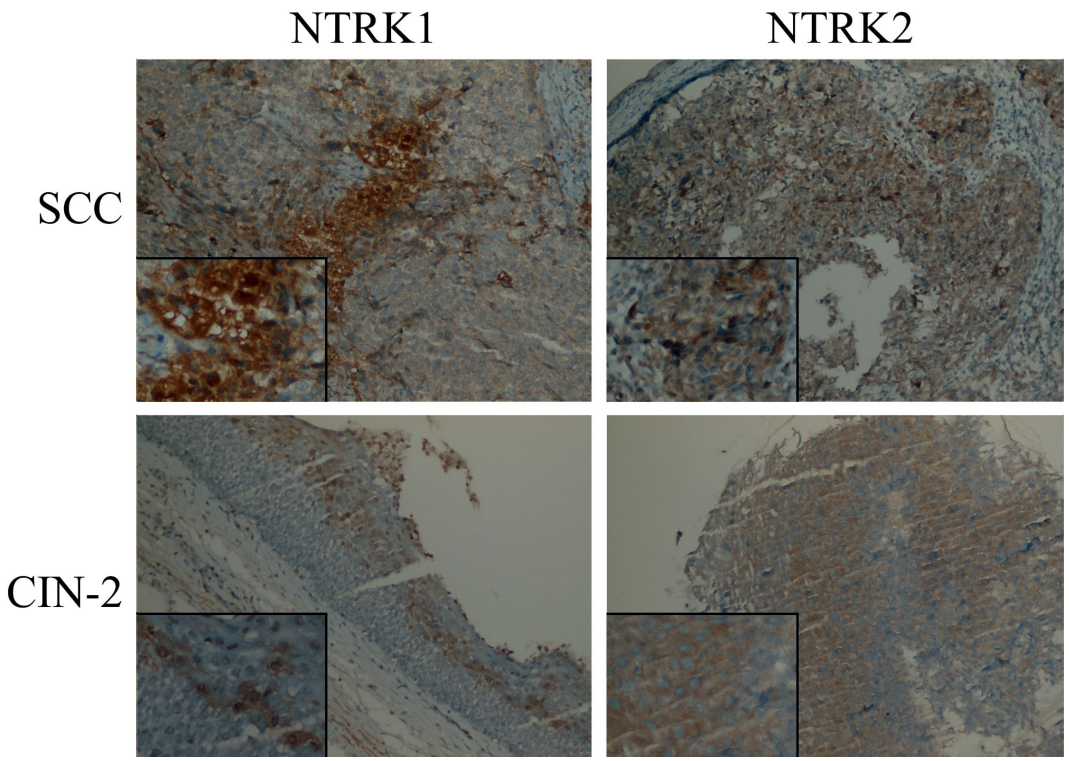

Fig. 1. SCC and CIN-2 cases showing positive NTRK1 and NTRK2 staining in IHC (200×, inset 400×).

In IHC analysis, positive NTRK1 staining was detected in 3.2% and 12.5% of SCC and CIN-2 samples and positive NTRK2 staining was detected in 6.5% and 6.3% of SCC and CIN-2 samples, respectively (Fig. 1). However, negative NTRK1/NTRK2 staining was detected in all samples of NCT, CIN-1, and CIN-3 (Fig. 2). Moreover, since the number of positively stained samples was remarkably low, no significant difference was found among the five main groups and among different grading and staging groups of SCC with regards to NTRK1/NTRK2 expression ($p > 0.05$) (Tables 2,3,4).

High p16 expression was detected in SCC (93.5%), CIN-3 (100.0%), CIN-2 (75.0%), and CIN-1 (27.8%), whereas no high p16 expression was detected in NCT. Additionally, p16 expression was significantly higher in SCC and SIL (CIN-1, CIN-2, and CIN-3) compared to NCT and was significantly higher in HSIL (CIN-2 and CIN-3) compared to LSIL (CIN-1) ($p < 0.05$). However, no significant difference was

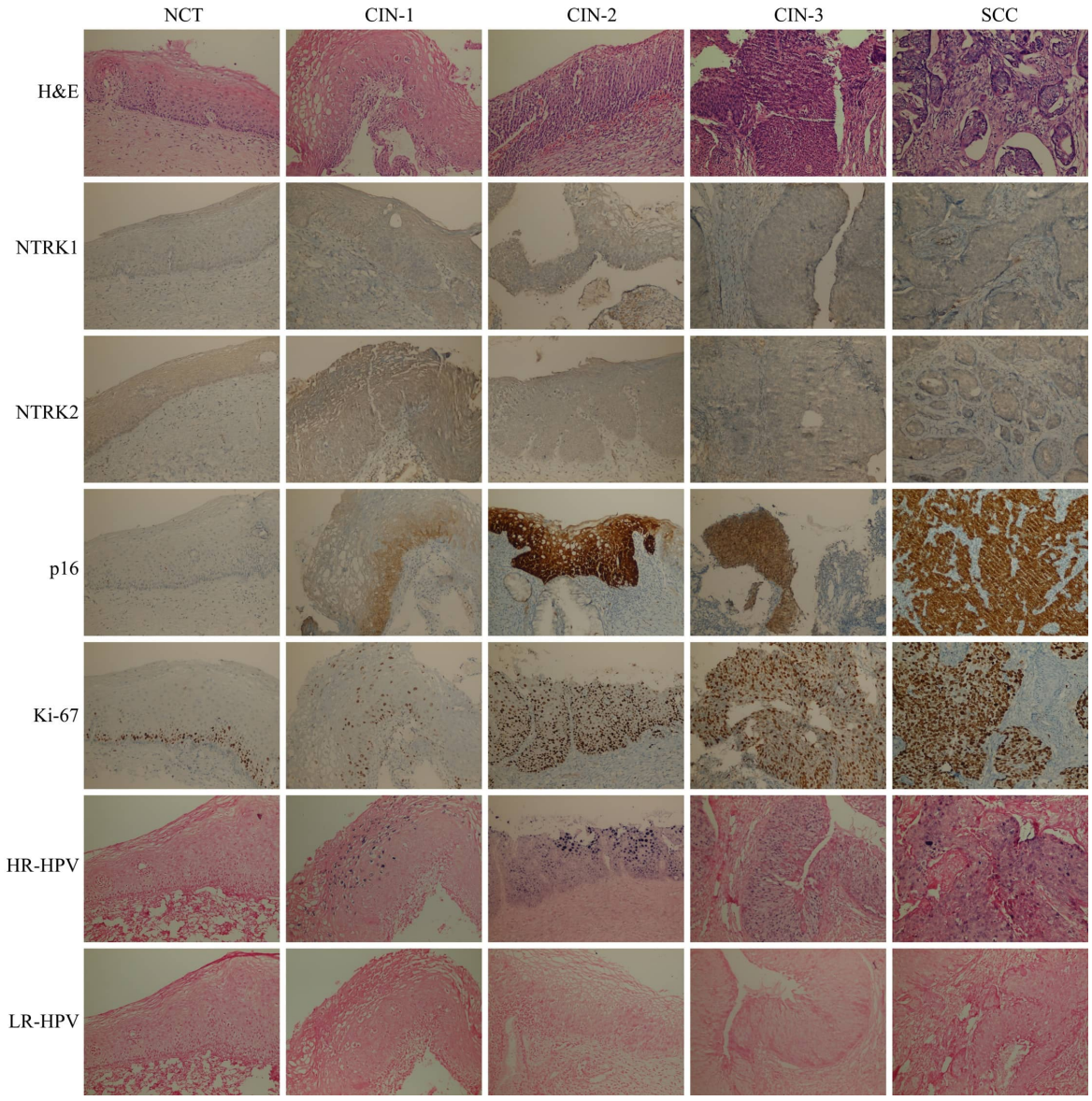

Fig. 2. H&E, IHC (NTRK1, NTRK2, p16, Ki-67), and ISH (HR-HPV, HL-HPV) staining (200×). NTRK and NTRK2 are negative. p16 low expression in NCT and CIN1. High expression of p16 in CIN-2, CIN-3 and SCC. Ki-67 low expression in NCT and CIN-1. Ki-67 high expression in CIN-2, CIN-3 and SCC. HR-HPV is negative on NCT. Episomal nuclear staining in CIN-1 and CIN-2. Integrated nuclear staining in CIN-3 and SCC. LR-HPV is negative in all.

found among different grading and staging groups of SCC with regard to p16 expression ($p > 0.05$). On the other hand, high Ki-67 expression was detected in SCC (83.9%), CIN-3 (92.9%), and CIN-2 (31.3%), whereas no high Ki-67 expression was detected in CIN-1 and NCT. Additionally, Ki-67 expression was significantly higher in SCC and CIN-3 compared to CIN-2, CIN-1, and NCT ($p < 0.05$). Nevertheless, no significant difference was found among different grading and staging groups of SCC with regard to Ki-67 expression ($p > 0.05$) (Fig. 2, Tables 2,3,4).

In ISH analysis, positive HR-HPV staining was detected in SCC (100.0%), CIN-3 (100%), CIN-2 (93.8%), CIN-1 (88.9%), and NCT (23.1%). Moreover, HR-HPV positivity was significantly higher in SCC and SIL compared to NCT ($p < 0.05$). However, no significant difference was found among different grading and staging groups of SCC with regard to HR-HPV positivity ($p > 0.05$). In contrast, positive LR-HPV staining was detected in 5.6% and 12.5% of CIN-1 and CIN-2 samples, respectively. However, negative LR-HPV staining was detected in SCC, CIN-3, and NCT. Additionally, no significant difference was found among the SCC, SIL, and NCT groups and among the different grading and staging groups of SCC with regard to LR-HPV staining ($p > 0.05$) (Fig. 2, Tables 2,3,4).

Six NTRK stained samples were positive for either NTRK1 or NTRK2 staining. All six samples were also positive for high p16 expression and HR-HPV staining and were negative for LR-HPV staining. The three NTRK positive

Table 2. Immunohistochemical and *in situ* hybridization findings.

	NCT	CIN-1	CIN-2	CIN-3	SCC	All	*p* value
	(n = 13) (%)	(n = 18) (%)	(n = 16) (%)	(n = 14) (%)	(n = 31) (%)	(n = 92) (%)	
NTRK1							0.295
Positive	0 (0.0)	0 (0.0)	2 (12.5)	0 (0.0)	1 (3.2)	3 (3.3)	
Negative	13 (100.0)	18 (100.0)	14 (87.5)	14 (100.0)	30 (96.8)	89 (96.7)	
NTRK2							0.302
Positive	0 (0.0)	0 (0.0)	1 (6.3)	0 (0.0)	2 (6.5)	3 (3.3)	
Negative	13 (100.0)	18 (100.0)	15 (93.8)	14 (100.0)	29 (93.5)	89 (96.7)	
p16	a	b	c	d	d		0.032
High	0 (0.0)	5 (27.8)	12 (75.0)	14 (100.0)	29 (93.5)	60 (65.2)	
Low	13 (100.0)	13 (72.2)	4 (25.0)	0 (0.0)	2 (6.5)	32 (34.8)	
Ki-67	a	a	a	b	b		0.001
High	0 (0.0)	0 (0.0)	5 (31.3)	13 (92.9)	26 (83.9)	44 (47.8)	
Low	13 (100.0)	18 (100.0)	11 (68.8)	1 (7.1)	5 (16.1)	48 (52.2)	
HR-HPV	a	b	b	b	b		0.001
Positive	3 (23.1)	16 (88.9)	15 (93.8)	14 (100.0)	31 (100.0)	79 (85.9)	
Negative	10 (76.9)	2 (11.1)	1 (6.3)	0 (0.0)	0 (0.0)	13 (14.1)	
LR-HPV							0.131
Positive	0 (0.0)	1 (5.6)	2 (12.5)	0 (0.0)	0 (0.0)	3 (3.3)	
Negative	13 (100.0)	17 (94.4)	14 (87.5)	14 (100.0)	31 (100.0)	89 (96.7)	

a,b,c,d: Assignment of a different letter indicates the presence of a significant difference between the group means ($p < 0.05$). NCT, non-neoplastic cervical tissue; CIN, Cervical intraepithelial neoplasia; SCC, squamous cell carcinoma; NTRK, neurotrophic tyrosine receptor kinase; HPV, Human papillomavirus; HR, high-risk; LR, low-risk.

SCC cases had poorly differentiated morphology and high Ki-67 expression, two had early and one advanced stage disease (Table 5). Alternatively, both the NTRK1 and NTRK2 stainings showed poor agreement with p16/Ki-67 expression and HR/LR-HPV positivity ($\kappa < 0.40$). p16/Ki-67 expression showed poor agreement with HR/LR-HPV positivity ($\kappa < 0.60$). However, a significant agreement was observed between p16 and Ki-67 expression ($\kappa > 0.60$) (Table 6).

4. Discussion

Squamous cell carcinoma of the cervix uteri is the most common malignant tumor of the female genital tract in many countries. In the present study, the median age of the cases with SCC and SIL, the HR/LR-HPV findings, and the good agreement between increased p16/Ki-67 expression and neoplastic progression were consistent with the literatüre [3–6, 22, 25–29]. However, we think that the unexpected 23.1% HR-HPV positivity in NCT is due to the fact that some of cases in this group included clinically suspected cervical lesions and HPV, as detected by the other health centers.

Literature reviews indicate a limited number of studies investigating *NTRK* gene fusions in cervical SCC. In a previous study, Gatalica ve *et al.* [9] investigated *NTRK* gene fusions in tissue samples from 11,502 patients and detected *NTRK1* gene fusions in CCs (1.5%; 1/68 cases), glial tumors (0.3%), soft tissue sarcomas (0.2%), colorectal carcinomas (0.2%), and lung adenocarcinomas (0.02%). Additionally, the authors detected *NTRK2* gene fusions in glial tumors (0.9%) and lung adenocarcinomas (0.02%). Lee *et al.* [14] detected positive TrkA expression in 2.7% and 1.5% of colorectal and gastric carcinomas, respectively. However, Moon *et al.* [15] found a remarkably high level of TrkB expression in the SCC of the uterine cervix (72.5%; 58/80 cases). In our study, positive NTRK1 staining was detected in 3.2% of SCC samples, as consistent with the study conducted by Gatalica *et al.* [9]. However, unlike the study by Gatalica *et al.* [9], positive NTRK2 staining was detected in 6.5% of SCC cases. In addition, positive NTRK1 and NTRK2 staining was detected in 12.5% and 6.3% of CIN-2 samples, respectively.

To our knowledge, there has been no study in the literature investigating *NTRK* gene fusions in cervical SIL. In cases of breast carcinoma, the HER2 (human epidermal growth factor receptor 2) overexpression is not considered in the assessment of normal breast tissue and in carcinoma *in situ* except for in invasive foci [30]. Similarly, NTRK1/NTRK2 expression, though its significance remains unknown, may also not be included in the assessment of cervical SIL.

In our study, both NTRK1 and NTRK2 showed positive staining in SCC and HSIL in a small number of cases and showed no positive staining in NCT and LSIL, and all the stained SCC samples showed poor differentiation. These findings imply that NTRK may have a role in CC progression, although it is challenging to draw such a conclusion due to the small number of positive stained samples. Alternatively, our findings also suggest that both NTRK1 and NTRK2 are not an auxiliary marker in grading cervical SCC and its differentiation from SIL. Nevertheless, we consider that Trk inhibitors could be a therapeutic option in these small num-

Table 3. Immunohistochemical and *in situ* hybridization findings among different grading groups of squamous cell carcinoma.

	WD	MD	PD	All	*p* value
	(n = 10) (%)	(n = 12) (%)	(n = 9) (%)	(n = 31) (%)	
NTRK1					0.471
Positive	0 (0.0)	0 (0.0)	1 (11.1)	1 (3.2)	
Negative	10 (100.0)	12 (100.0)	8 (88.9)	30 (96.8)	
NTRK2					0.109
Positive	0 (0.0)	0 (0.0)	2 (22.2)	2 (6.5)	
Negative	10 (100.0)	12 (100.0)	7 (77.8)	29 (93.5)	
p16					0.292
High	9 (90.0)	11 (91.7)	9 (100.0)	29 (93.5)	
Low	1 (10.0)	1 (8.3)	0 (0.0)	2 (6.5)	
Ki-67					0.588
High	8 (80.0)	10 (83.3)	8 (88.9)	26 (83.9)	
Low	2 (20.0)	2 (16.7)	1 (11.1)	5 (16.1)	
HR-HPV					0.999
Positive	10 (100.0)	12 (100.0)	9 (100.0)	31 (100.0)	
Negative	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
LR-HPV					0.999
Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Negative	10 (100.0)	12 (100.0)	9 (100.0)	31 (100.0)	

No significant difference was found among the groups ($p > 0.05$).

WD, well-differentiated; MD, moderately-differentiated; PD, poorly-differentiated; NTRK, neurotrophic tyrosine receptor kinase; HPV, Human papillomavirus; HR, high-risk; LR; low-risk.

bers of NTRK1/NTRK2 stained SCC since these inhibitors have been shown to provide effective outcomes in the target treatment of selected patients with *NTRK* gene fusions [7, 8, 10, 16].

There are a number of studies suggesting that NTRK1/NTRK2 show higher IHC expression in squamous differentiated tissues and SCC [15, 31–34]. Cho *et al.* [33] evaluated a total of 396 patients with oropharyngeal and non-oropharyngeal head and neck SCC and detected high expression of TrkA (40.9%) and panTrk (43.2%), which was relatively higher in patients with oropharyngeal, p16-positive, and HPV-positive SCC cases. Terry *et al.* [34] evaluated patients with lung tumors and reported that positive NTRK1/NTRK2 staining were detected in 72% and 51% of SCC patients and in 8.4% and 2.7% of the patients with adenocarcinomas, respectively. In our study, however, positive NTRK1/NTRK2 staining were both detected in 3.3% of the samples, although all our cases had squamous epithelium. Additionally, although all of these positive cases showed high p16 expression and HR-HPV positivity, both NTRK1/NTRK2 showed a poor agreement with p16 and HR-HPV ($\kappa < 0.40$).

Accumulating evidence suggests that IHC may not be as reliable as molecular methods in the detection of *NTRK* gene fusions [9, 16–18]. However, IHC is considered a cost-effective and practical method and has been shown to have a sensitivity of 75–100%, a specificity of 57.6–100%, a positive predictive value (PPV) of 9.3–100%, and a negative predictive value (NPV) of 96–100% in the detection of *NTRK* gene fusions [9, 11, 16, 17]. Gatalica *et al.* [9] evaluated samples from 28 different tumors with *NTRK* gene fusions and detected positive panTrk IHC staining in 21 cases. The authors also noted that the sensitivity of NTRK1, NTRK2, and NTRK3 staining in IHC were 87.5%, 88.9%, and 54.5%, respectively. Similarly, Rudzinsk *et al.* [17] reported that IHC had a sensitivity of 100%, specificity of 63.3%, PPV of 59.1%, and NPV of 100% in the detection of NTRK1 rearrangement.

Some previous studies suggested that IHC could be used as an auxiliary method and also as a screening tool in the detection of cases with *NTRK* gene fusions [11, 12, 16]. Similar to the HER-2 in breast carcinomas and the ALK [D5F3] IHC methods in lung cancers [30, 35], it may be useful to detect or screen *NTRK1/NTRK2* gene fusion by an IHC method in cervical SCC.

5. Conclusions

In conclusion, the results indicated that the increased p16 and Ki-67 staining in SCC and SIL of the uterine cervix showed a good agreement with tumor progression. Moreover, HR-HPV showed remarkably high expression in SCC and SIL. However, both NTRK1 and NTRK2 were found to have no contributory effect on the differentiation of cervical SCC from the SIL. For the small number of SCC cases with positive NTRK1/NTRK2 staining, Trk inhibitors may be a therapeutic alternative.

The most important limitation of our study is its relatively small sample size. In addition, in order to confirm positive NTRK1/NTRK2 expression in CIN-2 cases, further studies

Table 4. Immunohistochemical and *in situ* hybridization findings among different staging groups of squamous cell carcinoma.

	Early stage	Advanced stage	All	*p* value
	(n = 14) (%)	(n = 17) (%)	(n = 31) (%)	
NTRK1				0.299
Positive	1 (7.1)	0 (0.0)	1 (3.2)	
Negative	13 (92.9)	17 (100.0)	30 (96.8)	
NTRK2				0.888
Positive	1 (7.1)	1 (5.9)	2 (6.5)	
Negative	13 (92.9)	16 (94.1)	29 (93.5)	
p16				0.888
High	13(92.9)	16 (94.1)	29 (93.5)	
Low	1 (7.1)	1 (5.9)	2 (6.5)	
Ki-67				0.473
High	11 (78.6)	15 (88.2)	26 (83.9)	
Low	3 (21.4)	2 (11.8)	5 (16.1)	
HR-HPV				0.999
Positive	14 (100.0)	17 (100.0)	31 (100.0)	
Negative	0 (0.0)	0 (0.0)	0 (0.0)	
LR-HPV				0.999
Positive	0 (0.0)	0 (0.0)	0 (0.0)	
Negative	14 (100.0)	17 (100.0)	31 (100.0)	

No significant difference was found among the groups ($p > 0.05$).
NTRK, neurotrophic tyrosine receptor kinase; HPV, Human papillomavirus; HR, high-risk; LR, low-risk.

Table 5. Cases showing positive NTRK1 and NTRK2 staining.

	Diagnosis	Stage	Age	NTRK1	NTRK2	p16	Ki-67	HR-HPV	LR-HPV
Case-23	CIN-2		31	Positive	Negative	High	Low	Positive	Negative
Case-31	CIN-2		45	Negative	Positive	High	Low	Positive	Negative
Case-32	CIN-2		41	Positive	Negative	High	Low	Positive	Negative
Case-74	SCC (PD)	Early (2A1)	52	Positive	Negative	High	High	Positive	Negative
Case-75	SCC (PD)	Early (2A2)	50	Negative	Positive	High	High	Positive	Negative
Case-78	SCC (PD)	Advanced (2B)	49	Negative	Positive	High	High	Positive	Negative

NTRK, neurotrophic tyrosine receptor kinase; HPV, Human papillomavirus; HR, high-risk; LR, low-risk; CIN, cervical intraepithelial neoplasia; SCC, squamous cell carcinoma; PD, poorly-differentiated.

Table 6. Kappa (κ) statistics.

	NTRK1	NTRK2	p16	Ki-67	HR-HPV	LR-HPV
NTRK1	1.000					
NTRK2	–0.034	1.000				
p16	0.035	0.035	1.000			
Ki-67	–0.020	0.026	0.614	1.000		
HR-HPV	0.011	0.011	0.416	0.220	1.000	
LR-HPV	–0.034	–0.034	–0.032	–0.020	–0.041	1.000

NTRK, neurotrophic tyrosine receptor kinase; HPV, Human papillomavirus; HR, high-risk; LR, low-risk.

with larger samples are needed in SIL cases. If this study to be conducted confirms our results, it can contribute significantly to the NTRK1/NTRK2 information in these pre-malignant lesions.

Author contributions

RE: Study design, data collection and manuscript writing. FD: Data collection and revised the manuscript. HHA: Data analysis and revised the manuscript. IB: Supervision and data validation. IA: Data analysis and data validation. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in this study were performed in accordance with the ethical standards of Van Yuzuncu Yil University, Faculty of Medicine and the 1964 Helsinki Declaration and subsequent amendments or comparable ethical standards. This study was approved by Van Yuzuncu Yil University Noninterventional Clinical Research Ethics Committee (Approval No: 06; Date: 20 June 2017).

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Conflict of interest

The authors declare no conflict of interest.

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