# NtrkA Immunohistochemical Expression in Papillary Thyroid Carcinoma: A Clinicopathological Study

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## Abstract:

**Background:** Neurotrophin receptors are becoming more widely recognized as potential cancer therapeutic targets, although their clinical and pathological implications in thyroid cancer remain unclear. Examining neurotrophic tyrosine receptor kinase (Ntrk) fusions in papillary thyroid carcinoma (PTC) patient cohorts has offered information on the genetic basis of thyroid cancer.

**Methods**: This study included patients with PTC, thyroid adenoma, and multinodular goiter (MNG). The study aimed to investigate the association between the clinicopathological parameters and tropomyosin receptor kinase A (TrkA) expression in PTC patients and also to evaluate the immunohistochemical expression of TrkA in cases of thyroid adenoma and MNG, using immunohistochemistry streptavidin-biotin-technique.

**Results:** This study comprised 77 cases in total, 27 cases with PTC, 13 with thyroid adenoma, and 37 cases with MNG, TrkA expression was not observed in adenoma or MNG cases, while expression was observed in 29.6% of PTC cases. TrkA expression differed significantly between instances of PTC, thyroid adenoma, and MNG (P<0.001). In PTC, there was a strong positive relationship between TrkA expression and T stage (P=0.002), lymph node involvement (P=0.004), and distant metastasis. There was no significant statistical difference in TrkA expression among patients based on their age or gender.

**Conclusion:** TrkA expression is considered a negative prognostic marker, associated with tumor progression, advanced stages, and the presence of both lymph node and distant metastases, which may be valuable in assessing the aggressiveness of the tumor. TrkA could be a useful therapeutic target to reduce invasion and metastasis.

**Keywords:** NtrkA, PTC, Immunohistochemistry.

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## Background:

The most prevalent endocrine malignancy is PTC, which has a good prognosis in most instances. The rising global prevalence of PTC emphasizes the significance of early identification of individuals with aggressive tumors who will require more extensive surgery and/or postoperative treatment. The development of good prediction models may aid decision-making in specific instances [1]. PTC accounts for almost 84% of all thyroid malignancies [2].

Radiation exposure is a principal risk factor in PTC development; the incidence of PTC is higher in people with an exposure history to significant ionizing radiation. Familial Adenomatous Polyposis-Gardner Syndrome, Carney Complex Type 1, and Werner Syndrome are all familial syndromes associated with PTC [3].

The majority of PTC has a favorable prognosis, with a five-year overall survival rate exceeding 95%; however, tumor size, age, multifocality, nodal and distant metastases may point to more aggressive patients [4]. Anaplastic thyroid carcinoma (ATC), a highly lethal form of cancer, arises in a small subset of PTC cases [5]. Most ATCs arise from preexisting differentiated or poorly differentiated thyroid carcinoma [6].

NTRK1, NTRK2, and NTRK3 are genes that encode members of the Trk family, which includes

TrkA, TrkB, and TrkC receptors [7]. Neurotrophin receptors are excessively expressed in several human malignancies, and therefore play a role in tumor development and proliferation stimulation [8].

According to different research [9-11], NTRK rearrangements were found in up to 26.7% of pediatric instances of ary PTC. In contrast, data from The Cancer Genome Atlas (TCGA) and a substantial Chinese investigation found that the prevalence of NTRK rearrangements ranged between 2.3% and 3.4% across all age categories [12,13].

The identification of NTRK fusions in certain patients with PTC has enhanced our understanding of the genetic factors involved in thyroid cancer. As a result, there is growing interest in genomic testing for PTC patients to pinpoint individuals who could benefit from targeted treatments using Trk inhibitors [14].

Studies discussing the prognostic role of TrkA expression in PTC are limited. This study aimed to assess the relationship between TrkA immunohistochemical expression in patients with PTC and their clinicopathological characteristics, and also to elucidate the immunohistochemical expression of TrkA in cases of thyroid adenoma and MNG.

## Methods:

*Inclusion criteria:*

Formalin-fixed paraffin embedded tissue blocks were retrieved for this study from archived material in pathology department, Oncology department, Sohag university hospitals during period from January 2020 to December 2022

*Tissue samples*

Thyroid tissue samples included 77 adult patients; twenty-seven (27) cases of PTC, thirteen (13) cases of thyroid adenoma, and thirty-seven (37) cases of MNG. These samples were obtained from archives at the Pathology Department, Sohag University Hospitals, and the Sohag Oncology Center. All cases of PTC were obtained from total thyroidectomy surgery. Four cases of thyroid adenoma were removed by total thyroidectomy surgery and the other nine cases were removed by hemithyroidectomy, twelve cases of MNG were obtained from total thyroidectomy, and twenty-five cases were obtained from hemithyroidectomy. Clinical data for these instances were obtained from their medical records.

*Immunohistochemistry*

This study employed an antibody and chromogen detection equipment acquired from Boster Biological Technology. Four micrometer-thick slices of formalin-fixed paraffin-embedded tissue blocks from the test cases were removed of paraffin in xylene and hydrated once more with alcohols that had been down-regulated. To decrease endogenous peroxidase activity, tissue slices were rinsed with running water before incubating for ten minutes in 0.5% hydrogen peroxide. After washing, the samples were boiled in 10 mM citrate buffer (pH 6.0) in a high-power microwave for a total of twenty minutes to retrieve antigens.

Following a rinse in phosphate-buffered saline (PBS), various sections were exposed to a rabbit monoclonal anti-TrkA antibody at a 1:100 dilution. (catalog # M00706-1, Boster Biological Technology, Pleasanton, CA) at a temperature of 4°C overnight. After one day, they were rinsed with phosphate-buffered saline (PBS), and the sections were then incubated with streptavidin at room temperature for 10 minutes. Following a wash, they were exposed to 3,3′-diaminobenzidine tetrahydrochloride (DAB) solution, producing a brown precipitate. The sections were then counterstained with hematoxylin, dehydrated, and mounted using standard procedures. For the immunohistochemistry procedure, PBS was recorded in place of the primary antibodies as a negative control, while the normal appendix served as the positive control.

*Scoring*

Owing to the limited proportion of cells that showed positivity for TrkA immunohistochemistry, scoring as positivity versus negativity staining was recorded [15]. Tumors were regarded as positive if ≥1% of cells showed positivity at any intensity. Various staining patterns were likewise regarded as positive [16].

*Statistical analysis*

The version of the IBM SPSS Statistics 23.0 software was applied for statistical analysis. The test known as the Kolmogorov-Smirnov test was applied to determine the normality of continuous data, with findings reported as median, interquartile range, and full range. The categorical data was presented as frequencies and percentages and evaluated using Fisher's exact test. A p-value $< 0.05$ showed significance in statistics.

## Results:

This investigation included 27 cases of PTC, 13 cases of thyroid adenoma, and 37 cases of MNG; the age distribution of the studied patients is illustrated in (Table 1). TrkA expression was not observed in MNG or adenoma samples, while TrkA expressed as brownish cytoplasmic and membranous staining in 5-30% of tumor cells in 8/27 (29.6%) cases of PTC (Figure 1). There was a statistically significant difference in TrkA expression among cases of PTC, thyroid adenoma, and MNG ($P<0.001$) as shown in (Table 2).

The comparative examination of the various clinicopathological features of the examined patients of PTC revealed a statistically significant positive correlation between TrkA expression and T stage ($P=0.002$). One case, 1/16 (6.3%) with T1/T2 stage showed TrkA expression, while 7/11 (63.6%) cases with T3/T4 showed TrkA expression. Regarding the lymph node status, only 3/21 (14.3%) cases without lymph node metastasis revealed TrkA expression while the majority of cases with lymph node metastasis 5/6 (83.3%) revealed TrkA expression and this difference

was statistically significant (P=0.004). Additionally, there was a strong positive correlation involving TrkA expression and distant metastasis (P=0.017), where 4/5 (80%) of cases with distant metastasis showed TrkA expression versus 4/22 (18.2%) of cases without distant metastasis showed TrkA immunohistochemical expression. However, TrkA expression showed no significant association with patients' age and gender (Table 3).

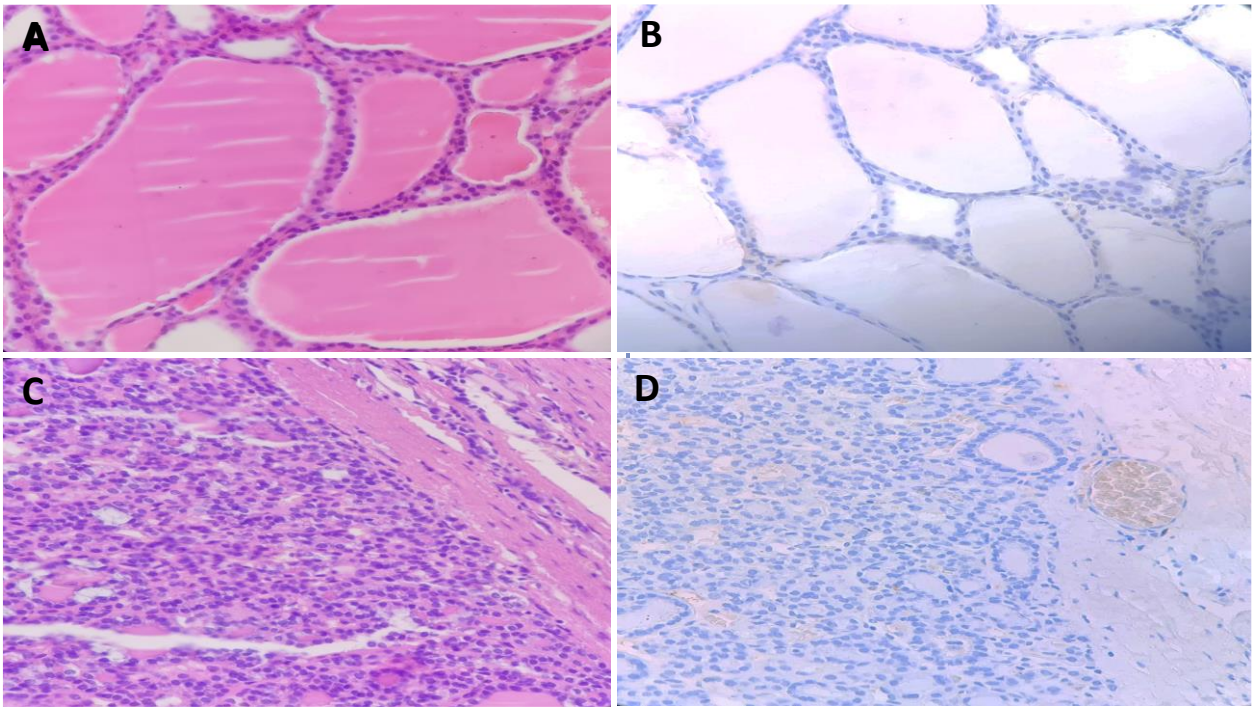


Figure (1): Multinodular goiter (A) and Follicular adenoma (C) show negative expression of NtrkA immunohistochemical (B & D), respectively (400X).

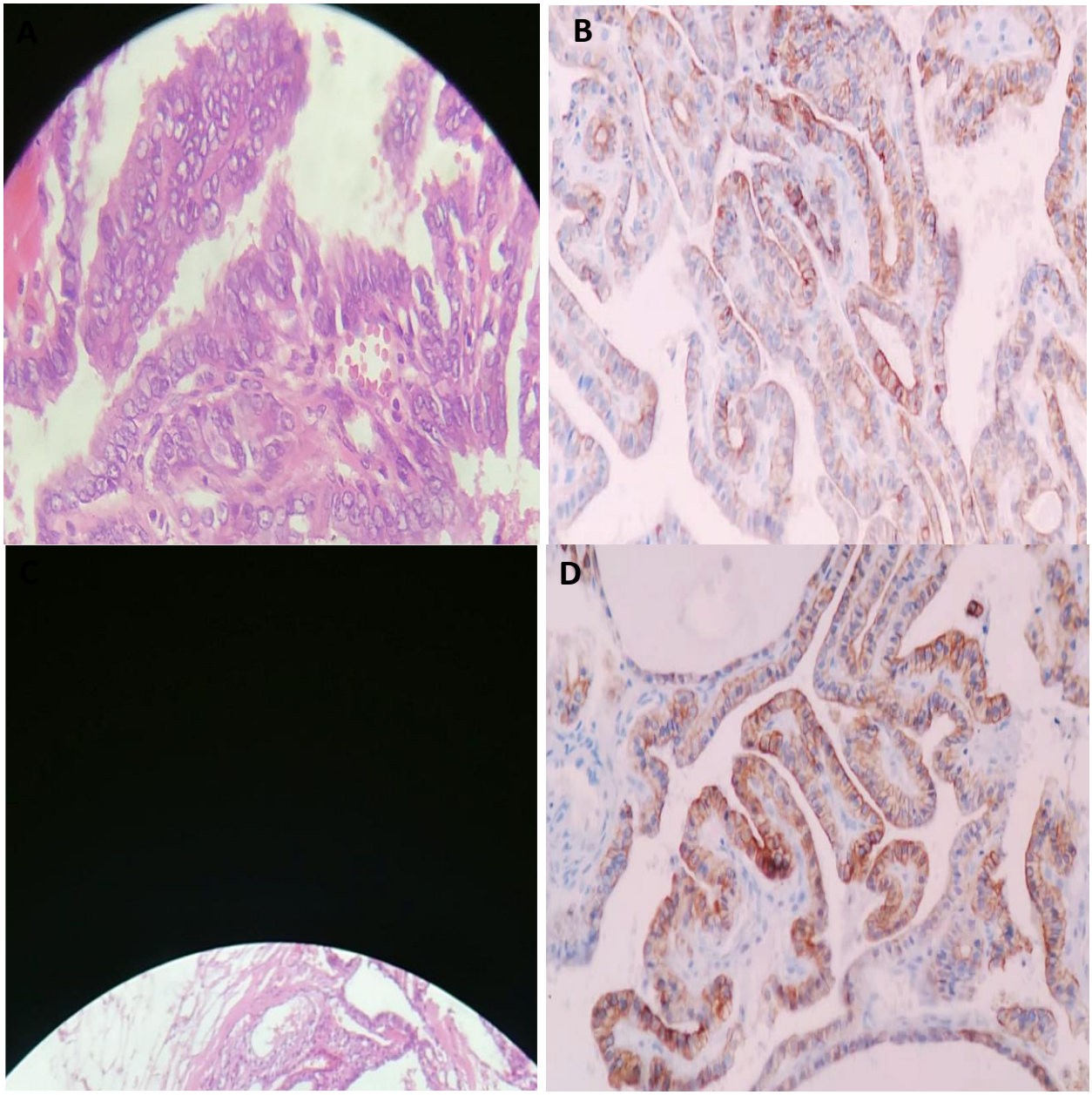


Figure (2): Papillary thyroid carcinoma (A and C) reveals positive cytoplasmic and membranous expression of NtrkA (B and D), 400X.

Table 1: Age distribution of the studied patients

| Histopathological type | No. of cases | Age (years) | |
|---|---|---|---|
| | | Mean ± SD | Median (Range) |
| Papillary thyroid carcinoma | 27 | 48.14 ± 8.10 | 49 (32-59) |
| Thyroid adenoma | 13 | 40.31 ± 8.18 | 40 (29-52) |
| Multinodular goiter | 37 | 43.49 ± 6.65 | 40 (33-61) |

Table 2: Association between TrkA immunohistochemical expression and cases of PTC, Thyroid adenoma, and Multinodular goiter

| Histopathological type | No. of cases | TrkA IHC Expression | | P-value |
|---|---|---|---|---|
| | | Positive | Negative | |
| Papillary Thyroid carcinoma | 27 | 8 (29.6%) | 19 (70.3%) | <0.001* |
| Thyroid adenoma | 13 | 0 | 13 (100%) | |
| Multinodular goiter | 37 | 0 | 37 (100%) | |

P- value was calculated by Fisher's Exact test.
*Statistically significant difference ($p<0.05$).

Table 3: Association between TrkA expression and the clinicopathological parameters in PTC patients

| Parameter | No. of cases of PTC (27) | TrkA IHC Expression | | P-value |
|---|---|---|---|---|
| | | Positive 8 (29.6%) | Negative 19 (70.3%) | |
| | | Age | | |
| ≤ 55 years | 16 (59.3%) | 5 (31.2%) | 11 (68.8%) | 1.000 (NS) |
| > 55 years | 11 (40.7%) | 3 (27.3%) | 8 (72.7%) | |
| | | Gender | | |
| Females | 18 (66.7%) | 6 (33.3%) | 12 (66.7%) | 0.676 (NS) |
| Males | 9 (33.3%) | 2 (22.2%) | 7 (77.8%) | |
| | | T stage | | |
| T1/T2 | 16 (59.3%) | 1 (6.3%) | 15 (93.7%) | 0.002* |
| T3/T4 | 11 (40.7%) | 7 (63.6%) | 4 (36.4%) | |
| | | LN status | | |
| N0 | 21 (77.8%) | 3 (25%) | 18 (75%) | 0.004* |
| N1 | 6 (22.2%) | 5 (83.3%) | 1 (16.7%) | |
| | | Distant Metastasis | | |
| M0 | 22 (81.5%) | 4 (18.2%) | 18 (81.8%) | 0.017* |
| M1 | 5 (18.5 %) | 4 (80%) | 1 (20%) | |

P- value was calculated by Fisher's Exact test.
*Statistically significant difference ($p<0.05$), NS = Non-Significant

## Discussion:

The NTRK1, NTRK2, and NTRK3 genes encode three transmembrane proteins known as Trk A, B, and C receptors. These receptors have lately been suggested as a prospective cancer therapeutic target [16].

NTRK rearrangements can be found in patients with PTC. Protein expression analysis with immunohistochemistry can be used to evaluate NTRK rearrangements [17].

Neurotrophin receptors are becoming more prominent as cancer targets, however, their clinical importance in PTC is unknown.

This study showed no immunohistochemical expression of TrkA in cases of thyroid adenoma or MNG, while TrkA was expressed in 8/27 (29.6%) of PTC specimens. Lack of immunohistochemical expression of TrkA could be explained by absence of NTRK gene fusions (NTRK1) to another gene partner which is an oncogenic driver.

Similarly, Faulkner et al., 2018 [15] found no immunohistochemistry expression of TrkA in normal thyroid tissue or adenoma samples, however, TrkA expression was detected in 27% of PTC cases. In contrast Brzeziańska et al., 2006 [18] and Musholt et al., 2000 [19] found a lower rate of rearrangements of NTRK1 in 12% and 12.6% of PTC cases, respectively. This may be due to differences in the method of detection of NtrkA. Ntrk fusion gene identification is mostly accomplished by immunohistochemistry, fluorescence in situ hybridization (FISH), RT-PCR, and

next-generation sequencing (NGS) determined by both DNA and RNA [20]. Pan-Trk immunohistochemical expression was found in 46.3% of cases of PTC as recorded by Macerola et al., 2022 [21], This increased prevalence might be attributed to changes in the antibody used and the number of cases.

Pan Trk immunohistochemical can be utilized as a screening tool and possible predictive marker in the detection/diagnosis of NTRK fusion cancers [22]. as recurrent NTRK fusions have been identified in a variety of cancers including salivary-gland tumors, soft-tissue sarcomas, infantile fibrosarcoma, as well as, colon, skin, and lung cancers, both in pediatric and adult patients [23].

The present investigation found no significant association between TrkA expression and patient age or gender. The results are consistent with those published by Faulkner et al., 2018 [15] and Brzeziańska et al., 2006 [18], who found no significant connection between patients' age and gender and NTRK1 rearrangement.

This study, revealed a statistically significant positive association between T stage/tumor size and TrkA immunohistochemical expression, In contrast to Faulkner et al., 2018 [15], who did not find a significant association; this might be related to discrepancies in the antibodies used and the number of cases.

In the present investigation, TrkA expression showed a significant positive association with LN status, similar to the result reported by Faulkner et al., 2018 [15] and in agreement with Kong et al., 2021 [23] who found that NTRK gene fusions were significantly associated with LN metastasis.

The current study revealed distant metastasis in 4/8 (50 %) of TrkA immunohistochemical -positive cases of PTC, a lower percentage was reported by Pekova et al., 2021 [24] who found distant metastasis in 3/10 (30%) of NTRK1 fusion-positive carcinomas, this may be due to differences in the method of detection of NTRKA, with a variable number of cases. Furthermore, this study uncovered a noteworthy link between TrkA immunohistochemical expression and the occurrence of distant metastasis. Notably, this correlation has not been explored in previous studies.

Although neurotrophin receptors are new targets in oncology, it is unknown how important neurotrophin receptors are clinicopathologically in thyroid cancer. According to this study, TrkA protein expression is linked to lymph node metastasis and is observed in about 20% of thyroid tumors. It serves as a marker of tumor aggressiveness. TrkA is increasingly being investigated as a therapeutic target in oncology. It is involved in the promotion of cancer cell invasion in numerous malignancies, including those of the breast [26], prostate [27], and pancreatic [28]. Trk inhibitors are undergoing clinical trials for lung cancer [29], while TrkA activation contributes to cancer cell invasion in breast cancer [26]. The available information validates NTRKA's function as an oncogenic protein and raises the possibility that treating thyroid cancer may benefit from targeting this pathway.

*Limitations*

Extrapolation of population-based data is challenging due to the small sample size.

## Conclusion:

In this study, TrkA immunohistochemical expression was detected in a proportion of PTC cases, while it was absent in cases of thyroid adenoma and MNG. TrkA expression is linked to higher T stages, lymph node metastasis, and distant metastasis. These findings suggest TrkA could serve as a valuable marker to assess the aggressiveness of tumors. TrkA could be a useful therapeutic target to reduce invasion and metastasis in PTC.

**Competing interest:**

The author(s) have nothing to declare

**Authors' contributions**

SF, WA, and RA contributed to the conception and design of the work. WD, SF, SM contributed to the acquisition, analysis, and interpretation of the data. WA, RA, and SF wrote the initial draft of the manuscript. SF, WA, RA, and SM revised and supervised the work. All authors approved the final version of the manuscript.

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