Immunohistochemical Expression of WT1 in Nasopharyngeal Carcinoma Among Filipino Patients in a Tertiary Hospital*

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ABSTRACT

Background. Nasopharyngeal carcinoma (NPC) is endemic in Southeast Asia and the Philippines. Novel treatments are desirable due to the high disease burden and adverse effects of existing modalities. Detection of WT1 expression via immunohistochemistry has been reported in many tumors. Moreover, immunotherapy via WT1 peptide vaccination has shown promising results in a wide range of malignancies. No studies on WT1 expression in NPC have been published in any population.

Objective. Documenting WT1 expression in NPC via immunohistochemistry may provide insight into the possibility of using WT1 vaccination for this disease.

Methodology. This was a retrospective descriptive study. All newly-diagnosed cases of NPC from 2016 to 2017 with samples stored in the Department of Laboratories of the Philippine General Hospital were considered. Cases were included based on specific criteria. The tumor classification of each case was reviewed and WT1 immunohistochemistry staining was performed. Assessment of the strength of WT1 immunostaining was conducted. The results were analyzed using Chi-square test for association with fisher exact correction.

Results. A total of 57 cases were included, all of which were non-keratinizing squamous cell carcinomas (NK-SCCs). Forty-nine were undifferentiated type while eight were differentiated type. The mean age was 48 years. Two thirds were male, one third were female. Seventeen of the 57 cases (29.8%) were positive for WT1 immunostaining, and all were undifferentiated type. The majority (82.32%) of the positive cases showed cytoplasmic expression. There was a significant association between tumor classification and WT1 staining.

Conclusion. Similar to studies conducted in other carcinomas, a considerable subset of NPCs express WT1. This finding opens other avenues for exploration, including the feasibility of WT1 peptide vaccination as a treatment option. Further studies on the associations between WT1 and NPC are recommended.

Key words: nasopharyngeal cancer, Wilms' tumor, wt1, immunohistochemistry, immunotherapy

INTRODUCTION

Nasopharyngeal carcinoma (NPC), while rare in most parts of the world (<1 per 100,000), is endemic in Southeast Asia, with an estimated incidence ranging from three to 30 per 100,000.[1] The highest incidences (15-50 per 100,000) have been recorded in China, particularly in the southern regions, and it is uncommon among Caucasians.[2] The pathogenesis is complex, but Epstein-Barr virus (EBV) infection is a major predisposing factor especially in endemic areas. EBV-LMP1 is the primary oncogene identified and is present in up to 90% of tumors.[3] EBV infection along with genetic predisposition and environmental factors altogether contribute to tumorigenesis.[4]

A preliminary analysis by Mejia and Sarmiento in 2014 based on data from 49 patients from four centers in one year estimated the disease burden in the Philippines to be 2.07 per 100,000.[1] This study did not include data from the Philippine General Hospital (PGH). The mainstay of treatment in NPC is radiotherapy, with 10-year survival rates of up to 43% overall.[3] Country-specific data on survival and remission rates for the Philippines is lacking.

Given the high disease prevalence and the side effects of radiotherapy, including the risk for development of second primary tumors,[5] new anti-cancer treatments are desirable. The role of the immune system in cancer progression and control has been known for years.[6] The field of immunotherapy has emerged as an important front in the development of novel anti-cancer therapies. Interest in cancer immunotherapy has grown considerably since the discovery of the T-cell receptor in 1982.[7] Subsequent research and clinical trials gave way to the approval by the United States Food and Drug Administration of anti-cancer immune checkpoint antibodies targeting CTLA-4 (Ipilimumab) in 2011[7] and PD-1 (Pembrolizumab) in 2014.[8]

Historically, immunotherapeutic research on NPC has been focused on the EBV antigens LMP1, LMP2, EBNA1, EBER and EBV-encoded RNA.[9] These antigens have shown limited immunogenicity, play a role in tumor oncogenesis and contribute to viral latency and immune evasion. Together, these attributes constitute major challenges in harnessing EBV-related antigens as immunotherapy targets.[9] Studies on non-EBV antigens, such as immune checkpoint antibodies, have also been performed. Recently, various clinical trials using EBV-related (anti-LAG3, anti-LMP2 vaccine) and non-EBV immunotherapeutic agents (Pembrolizumab, Nivolumab) have started. A phase Ib trial published in 2017 showed an objective response rate of 25.9% to Pembrolizumab in 27 patients. Eligibility in this trial included unresectable or metastatic disease, failure of prior standard therapy, and immunohistochemical PD-L1 expression in 1% or more of either the tumor cells or tumor-infiltrating lymphocytes.[10] Even more recently, cell-based treatments using autologous cytotoxic T-lymphocytes (CTLs) are underway.[9]

Wilms' Tumor 1 (WT1) peptide vaccines are among the immunotherapies in development outside of current NPC-related research. The earliest was developed in Japan by Oka et al.,[11] which was followed by a series of further investigations and clinical trials by various authors.[12,13] The vaccine-based therapy indirectly enhances the immune response against malignant tumors through stimulating the action of WT1-specific CD8+ CTLs.[11,12] The WT1-specific CTLs do not induce cell lysis in normal tissues that express wild-type WT1 due to complex immunologic factors,[11,12] thus greatly limiting toxicity. Central to the selection of cases for WT1 peptide immunotherapy is the detection of WT1 protein in tumor cells using polymerase chain reaction, Western blot, immunohistochemistry and/or other methods.[11,14,15] Evidence accrued from early trials have solidified immunohistochemistry, an accessible and relatively inexpensive technique, as a reliable index of WT1 expression.[11,13,15]

The preliminary clinical trials of WT1 peptide vaccination focused on hematologic malignancies. The results for leukemia and myelodysplastic syndromes were promising in terms of slowing disease progression.[12,13] WT1 was found to be highly-immunogenic in these trials. Trials have also been conducted on cases of glial tumors, soft tissue malignancies and various solid cancers.[6,7,13] In 2009, the American National Cancer Institute identified WT1 as the top priority antigen in cancer treatment research among 75 different antigens.[16] There has been considerable interest in documenting WT1 expression in various tumors for the purpose of identifying possible candidates for WT1 peptide immunotherapy.[6,7,12,15]

At the time that this study was conducted, there were no published studies in the English literature documenting WT1 expression in NPC using immunohistochemistry. Literature search was performed in PubMed and Google Scholar which yielded no published articles. Given the high immunogenicity of WT1 as an immunotherapeutic target in other malignancies, it would be worthwhile to determine the degree of WT1 expression in NPC. Subsequently, this could potentially provide a rationale for utilizing WT1 immunotherapy for the treatment of this malignancy.

Review of Related Literature

The Wilms' Tumor 1 gene (WT1) was the first discovered gene associated with Wilms' Tumor (WT).[17] Located at chromosome 11p13 and initially cloned in 1990,[18] the gene plays an important role in normal human embryonic development.[19] Mutations are associated with Wilms tumor-aniridia-genitourinary anomalies-mental retardation (WAGR) syndrome, Denys-Drash syndrome and Frasier syndrome.[17] Initially discovered as a tumor suppressor, mutations in WT1 are found in up to 15% of sporadic cases of WTs.[19] Successive studies revealed that WT1 is overexpressed in a range of benign[20] and malignant neoplasms such as hematologic malignancies,[19,21] a broad range of carcinomas,[15,22] soft tissue tumors[23-25] and glial neoplasms.[26] Genomic sequencing in these cases did not reveal mutations in the WT1 gene. This evidence suggests that wild-type WT1 may have a possible oncogenic role in malignancies aside from WT.[15,19,25] WT1 is widely-regarded to function as a regulator of transcription but it has become apparent that its full function is more complex.[27] Evidence has accumulated that WT1 can be a tumor suppressor and an oncogene depending on which cell types express it.[19]

In routine histopathology, the WT1 gene product is detected using immunohistochemistry. In established practice, it has been used as a supportive marker in the diagnosis of Wilms' tumor,[3] ovarian serous tumors,[28] mesothelioma,[29] and various other tumors. Generally, only nuclear staining was considered positive, and cytoplasmic expression was initially thought of as due to cross-reactivity of the antibody with unknown proteins.[29] Subsequent studies have increasingly uncovered evidence that both nuclear and cytoplasmic WT1 immunostaining in a wide range of neoplasms is in fact due to the presence of the WT1 protein,[15,23,24] and thus can be used as an index of WT1 protein expression. This finding accounted for the cytoplasmic expression previously seen in tumors that were generally regarded to express only nuclear staining such as in malignant mesothelioma. The presence of the WT1 peptide within the cytoplasm was confirmed in several studies using Western Blot and other methods.[15,23,27] Ye et al. discussed that WT1 expression in the cytoplasm is mainly due to post-translational phosphorylation at zinc fingers leading to loss of the ability to bind DNA.[30] This then results to retention of WT1 in the cytoplasm. Niksic et al.[27] also reported that WT1 shuttles between the nucleus and the cytoplasm in association with active polyribosomes, suggesting a role for it in translation regulation.

Nuclear and/or cytoplasmic expression of WT1 has been documented using immunohistochemistry in gastrointestinal, breast, lung, prostatic, kidney, urothelial and gynecologic cancers,[15,22,31] as well as soft tissue sarcomas,[15,23-25] pediatric small round blue cell tumors,[14] and gliomas.[26] Among soft tissue tumors, rhabdomyosarcomas have shown consistent cytoplasmic expression.[24]

Yang et al.[19] reviewed several studies of WT1 immunohistochemistry expression in hematologic malignancies. WT1 was found to be increased in 354 of 476 (74%) cases of acute myeloid leukemia (AML) and 86 of 131 (66%) cases of acute lymphoblastic leukemia (ALL). Some types of myelodysplastic syndromes (MDS) also had increased levels of WT1.

Studies documenting WT1 expression in head and neck carcinomas are more limited. Mikami et al.[32] analyzed tissues from six cell lines of oral squamous cell carcinoma (SCC) and one showed overexpression of WT1 protein while two out of 29 cases showed positive WT1 expression using immunohistochemistry. Xingru et al.[33] demonstrated that WT1 promotes cell proliferation in vitro in a study that used cells derived from hypopharyngeal SCC. Leader et al.,[20] in a study of 80 salivary gland tumors, found that WT1 was expressed in most benign salivary gland neoplasms while it is lost in malignant neoplasms with the exception of polymorphous low-grade adenocarcinomas (now called polymorphous adenocarcinoma in the 2017 WHO classification[34]) in which 11 out of 12 cases were positive. In 2002, a study by Oji et al.[35] showed WT1 gene expression in 42 out of 56 (75%) head and neck SCCs using real-time reverse transcriptase-polymerase chain reaction (RT-PCR); none of which were nasopharyngeal cancers. In addition, only six cases underwent immunohistochemical staining, and all were positive. A study by Fattahi et al.[36] in 2015 contrasted with the initial findings of Oji et al., wherein only three out of 45 (6.2%) cases of oral SCCs stained positive for WT1.

Across all the studies mentioned, gene sequencing findings in cases with positive immunohistochemistry results did not show mutations of the WT1 gene. This suggests a role for wild-type WT1 in tumorigenesis or possible epigenetic modifications which led to increased WT1 expression in various tumors.

In summary, increased wild-type WT1 expression has been demonstrated in a wide range of malignant tumors, with promising implications in the realm of cancer immunotherapy. There were no published studies analyzing the extent of WT1 expression via immunohistochemistry in NPCs in the English literature, even among studies focused on head and neck cancers. This dearth of information is what this study aimed to address, and demonstration of WT1 activity in NPC would put forward the possibility of WT1-specific cancer immunotherapy for this tumor.

Objectives

The study aimed to evaluate the immunohistochemistry staining patterns for WT1 in nasopharyngeal carcinomas diagnosed in the Philippine General Hospital from 2016 to 2017.

Specifically, the study aimed to:
1. Determine the basic demographic information of patients diagnosed with nasopharyngeal carcinomas in the hospital, namely: age, sex and tumor histologic classification.
2. Determine the rates of positive and negative expression of WT1 among the various histologic classifications.
3. Determine the sub-cellular localization, extent and intensity of WT1 staining among nasopharyngeal carcinomas according to histologic classification.
4. Identify and describe the predominant WT1 staining patterns for each histologic classification of nasopharyngeal carcinoma.

METHODOLOGY

Ethical Considerations

The study was approved by the University of the Philippines-Manila Research Ethics Board (UPM REB) prior to being conducted. A waiver of consent was requested and approved as there were no risks to the study participants. The methods of data collection, handling and storage ensured anonymity and confidentiality of the participants.

Study Design

The study was a descriptive, retrospective study and involved slide reviews of patients who were diagnosed with NPC in accordance with the inclusion criteria below. All recent cases (2016 to 2017) were included. As this is a preliminary study, the use of recent tissue samples ensured the most optimal immunohistochemistry staining results.

Only the patients' age and sex were collected. The formalin-fixed, paraffin-embedded tissue blocks of each case were retrieved and processed for immunohistochemistry staining with WT1.

Inclusion Criteria

The study included all newly-diagnosed cases of nasopharyngeal carcinoma from 2016 to 2017 at the Philippine General Hospital that have been confirmed using histomorphologic assessment and immunohistochemistry staining with at least a Pan-Cytokeratin.

Exclusion Criteria

The following were excluded: cases of recurrent or persistent nasopharyngeal carcinoma that have already undergone radiotherapy and/or other treatments; cases of nasopharyngeal carcinoma metastatic to other sites that do not have a nasopharyngeal tissue sample in storage; cases that have concomitant malignant tumors elsewhere; cases that have deteriorated and unsalvageable slides and paraffin-embedded tissue blocks.

Data Collection

All diagnosed NPCs in the PGH from 2016 to 2017 that fulfilled the criteria were included in the study. The patients' age and sex were collected from the records of the surgical pathology and outpatient sections of the Department of Laboratories. All patients were anonymized.

Slide Review

The diagnosis for each case was classified in accordance with the WHO Classification of Tumors recommended by 8th edition (2017) of the American Joint Committee on Cancer (AJCC) Staging Manual[34] (Table 1).

Table 1. Classification of tumors according to the 8th edition of the AJCC staging manual

AJCC/WHO 2017 Classification	Former Terminology
Keratinizing squamous cell carcinoma	WHO Type I (squamous cell carcinoma)
Non-keratinizing squamous cell carcinoma, Differentiated	WHO Type II (transitional cell carcinoma)
Non-keratinizing squamous cell carcinoma, Undifferentiated	WHO Type III (lymphoepithelial carcinoma)
Basaloid squamous cell carcinoma	No synonym exists (recently described)

The stained biopsy slides and blocks were retrieved for review. Each case had at least a Hematoxylin and Eosin (H&E) stained slide and an immunohistochemistry slide for Pan-Cytokeratin. A consensus diagnosis was generated by three pathologists, with at least two out of three (2 out of 3) pathologists concurring.
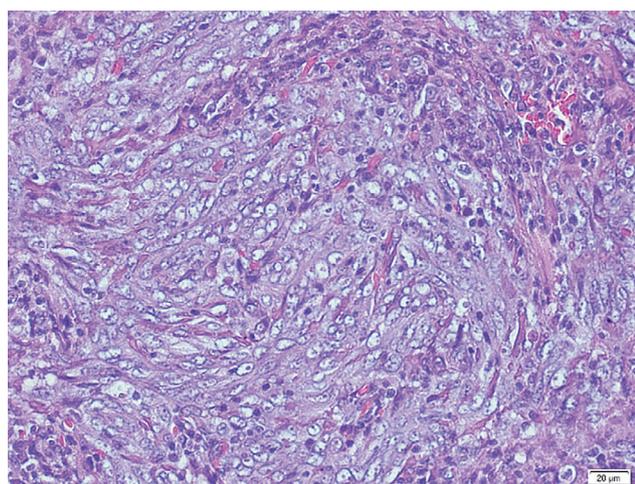
Immunohistochemistry with WT1

New slides were prepared from the formalin-fixed and paraffin-embedded tissue blocks for immunohistochemistry. Immunohistochemistry staining was performed using the standardized protocols established by the section of surgical pathology (Autostainer Link 48, DAKO, CALIFORNIA, USA). 3-um sections were prepared and placed on glass slides. Each slide was deparaffinized, rehydrated and subjected to heat-induced epitope retrieval for 10 minutes using an automated system (PT Link Instrument, DAKO, CALIFORNIA, USA). The sections were then treated with a peroxidase-blocking solution (FLEX) for five minutes. Subsequent incubation with the ready-to-use anti-WT1 antibody was done at room temperature for 15 minutes. A monoclonal antibody against WT1 (6F-H2 DAKO, CALIFORNIA, USA) was used. Visualization of signals was done using HRP Labelled Polymer (DAKO, CALIFORNIA, USA) for 20 minutes, followed by washing with a buffer for 10 minutes, and then incubation in DAB+Chromogen (DAKO, CALIFORNIA, USA) for 10 minutes. The slides were counterstained with Hematoxylin. Positive controls were included with each case: either Wilms' tumor
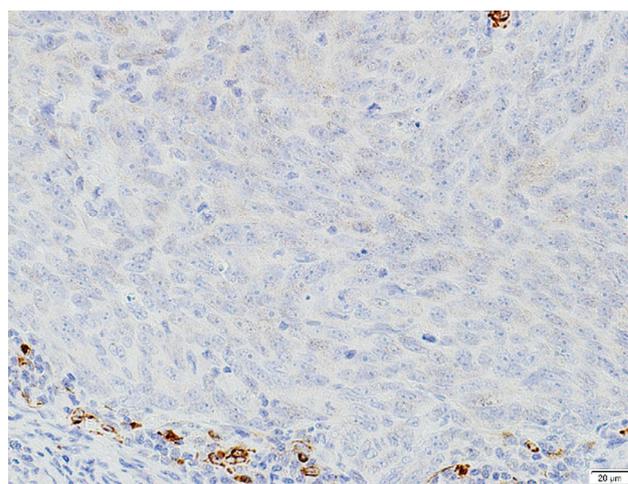

Figure 1. Sample photomicrograph of a non-keratinizing, squamous cell carcinoma of undifferentiated type (H&E, 400X).

Figure 2. Sample photomicrograph of a case which was negative for WT1 immunostaining (400X).
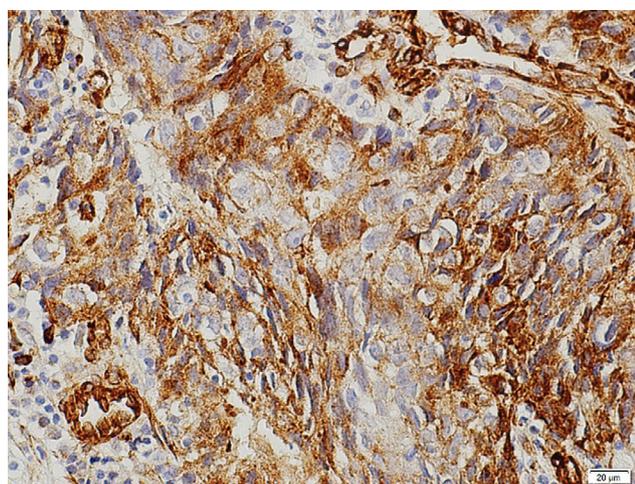

Figure 3. Sample photomicrograph of a case which showed granular, cytoplasmic immunostaining for WT1 (400X).
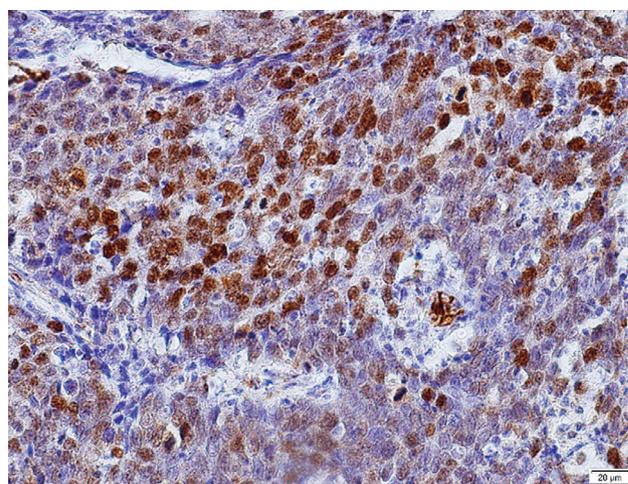

Figure 4. Sample photomicrograph of a case which showed positive WT1 nuclear immunostaining (400X).

or ovarian serous carcinoma. The WT1 antibody also stained lymphocytes and endothelial cells which served as internal controls.

Assessment of WT1 Immunostaining

Assessment of WT1 immunostaining was performed by three different pathologists, all of whom were blinded in terms of clinicopathologic information. Criteria for assessment was based on a modified version of the assessment done by Kim et al.[23] The intensity of the staining and the proportion of the positively-staining area were considered together and evaluated semi-quantitatively.

Both nuclear and cytoplasmic staining were considered. For staining intensity, a score of 0 was assigned if there was no staining or if it is barely perceptible. Clearly-perceptible but faint staining was assigned a score of 1. Distinct staining that is not as strong as the control was assigned a score of 2. Staining intensity equal to or stronger than the positive controls was assigned a score of 3. In cases where the staining pattern was heterogeneous, the more frequent intensity was considered. Afterwards, the percentage of tumors cells that stained positive were estimated (1-100). The intensity and percentage were multiplied and assigned a final strength based on the product. The final strengths were negative (0-20), weak (21-80), moderate (81-180) and strong (181-300).

RESULTS

A total of 79 new cases of NPC were diagnosed from 2016 to 2017. Fifty-seven (57) out of these 79 cases were eligible for review and immunohistochemistry testing. Twenty-two (22) cases were not included in the review due to the following: (a) irretrievable tissue blocks (n=15), (b) cases that were sampled from outside hence the tissue blocks were not available (n=6) and (c) one case with a non-usable block. The diagnosis of each case was reviewed using the H&E slide and corresponding immunohistochemistry slide for Pan-Cytokeratin.

Of the 57 cases reviewed, exactly two-thirds (n=38; 67%) were male and the remaining one-third (n=19; 33%) were female. The median age was 48 years old. The females averaged older at 53.53 years while the average age of males was 45.2 years. The youngest patient was a 10-year-old male while the oldest was a 79-year-old female.

All of the cases were non-keratinizing squamous cell carcinomas (NK-SCC). The majority of cases (n=49, 86%) were of the undifferentiated subtype while the remainder (n=8; 14%) were of the differentiated subtype. None of the cases were keratinizing squamous cell carcinomas (K-SCC) or basaloid squamous cell carcinomas (B-SCC) (Table 2).

Table 2. Overview of results per tumor differentiation and sex

Tumor Classification	Sex		Subtotal
	Male	Female	
Keratinizing squamous cell carcinoma	0	0	0
Non-keratinizing squamous cell carcinoma, *Differentiated*	6	2	8
Non-keratinizing squamous cell carcinoma, *Undifferentiated*	32	17	49
Basaloid squamous cell carcinoma	0	0	0
Grand Total	38	19	57

Analysis of WT1 Staining

Seventeen out of 57 cases (29.82%) stained positive for WT1 while 40 cases (70.18%) were negative (Table 3). Among the 40 cases which were negative, 32 (80%) were undifferentiated while eight (20%) were differentiated. Conversely, all 17 (100%) positive cases were undifferentiated. The distribution of the 17 positive cases in terms of sex followed the overall distribution: 11 (64.7%) were male, and 6 (35.3%) were female. The positive cases accounted for a third (34.7%) of all the cases classified as undifferentiated type (17 out of 49). In terms of intensity, most cases were assigned scores of 1 and 2. In terms of tumor cell population stained, an average of 45% of tumor cells expressed WT1. Five cases expressed the protein in more than 70% of the tumor cell population.

Fourteen out of the 17 (82.35%) positive cases showed diffuse to granular cytoplasmic WT1 expression. Two cases showed nuclear expression, and one showed both nuclear and cytoplasmic expression (mixed). In terms of strength of staining, the positive cases were distributed almost evenly between weak (n=8) and moderate (n=9) expression. None of the cases showed strong expression as defined by the assessment protocol.

Table 3. WT1 Immunohistochemistry staining profile of nasopharyngeal carcinoma per histologic classification and localization of staining

Classification	Localization	Positive		Total positive	Total negative	Grand total
		Weak	Moderate			
Non-keratinizing squamous cell carcinoma, *Differentiated*	Cytoplasmic	0	0			
	Mixed	0	0			
	Nuclear	0	0	0	8	8
Non-keratinizing squamous cell carcinoma, *Undifferentiated*	Cytoplasmic	8	6			
	Mixed	0	1			
	Nuclear	0	2	17	32	49
Grand Total		8	9	17	40	57

Statistical tests were performed using Chi-square test of association with fisher exact correction. STATA 14 (StataCorp, Texas, USA) was used for all statistical analysis. There was a statistically significant association (*p-value*=0.047) between the presence of staining and tumor classification (Table 4).

Table 4. Association of WT1 Immunostaining with tumor classification

Tumor Classification	Staining		p-value
	Positive	Negative	Fisher's Exact
Non-keratinizing squamous cell carcinoma, *Differentiated*	0	8 (20.00)	0.047
Non-keratinizing squamous cell carcinoma, *Undifferentiated*	17 (100.00)	32 (80.00)	

There was no statistically significant association between tumor type and strength of staining (*p-value*=0.221). There was also no statistically significant association between the strength of staining and localization (*p-value*=0.329) among the positive cases classified as undifferentiated-type, NK-SCC (Table 5).

Table 5. Association of WT1 immunostaining with strength of staining and localization

| Variable | Strength of Staining | | | p-value |
	Weak (n=8)	Moderate (n=9)	Negative (n=40)	Fisher's Exact
Tumor Classification				
Differentiated	0	0	8 (20.00)	0.221
Undifferentiated	8 (100.00)	9 (100.00)	32 (80.00)	
Localization (Differentiated)				
Cytoplasmic	0	0	0	–
Mixed	0	0	0	
Nuclear	0	0	0	
Localization (Undifferentiated)				
Cytoplasmic	8 (100.00)	6 (66.67)	0	0.329
Mixed	0	1 (11.11)	0	
Nuclear	0	2 (22.22)	0	

DISCUSSION

Cancer immunotherapy using WT1 peptide vaccination has been undergoing trials over the past decade. Taking the results of several investigations together, researchers have acknowledged the challenges in assessing the potential of WT1 as an anti-cancer antigen. Most of these factors are inherent to the time-consuming nature of clinical trials and the slow action of anti-cancer vaccines, in general, relative to other anti-cancer treatments such as chemotherapy.[6,37] Nevertheless, considerable developments have been achieved. Limited early trials in hematologic and other malignancies have found the method to be safe and efficacious.[11] The trials showed that the treatment enhances the body's immune response against cancer cells through the action of WT1-specific CD8+ CTLs.[6,13] Experience with the vaccinations for AML, MDS and other hematologic malignancies in Japan has advanced to the point where WT1 levels in peripheral blood are being utilized as a marker for minimal residual disease. Complete remission (CR) has been achieved via WT1 peptide vaccination in combination with other treatments for some cases of AML and MDS.[12,38] Recent proposals for further trials focusing on hematologic malignancies have called for cure-oriented approaches.[38]

Trials in carcinomas have also been ongoing. A Phase I trial conducted by Ohno et al. in 2012 with 28 patients showed that WT1 peptide vaccination combined with other treatments was well-tolerated and showed 60% improved clinical response in patients with advanced cervical, ovarian, lung, colorectal, pancreatic or biliary tract cancers.[37] More recently in 2018, a Phase II randomized study of a WT1 vaccine combined with Gemcitabine conducted by Nishida et al. showed improved one-year progression-free survival in 85 evaluated patients with advanced pancreatic ductal adenocarcinoma versus Gemcitabine alone.[39] Overall survival was not significantly altered.

Aside from its therapeutic potential in cancer immunotherapy, the interest in WT1 has extended to its value as a prognostic marker. Kim et al. examined the prognostic value of WT1 expression in 63 patients with soft tissue sarcomas. They found that strong WT1 expression was associated with improved outcomes among patients with high-grade soft tissue sarcomas, but not in other groups.[23] A 2015 meta-analysis conducted by Qi et al.[40] on the association of WT1 and prognosis in patients with solid cancers included 22 studies and 3,620 patients.

Their findings contrasted with Kim et al., as they found that WT1 expression seemed connected to increased risk for disease relapse and progression. The differences in results illustrates the limited knowledge regarding WT1 activity in various tumors de novo. The data on the usefulness of WT1 as a prognostic marker in cancers is still being accumulated and remains controversial.

There have been no published studies on WT1 expression in NPC. Our study of 57 cases of NPC all consisted of NK-SCCs. Eight cases were of the differentiated subtype, and 49 were of the undifferentiated subtype in accordance with the WHO classification. The undifferentiated subtype of NK-SCC is the most common in endemic areas[2] and so the distribution in our study seems consistent with trends observed in the literature. K-SCCs are less frequent in endemic areas, and are less frequently associated with EBV.[2] B-SCCs of the nasopharynx are similar to basaloid SCCs elsewhere in the head and neck and may also be associated with EBV in endemic areas. The literature on the differences in behavior, tumor spread and prognosis among the different tumor classifications of NPC has been inconclusive thus far.[2]

Seventeen out of 57 cases (29.82%) stained positive for WT1, and all were of the undifferentiated subtype. In addition, the majority (n=14; 82.35%) of the positive cases showed diffuse to granular cytoplasmic expression. Two cases showed nuclear expression, and one case exhibited both nuclear and cytoplasmic expression. Approximately half of cases (n=8) stained weakly for WT1, and a slightly higher number stained with a moderate intensity (n=9). None of the cases stained with a strong intensity.

No equivalent comparisons of the results can be made in the literature due to the lack of studies documenting WT1 immunostaining in NPC. As such, the results were compared with studies done on cancers of the head and neck as well as cancers in various other organ systems. The degree of WT1 expression in NPC seen in our study was higher than the results obtained by Leader et al.[20] in their study on salivary gland neoplasms. The exception is polymorphous adenocarcinoma which showed positive WT1 expression in 11 out of 12 cases. Our study also showed higher WT1 expression rates in NPC compared with oral SCCs as studied by Mikami et al. (6.9%)[32] and Fattahi et al. (6.2%).[36] Conversely, our results differ from the findings of Oji et al., wherein six out of six cases of oral SCCs were reportedly positive for WT1 via immunohistochemistry.[35]

A study conducted by Nakatsuka et al.[15] in 2006 included a wide variety of cancers. They used polyclonal (C-19) and monoclonal (6F-H2) antibodies for assessing WT1 immunostaining; this monoclonal antibody was the same used in our study. Both nuclear and cytoplasmic staining were considered. They found discrepant immunostaining results between the two antibodies in 129 out of 338 cases (38%) studied. For the 6F-H2 antibody, they found a wide range of expression rates (5-81%). The cancer types that showed less than 50% expression rates among cases were cervical (5%), prostate (25%), lung (30%), urothelial (33%), renal (36%), gastric (42%) and esophageal (45%). The cancer types which showed greater than 50% expression

rates were breast (52%), pancreatic (65%), ovarian (66%), biliary (68%), colorectal (69%) and endometrial (81%).

Overall, the degree of WT1 expression in NPC is higher compared to head and neck cancers and lower compared to cancers of other organ systems; exceptions being cancers of the uterine cervix, lung and prostate.

Our study also found a significant association between tumor classification and positive WT1 immunostaining. Current evidence shows no clinical difference in the behavior between the differentiated and undifferentiated subtypes of NK-SCC of the nasopharynx.[2] As such, whether or not the association has any relevance needs further study given the small sample size and limited knowledge regarding the role of WT1 in NPC. A comprehensive NPC-related genomic survey conducted by Hu et al.[41] did not specifically-include WT1.

No other statistically significant associations were found.

Limitations

The study was inherently limited by its retrospective nature. Due to the dearth of information on WT1 expression in nasopharyngeal carcinoma, the study aimed to offer only an initial glimpse into a possible role of the gene in this subset of head and neck cancers. The small sample size (N=57) also limited the analysis which could be made due to the limited NPC tumor classifications represented. The study is also limited to documenting the strength and localization of WT1 immunostaining and the number of positive versus negative cases. Correlating these results with clinical factors, morphologic features and other variables is beyond the scope of this study.

CONCLUSION

To the best of our knowledge, this is the first study in the English literature that has studied the immunohistochemical expression of WT1 in NPC. Expression of WT1 was documented in a considerable proportion (29.82%) of NPC cases included in our study. All of the positive cases were NK-SCCs of undifferentiated subtype. The vast majority of the positive cases showed cytoplasmic staining.

Recommendations

Additional data collection is needed to expand on the preliminary information from this study. Prospective studies with larger sample sizes are strongly recommended.

Specifically, the results and observations made during the conduct of the study point to meaningful avenues for further exploration along several possible routes:

1. Confirmation of WT1 gene products – Previous studies have shown that WT1 immunostaining is specific for detecting WT1 protein in malignant tumors. This was in the form of mRNA detected via RT-PCR or WT1 peptide isolated via Western Blot. This has yet to be proven for NPC specifically, and confirmation would be ideal.

2. Antibody type – The 6F-H2 antibody used in this study recognizes the N terminus of the WT1 protein. If the N terminus is lacking, then sensitivity would be affected. Nakatsuka et al. found different staining results between polyclonal and monoclonal WT1 antibodies in 129 out of 338 cases (38%) studied.[15] Further studies using a polyclonal antibody to detect other isoforms of the WT1 protein may provide additional useful information.

3. WT1 allele – In prior studies of WT1 expression in other cancers, the WT1 gene was usually of the wild-type allele. This was revealed through genomic sequencing of the WT1 gene in cancers with WT1 overexpression. Genomic sequencing of the WT1 gene in cases of NPC, in correlation with immunohistochemistry results, might provide useful information. Current knowledge about the complex nature of NPC oncogenesis does not mention a role for WT1.[41] The complex pathology of NPC is still unravelling and yet to be fully understood.

4. Tumor classification and WT1 staining – Our study showed a statistically significant association between tumor classification and WT1 immunostaining. The limited literature regarding the role of WT1 in NPC and the study limitations preclude further interpretation of this finding. Additional studies investigating this association are recommended.

5. Histomorphology and WT1 staining – Our study did not correlate between WT1 staining and histomorphologic features. Analyzing the extent, localization and intensity of WT1 staining and their association with morphologic features is recommended.

6. Recurrent and resistant cases of NPC – Our study included only newly-diagnosed cases of NPC that have not yet undergone treatment. The pattern of WT1 expression may be different in cases of metastatic, recurrent or cases that are non-responsive to conventional treatment. Given the possible changes at the genetic and molecular level that have occurred in this subset of NPCs, studying WT1 expression in this population may provide valuable insight.

7. Tumor microenvironment – We have observed WT1 staining among some tumor-related elements, namely the endothelial cells of the tumor blood vessels and the associated lymphocytes. Given the lymphocyte-rich morphology of NPC and association with EBV, further study of this observation and its possible therapeutic implications is recommended. Other authors have observed similar WT1 staining in the tumor-related elements in other tumor types.[15,23,24]

8. Prognosis and correlation with other antigens – To date, only the presence of EBV viral DNA has been incorporated clinically as a distinct prognostic marker for NPC.[2] Previous studies have suggested a correlation between WT1 expression in solid cancers and poorer prognosis, though this remains unsettled. Further study may be done to determine if WT1 expression in NPC is connected with tumor behavior and whether correlation with EBV-related antigens (such as LMP1, LMP2, EBNA1 and EBER) is present.

9. WT1 peptide vaccination in NPC – Early trials using various immunotherapy agents for cases of advanced NPC are ongoing.[9,10] None are currently for WT1. Our study has shown that some cases of NPC express WT1. Further data is needed in order to determine the feasibility of WT1 peptide vaccination for NPC.

This study provided a glimpse into the role of WT1 in NPC. The results indicate that a subset of NPCs express WT1. Additional studies examining this relationship in larger populations are recommended. In addition, the results presented here provided potential rationales for the further study of WT1 and its association with NPC.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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