Immune Expression of p53 and Its Association with Histological Risk Classification in Wilms Tumor

*Akter J,[1] Jeba R,[2] Karim SS,[3] Islalm MJ,[4] Shahana Sultana,[5]Yeamin MA,[6] Rashid HO,[7] Nupur FP,[8] Khatun J[9]

Abstract

Background: Wilms' tumor, a type of kidney cancer that occurs in children. The p53 gene serves a crucial function as a tumor suppressor. Mutations in p53 are closely linked to the emergence of anaplastic features, which are notably aggressive and suggest an unfavorable prognosis.

Methods: This study was conducted at the Department of Pathology, Dhaka Medical College. A total 30 samples were collected from Dhaka Medical College and a private laboratory. The histological assessments were conducted at the same institutes. Immunohistochemistry was conducted at the Private Diagnosis Centre. Qualitative data were expressed as frequency and percentage. Fisher's exact test was used to examine the relation between qualitative variables. A p-value less than 0.05 was considered significant.

Results

The age of the patients ranged from 5 months to 14 years, with a mean of 4.9 ± 3.4 years. The male-to-female ratio was 1:1.1. The mean size of the tumor was 8.5 ± 3.4 cm. Tumor laterality varied, with 40.0% on the left, 36.7% on the right, and the remaining 20.0% was not available. Histologically, 83.3% were favorable, and 16.7% were unfavorable. Risk classification showed that 33.3% were low-risk, 50.0% intermediate-risk, and 16.7% high-risk. P53 expression was observed in 23.3% of cases. Favorable histology was associated with negative p53 (100.0%), unfavorable histology with positive p53 (71.4%). Low-risk histology was linked to negative p53 (43.5%), intermediate risk showed mixed patterns (28.6% positive, 56.5% negative), and high-risk histology strongly correlated with positive p53(71.4%).

Conclusion

The presence of positive p53 protein in Wilms' tumor could serve as a marker for high-risk tumors as it is associated with an unfavorable prognosis.

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1. **Dr. Jhuma Akter, MBBS, MD (Pathology), Pathologist, Dhaka Medical College, Dhaka. jhuma.kmc@gmail.com*
2. *Dr. Ruksana Jeba, MBBS, MD (Pathology),Professor and head, Department of pathology, Dhaka Medical College, Dhaka,ruksanajeba67@gmail.com*
3. *Dr. Saiyeda Sinthia Karim, MBBS, MD (Pathology), Associate professor, National Institute of Laboratory Medicine and Referral centre, Dhaka, sinthiakarim330@gmail.com*
4. *Dr. Mohammad Jahirul Islam, Junior consultant, Department of Surgery, Shaheed Suhrawardy Medical College and Hospital, dr.jahir2020@gmail.com*
5. *Dr. Shahana Sultana, MBBS, MD (Pathology), Assistant Professor (cc), Green life Medical College, Dhaka, shahanashova935@gmail.com*
6. *Dr. Md. Abdullah Yeamin, MBBS, MD (Pathology),Lecturer, Jashore Medical College, Jashore, peyal4794@gmail.com*
7. *Dr. Harum Or Rashid, MBBS, MD (Pathology),Lecturer, Shaheed M Monsur Ali Medical College, Sirajgonj, drharunorrashidjewel@gmail.com*
8. *Dr. Farjana Pervin Nupur, MBBS, MD (Pathology), Lecturer, Pathology, Dhaka Medical College, Dhaka, pervin_farjana@yahoo.com*
9. *Dr. Julekha Khatun, MBBS, MD (Pathology), Lecturer, Pathology, Dhaka Medical College, Dhaka, julekhacmc48@gmail.com*

** For correspondence*

Introduction

Wilms tumor comprises 90% of malignant renal tumors in the pediatric population and constitutes 6% of all pediatric malignancies.[1] In Bangladesh, Wilms' tumor comprises about 7% of all childhood malignancies.[2] Approximately two-thirds of cases are identified before the age of 5, and nearly all instances are diagnosed before reaching a more advanced age.[3] Wilms tumor, another name Nephroblastoma, is the most frequent type of kidney cancer in children, which usually affects children aged 2-5 years old; however, it can also occasionally affect older children. Based on histology, Wilms tumor is categorized into three risk groups: low, intermediate, and high-risk groups. As components of a larger risk stratification system, these classifications offer important information regarding the tumor's possible aggressiveness.[1] But Wilms tumor classified as low risk can be lethal and non-responsive to treatment. On the other hand, some classified as high-risk tumors are responsive to therapy. This dynamic clearly indicates the need for new prognostic factors that will be able to discriminate between lethal and non-lethal Wilms tumor.[4] Treatment for these Wilms tumor often involves more intensive chemotherapy and radiation therapy in addition to surgery.[5]

An important tumor suppressor called p53 can be found on human chromosome 17p13.1 and is one of the prognostic factors that have been researched.[6] When p53 is mutated, it loses its ability to regulate the cell cycle and apoptosis in response to DNA damage. This can lead to uncontrolled cell division and the formation of tumors. [1] A review found that patients with Wilms tumor with p53-negative cases were found to live longer than those with p53-positive cases.[4] Some of these studies have confirmed the correlation of TP53 overexpression with anaplasia and prognosis in Wilms tumor.[4] Others revealed no correlation between p53 and Wilms tumor development.[7] A review by Atwa et al. found that p53 expression was linked to clinically advanced stages of nephroblastoma and unfavorable histological types.[8]

Wilms tumor is the commonest primary renal neoplasm in children. It is a mixed tumor of heterogeneous histology, morphological features, and invasive behaviors. To adopt a better therapeutic strategy and determine the prognosis, it is necessary to differentiate between high and low-risk types of nephroblastoma. The tumor suppressor marker p53 stands out as a key element extensively examined in various cancers. Study of p53 expression might aid in the diagnosis of different risks of Wilms tumor, guiding therapeutic strategies and improving the outcome of the patient. The aim of this study was to find out the association of the immune expression of p53 with histological risk classification in Wilms tumor.

Methods

It was a cross-sectional study. The study was carried out from March 2022 to February 2024. The study was conducted in the Department of Pathology, Dhaka Medical College (DMC), Dhaka. An immunohistochemical study was conducted in a private diagnostic center in Dhaka. Histomorphologically diagnosed Wilms tumor cases were collected. Patients with nephroblastoma in <14 years old and histologically confirmed as Wilms tumor were included. Recurrent or metastatic nephroblastoma and patients who received pre-operative chemotherapy were also included. Autolyzed samples were excluded from this study. The sample size was 30. A purposive sampling technique was done.

After getting permission from the ethical review committee, a total of 30 study materials were collected. Among 30 cases, 18 nephrectomy samples were collected from DMC, and 12 formalin-fixed paraffin-embedded blocks were collected from a private laboratory in Dhaka.

After proper grossing of nephrectomy specimens, tissues were fixed in 10% formalin, processed, and embedded in paraffin. Then, formalin-fixed, paraffin-embedded blocks were sectioned at 4-5 micrometers thickness and stained with hematoxylin & eosin.

Hematoxylin & eosin-stained slides were examined thoroughly to evaluate histological subtypes and histological risk classes.

National Wilms Tumor Study Group (NWTSG) studies show [9,13]

- Favorable histology.
- Unfavorable histology.
  - Blastemal.
  - Diffuse anaplasia.

Features of anaplasia

- Nuclear enlargement.
- Hyperchromatism.
- Enlarged abnormal mitotic figure.

According to the revised SIOP working classification[14]

A. Low-risk tumor
   a. Cystic, partially differentiated nephroblastoma.
   b. Completely necrotic nephroblastoma.
B. Intermediate-risk tumor:
   a. Nephroblastoma-epithelial type.
   b. Nephroblastoma-stromal type.
      - Nephroblastoma-mixed type.
      - Nephroblastoma-regressive type.
      - Nephroblastoma-focal anaplasia.
C. High-risk tumor:
   a. Nephroblastoma-blastemal type.
   b. Nephroblastoma-diffuse anaplasia.

Immunohistochemical analysis for p53

Sections were cut at 3-4 micrometers thick from a representative paraffin block, mounted on a poly-L-lysine-coated slide. Paraffin-embedded sections were immunostained with monoclonalmouse anti-human p53 protein, clone DO-7 Ready-to-Use (Dako, Glostrup, Denmark).

Immunohistochemical evaluation of p53

Sections stained for p53 were examined and assessed for density and intensity of stain. And the percentage of immunostained cells was determined. For assessment of p53 immunostaining, tumor cells with a clearly brown reaction in the nuclei were counted. By monitoring at least 1000 tumor cells from more than five high-power fields was counted. Positive cells were present at relatively uniform density, and the percentage was calculated.[9]

Table I: Scoring system for assessment of density and intensity of stain

Density	Intensity	Score
0-5%	Absent	0
5-50%	Mild	1*
>50%	Moderate	2*

* If the total score was more than 0 (i.e., 1 or 2), then expression of p53 was considered as positive.[4]

Data Management and Analysis

Data were analyzed using the IBM SPSS (Statistical Package for the Social Sciences statistical package (version 26). Numerical data were expressed as mean±SD (standard deviation), maximum, and minimum. Qualitative data were expressed as frequency and percentage. Fisher's exact test was used to examine the relation

between qualitative variables. A p-value less than 0.05 was considered as significant.[4]

Ethical Implication

Every ethical issue was discussed with the patients regarding the study, and informed written consent was obtained. The research protocol was approved by the Institutional Review Board (IRB) of DMC, Dhaka.

Results

A total of 30 samples confirmed as cases of Wilms tumor were included in the study. The results are presented in tables in this chapter.

Table II. Demographic and histologic variables

Age (years)	No. of cases	Percentage (%)
0-1	3	10.0
1-4	14	46.7
5-9	9	30.0
10-14	4	13.3
Sex		
Female	16	53.3
Male	14	46.7
Laterality		
Left	12	40.0
Right	11	36.7
Not available	6	20.0
Retroperitoneal mass	1	3.3
Tumor size		
0-5 cm	5	16.7
5-10 cm	17	56.7
> 10 cm	8	26.7
Histology		
Favorable	25	83.3
Unfavorable	5	16.7
Only blastemal	4	
Both blastemal and diffuse anaplasia	1	
Histological risk group		
Low	10	33.3
Intermediate	15	50.0
Nephroblastoma Epithelial type	2	
Nephroblastoma Mixed type	10	
Nephroblastoma Regressivetype	3	
High	5	16.7
Only blastemal	4	
Both blastemal and diffuseanaplasia	1	
Expression of p53		
Positive	7	23.3
Negative	23	76.7
Total	30	100.0

Table **III** illustrates the association between tumor histology and p53 expression in 30 cases. Notably, favorable histology aligns with negative p53 expression (100.0%), while unfavorable histology corresponds to positive p53 expression (71.4%). The obtained p- value (<0.001) through Fisher's Exact test signifies the statistical significance of this association.

Table **III** also shows the connection between histological risk groups and p53 expression in 30 cases. The data show that low-risk histology is associated with negative p53 expression (43.5%), intermediate risk exhibits a mixed pattern (28.6% positive, 56.5% negative), and high-risk histology aligns strongly with positive p53 expression (71.4%). The calculated p-value (<0.001) using Fisher's Exact test emphasizes the statistical significance of this association.

Table III: Association of p53 expression with tumor histology, histological risk group (n=30)

Tumor histology	p53 expression		p-value
	Positive N (%)	Negative N (%)	
Favorable	2 (28.6)	23 (100.0)	<0.001
Unfavorable	5 (71.4)	0 (0.0)	
Histological risk group			
Low	0 (0.0)	10 (43.5)	<0.001
Intermediate	2 (28.6)	13 (56.5)	
High	5 (71.4)	0 (0.0)	

p-value obtained by Fisher's Exact test

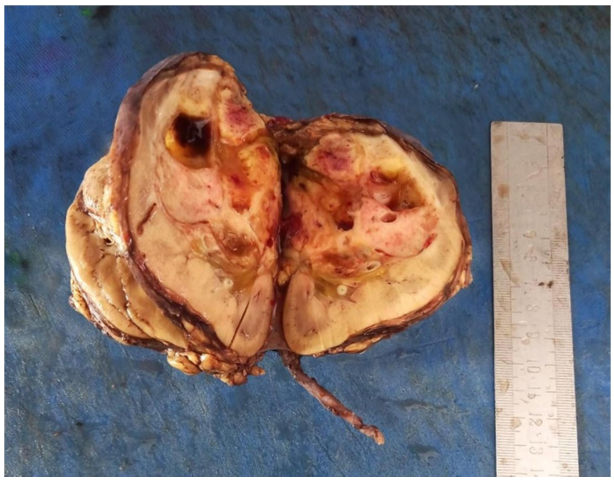

Figure 1. Photomicrograph showing Gross pathology of Wilms tumor

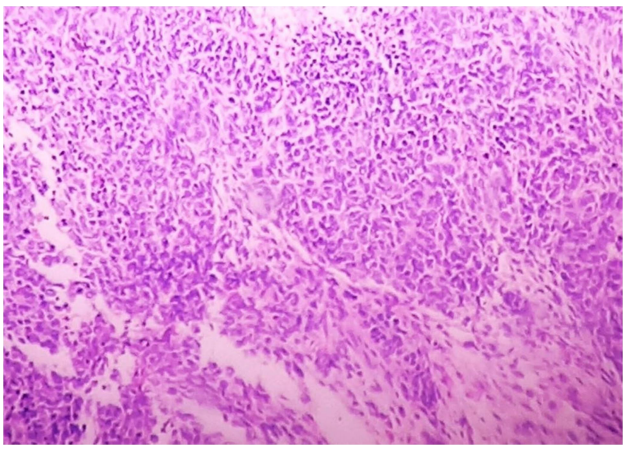

Figure 2. Photomicrograph showing high risk histology in Wilms tumor (IHC 200X)

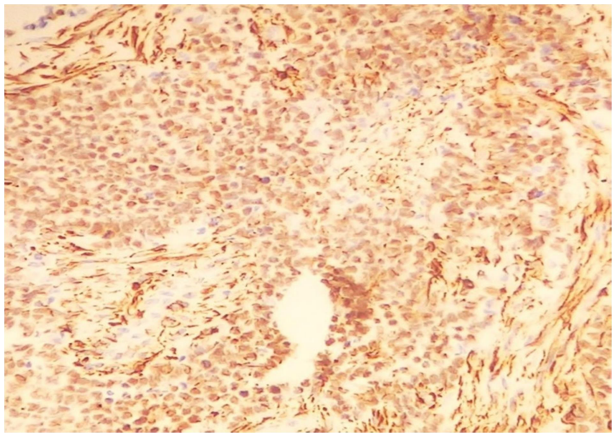

Figure 3. Photomicrograph showing high-risk case with strongly positive expression of p53 immunostain in Wilms tumor (IHC 200X)

Discussion

In this current study, the age range of the patients was from 0.5-14.0 years, with a mean age of 4.9±3.4 years,and the majority (46.7%) belonged to the age group of 1-4 years. In this study, the male to female ratio was 1:1.1. Almost similar patterns were observed in other study.[10]Regarding laterality of tumors, 40.0% occurred on the left, 36.7%on the right, 20.0% laterality was not available and 3.3% were retroperitoneal masses. In the majority (56.7%) of cases, tumor size was between 5-10 cm, with a mean size of 8.5 ± 3.4 cm. According to Darwish, the mean size of the tumor was 10.5cm, and the predominant case with larger than 7cm. Among the cases, 83.3% exhibit favorable histology, while 16.7% show unfavorable features. 50.0% of cases fell into the intermediate-risk group, 33.3% and 16.7% fell into the low-risk and high-risk category, respectively. Wilms tumors with "favorable" histology may present poor prognosis or aggressive behavior, the underlying biologic mechanisms of which is unknown or may be due to genetic mutations.[4]

About 76.7% of cases showed negative expression for p53. Cases with unfavorable histology and high-risk group of tumors showed a significant association with positive expression of p53. Franken et al. (2013) reported a higher score for immunohistochemical p53 expression associated with unfavorable Wilms tumor histology and predicts poorer survival (4). This finding is similar to the present study. Unfavorable histology was associated with positive p53 expression (71.4%). Additionally, in alignment with present study, it was noted that tumors expressing weak to high levels ofp53 had significantly reduced overall survival compared to those negative for p53, as reported by Jadali et al. in 2011.[11] In contrast to the present finding favorable histology can lead to aggressive tumors, vigorous growth in some of these neoplasms could be dysfunction of the p53 tumor suppressor.

In this study, the data showed that low-risk histology is associated with negative p53 expression (43.5%), intermediate risk exhibited a mixed pattern (28.6% positive, 56.5% negative), and high-risk histology aligned strongly with positive p53 expression (71.4%). The high percentage of p53 expression in higher degrees of malignancy (mainly with diffuse anaplasia) is probably linked to the appearance of cells that have lost the p53 function and become aneuploid with various chromosomal transformations. It has been suggested that a high level of aneuploidy contributes to resistance to chemotherapy and poor prognosis.[11]

Franken et al. (2013) reported a higher score for immunohistochemical p53 expression associated with unfavorable Wilms tumor histology and predicts poorer survival.[4] This finding is similar to the present study.

On the other hand, D'Angelo et al. reported that the presence of p53 immunopositivity did notexhibit a significant association with

the pathological stage at the time of diagnosis in patients with histologically favorable Wilms tumor.[12] It did not support the findings of the present study. The dissimilarities could be influenced by factors such as the specific criteria used for histological classification, the genetic makeup of the studied populations, and the potential presence of distinct subtypes within each histological category. Conversely, individuals with histologically unfavorable disease were found to have a higher likelihood of testing positive for p53 compared to those with favorable histology. Specifically, all tumors of unfavorable histology showed a positive p53 expression.[1]

Conclusion

This study revealed that p53 expression is associated with unfavorable histology and poor prognosis, with increased risk. So, p53 protein in the Wilms tumor may serve as a marker in the evaluation and treatment of pediatric malignancies for better prognosis.

Limitation

The number of samples of the individual category was low.

Recommendation

Other molecular markers and genetic factors will provide a more holistic understanding of Wilms tumor pathogenesis.

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