Role of expression of p53 and Ki67 in the progression of Wilms tumor: Correlation with patients’ survival

Ahmad Darwish, Heba Sheta and Doaa Alemam
Welcome letter from Editor-in-Chief

Welcome to the Int J Cancer and Biomedical Research (IJCBR)!

It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

The IJCBR relies on a distinguished expert of the Advisory and Editorial Board Members from the top international league covering in depth the related topics. They timely review all manuscripts and maintain highest standards of quality and scientific methodology and ethical concepts. Meanwhile, we take all possible means to keep the time of the publication process as short as possible.

I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

Mohamed L. Salem,
Editor in Chief
Role of expression of p53 and Ki67 in the progression of Wilms tumor: Correlation with patients’ survival

Ahmad Darwish¹, Heba Sheta² and Doaa Alemam³

¹Pediatrics Department, Faculty of Medicine, Mansoura University, Egypt
²Pathology Department, Faculty of Medicine, Mansoura University, Egypt
³Public Health and Community Department, Faculty of Medicine, Mansoura University, Egypt

ABSTRACT

Background: Wilms tumor (WT) is the most frequent renal tumor of childhood and is a highly responsive tumor to chemotherapy. P53 and ki67 are two of the most important markers that have been evaluated in many cancers. The role of p53 in the pathogenesis and progression of WT is only partly understood. Over the past decade, the importance of Ki-67 in the prognosis of breast cancer has been widely studied. On the other hand, fewer studies are available for this in WT. Aim: To study the possible role of p53 and ki67 in the progression and outcome of Wilms tumor cases. Methods: Immunohistochemical (IHC) staining for p53 and ki67 were done and correlated with clinicopathological data of Wilms tumor cases. Results: There was a positive correlation between p53 and ki67 expression and unfavorable histology, higher tumor stage, shorter disease-free survival (DFS) and overall survival (OS). Conclusion: Ki67 and p53 expression was positively correlated with aggressive behavior of Wilms tumor.

Keywords: Wilms tumor, anaplasia, p53, ki67.

INTRODUCTION

Primary renal tumors account for 4–7% of all childhood cancers. In the Western world, Wilms tumor (WT) or nephroblastoma represents about 90% of these cases. Renal tumors are common in children aged less than 4 years but their relative frequency decreases in older age groups (Stelianova-Foucher et al., 2017). According to the cancer pathology registry, national cancer institute, Cairo University; nephroblastoma represents 31.03% of renal neoplasms (Mokhtar et al., 2016). Moreover, the incidence of Wilms tumor in Africa based on WHO data for children ages 0-14 y., reported an incidence of 6 per million in Egypt (Cunningham et al., 2020).

Treatment modalities of WT include surgery, chemotherapy and, for some patients, radiotherapy. Long-term cure rates have improved to >90% with the introduction of multimodal treatment (Brok et al., 2016). Tumor histology either favorable or unfavorable and tumor stage are the main determining factors of chemotherapy protocol of WT (Oostveen and Pritchard-Jones, 2019). Despite the excellent prognosis for most children with WT, about 15% of patients will relapse, usually within 2 years of diagnosis (Pritchard-Jones et al., 2015). Furthermore, a proportion of patients will display severe early and late treatment-related complications, e.g. cardiotoxicity secondary to doxorubicin or radiotherapy-induced organ dysfunction, musculoskeletal abnormalities, infertility and secondary malignancies (Termuhlen et al., 2011). Therefore, the identification of ideal cost-effective prognostic markers that reduce these complications would be beneficial (Krishna et al., 2016).

Wilms tumor represents a genetically heterogeneous group, displaying a high degree of intra-tumoral heterogeneity (Malogolowkin et al., 2008). The tumor suppressor marker p53 and tumor proliferation marker (Ki67) are two of the most important markers that have been evaluated in many cancers. Ki67 is a nuclear
antigen associated with cell proliferation which is present throughout the cell cycle and is absent in resting cells (Berrebi, 2008).

The p53 tumor suppressor gene (Tp53) is located on chromosome 17p13.1. In a normal healthy cell, P53 behaves as a multifunctional transcription factor involved in the control of the cell cycle, programmed cell death, senescence, differentiation, deoxyribonucleic acid (DNA) replication and DNA repair. p53 prevents neoplastic transformation by three interlocking mechanisms: activation of temporary cell cycle arrest (quiescence), induction of permanent cell cycle arrest (senescence), or triggering of programmed cell death (apoptosis) (Wei et al., 2006).

The role of p53 in the pathogenesis and progression of WT is only partly understood. The significance of p53 expression in WT is conflicting. Several studies have been carried out to determine the role of this protein expression as a prognostic factor in patients harboring WT. Some of these studies have confirmed the correlation of TP53 overexpression with anaplasia and prognosis in WT (Franken et al., 2013; Ooms et al., 2016); others revealed no correlation between p53 and Wilms tumor development (Krishna et al., 2016). Over the past decade, the importance of Ki67 prognosis of breast cancer has been widely studied as regard prognosis and treatment (Abubakar et al., 2019). On the other hand, with respect to WT, several studies are conducted (Menon et al., 2019).

**PATIENTS AND METHODS**

**Patients**

This is a retrospective study including 38 patients with Wilms tumor who were presenting to the pediatric oncology unit, Oncology Center-Mansoura University-OCMU, from January 2010 to July 2018. Formal consent was obtained from the children’s guardians/parents before data and sample collection. This study was approved by the Institutional Review Board (IRB code no: R/18.08.258).

**The study groups**

The International Society of Pediatric Oncology treatment policy of patients with WT was followed for our cases. This included preoperative chemotherapy then followed by surgery and further chemotherapy with or without radiotherapy, if necessary. Histologically, three risk groups are identified (a) low risk (cystic partially differentiated nephroblastoma), (b) intermediate-risk (regressive, epithelial, stromal, or mixed), and (c) high risk (blastemal or diffuse anaplasia). A diagnosis of anaplasia was made according to National Wilms’ Tumor Study Group criteria (Beckwith, 1983).

Clinical data were obtained from the patient’s clinical sheets including the patient’s age, gender, tumor stage & tumor recurrence if present. Hematoxylin & Eosin (H&E) slides were reviewed to confirm the diagnosis and assess other histopathological parameters such as anaplasia and blastemal predominant histology by blinded review. Exclusion criteria include complete necrosis of the tumor following chemotherapy and incomplete clinical data.

**Immunohistochemistry**

Immunohistochemical staining of the formalin-fixed, paraffin-embedded tissue sections (4-μm thick) was performed following the technique of Hsu et al., 1981). Immunohistochemical staining was done of resection specimens. Epitope retrieval was performed with the use of heat-induced epitope retrieval technique using Cell Marque triology in conjunction with a pressure cooker. Each antibody was supplied in a concentrated form (Genemed; Biotechnologies, Mouse monoclonal anti-p53, clone BP-53-12; Mouse monoclonal anti-ki67, clone GM010) and diluted in the ratio of 1:100 in Phosphate-buffered saline. Then, they were incubated with UltraVision One AP Polymer for 30 min at room temperature. Then, subsequent ordinary steps were performed. Only cases with clear nuclear staining for p53 and ki67 were considered positive. ki67 was graded as low, moderate, and high. The expression of proliferation index Ki-67 is categorized into 3 groups: low (Ki-67 ≤15%), moderate (Ki-67:16-30%), and high (Ki-67 > 30%) according to the recommendations of the St Gallen International Consensus of Experts (Goldhirsch et al., 2011). Immunohistological upregulation of p53 was noted to be present if 20% or more of the nuclei stained positive (Krishna et al., 2016).
Statistical analysis

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 21). The normality of data was first tested with a one-sample Kolmogorov-Smirnov test. Qualitative data were described using the number and percent. Association between categorical variables was tested using Chi-square test while Fischer exact test and Monte Carlo test were used when expected cell count less than 5.

Continuous variables were presented as mean ± standard deviation for parametric data and median for non-parametric data. The two groups were compared with the Student t-test for parametric data and Mann Whitney test for non-parametric. ANOVA test was used to compare more than 2 means, while Kruskal-Wallis test was used to compare more than 2 medians.

Cox regression analysis was used to predict the most significant determinants of predictors of mortality among significant variables on univariate analysis using the enter statistical technique. Survival analysis was tested by Kaplan-Meier and Log-rank test to determine the statistical significance of differences among curves. For all above mentioned statistical tests done, the threshold of significance is fixed at ≤ 5% level (p-value).

RESULTS

Study group

In total, 38 cases of Wilms tumor were identified from hospital records between January 2010 to July 2018. Two cases were excluded due to defective medical reports and another 2 cases were due to complete necrosis of the tumor after receiving chemotherapy.

Clinicopathological criteria

The clinicopathological features of the studied patients are listed in Table 1. There was a female predominance (64.7%). Age at presentation ranged from 1 to 13 years with a median age of 3 years. Clinical presentations were variable, with abdominal mass being the most common (73.5%), followed by incidentally discovered (32.4%), hematuria (20.6%) and abdominal pain (20.6%). Distribution of the tumor stage was 38.2% for stages I and II, 61.8% for stages III and IV. About 60% of the cases were left-sided. Of the studied 34 cases, 15 (44.1%) cases were alive at the end of the current study, and 19 (55.9%) succumbed to the disease. As regard tumor characteristics, the tumor size mean was 10.67 cm. The predominant histology was the triphasic histology (97.1%) in the form of blastemal, epithelial, and mesenchymal elements (Figure 1d). There was predominance for favorable histology 65% (Figure 1d) as compared to 35% of the cases that showed anaplasia; either focal or diffuse (Figure 1a).

Immunohistochemical expression and correlation with patients’ clinicopathological parameters

Regarding p53 immunoreactivity, about 60% of the studied cases showed a positive reaction (Fig. 1b), as compared to 40% with a negative reaction (Fig. 1e). When comparing p53 expression with histology, all tumors with unfavorable histology showed a positive reaction for p53. In respect to tumors with favorable histology, 68.2% showed negative reactions while only 31.8% of these tumors were positives (p <0.001). Moreover, there was a positive correlation between p53 positivity and the presence of abdominal pain (p=0.011), higher tumor stage (p=0.002), and higher Ki67 immunoreactivity (p <0.001). Moreover, there was a significant relation between positive p53 expression and decreased both overall survival and disease-free survival of our studied cases (Figure 2a, b).

Regarding ki67 immunoreactivity; 29.4% showed mild positive reaction for ki67, 35.3% showed moderate reaction (Fig. 1f) and the same percentage showed high reaction (Fig. 1c). Moreover, there was a positive correlation between increased ki67 positivity and higher risk group (p=0.005) and higher p53 immunopositivity (p=0.014). When comparing ki67 expression with histology, 66.7% of tumors with unfavorable histology showed high ki67 expression in comparison to 8.3% that showed low expression. On the other hand, only 18.2% of tumors with favorable histology showed high expression with an equal percentage for low
and moderate expression (each represented 40.9%) (p=0.014). As noted in p53, there was a significant relationship between increased ki67 expression and decreased both overall survival and disease-free survival of our studied cases (Figure 2c,d).

Table 1. Descriptive data of the studied cases.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/ year; Median (Min-Max)</td>
<td>3.00 (1-13)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (35.3)</td>
</tr>
<tr>
<td>Female</td>
<td>22 (64.7)</td>
</tr>
<tr>
<td>Clinical Presentation, n (%)</td>
<td></td>
</tr>
<tr>
<td>Abdominal mass</td>
<td>25 (73.5)</td>
</tr>
<tr>
<td>Hematuria</td>
<td>7 (20.6)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>7 (20.6)</td>
</tr>
<tr>
<td>Accidental</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td>Tumor stage, n (%)</td>
<td></td>
</tr>
<tr>
<td>Stage 1 &amp; 2</td>
<td>13 (38.2)</td>
</tr>
<tr>
<td>Stage 3 &amp; 4</td>
<td>21 (61.8)</td>
</tr>
<tr>
<td>Laterality, n (%)</td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>19 (55.9)</td>
</tr>
<tr>
<td>Right</td>
<td>15 (44.1)</td>
</tr>
<tr>
<td>Tumor Size (cm); Mean ± SD</td>
<td>10.67±3.30</td>
</tr>
<tr>
<td>Histology, n (%)</td>
<td></td>
</tr>
<tr>
<td>favorable</td>
<td>22 (64.7)</td>
</tr>
<tr>
<td>unfavorable</td>
<td>12(35.3)</td>
</tr>
<tr>
<td>Histologic subtype, n (%)</td>
<td></td>
</tr>
<tr>
<td>Triphasic</td>
<td>33(97.1)</td>
</tr>
<tr>
<td>Blastema only</td>
<td>1(2.9)</td>
</tr>
<tr>
<td>Risk group, n (%)</td>
<td></td>
</tr>
<tr>
<td>low</td>
<td>1(2.9)</td>
</tr>
<tr>
<td>intermediate</td>
<td>28(82.4)</td>
</tr>
<tr>
<td>high</td>
<td>5(14.7)</td>
</tr>
<tr>
<td>Recurrence, n (%)</td>
<td>7 (20.6)</td>
</tr>
<tr>
<td>Mortality, n (%)</td>
<td></td>
</tr>
<tr>
<td>Died</td>
<td>19(55.9)</td>
</tr>
<tr>
<td>Survived</td>
<td>15(44.1)</td>
</tr>
<tr>
<td>P53, n (%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>15(44.1)</td>
</tr>
<tr>
<td>Positive</td>
<td>19(55.9)</td>
</tr>
<tr>
<td>ki67, n (%)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>10 (29.4)</td>
</tr>
<tr>
<td>Moderate</td>
<td>12(35.3)</td>
</tr>
<tr>
<td>high</td>
<td>12(35.3)</td>
</tr>
</tbody>
</table>

The relationships between the p53 and ki67 expression and clinicopathological data of the studied cases were represented in Table 2. By applying multivariate Cox regression analysis to test the prognostic yield of these prognostic factors; higher tumor stage (p=0.025), p53 positivity (p=0.001) and high ki67 positivity (p=0.007) were independent predictors of mortality (Table 3).

DISCUSSION

In this study, 34 cases of WT were studied. The male to female ratio was 1:1.8. This result is in discordance with most of the previous studies in which there was male predominance that ranged from 1.1 to 2 (table 4). However, other studies that reported female predominance like our study (Krishna et al., 2016). Age at presentation was wide-ranged from 1-13 years with median age of 3 years which is in concordance with some previous studies (Sredni et al., 2001; Mitchell et al., 2006; Chan et al., 2014). On the other hand, other studies reported lower mean age at presentation (Trehan et al., 2012; Sangkhathat et al., 2018) and others reported higher age (Anyanwu et al., 2015; Pribnow et al., 2017). High age at presentation may be attributed to the late age of presentation of the patient to the health care center, especially in less developed countries (Cunningham et al., 2020). The most common clinical presentation was abdominal mass (73.5%). This result was in concordance with other studies (Levitt et al., 2012; Illadea et al., 2018; Seminara et al., 2019).

About tumor laterality, all cases were unilateral with about 60% being left-sided. This contrasted with the study conducted by Seminara, et al. 2019 that found more prevalent right-sided tumors of their studied cases (57%) (Seminara et al., 2019). Right-sided predominance was also observed by Tang et al. (2019) (Tang et al., 2019). Moreover, there was predominance for higher tumor stages (Stage III and IV). These findings are like some studies (Sredni et al., 2001; Jadali et al., 2011; Salama and Kamel , 2011). However, patients of other studies were presented in earlier stages (Beniers et al.,2001; Skotnicka-Klonowicz et al., 2002; Franken et al., 2013; Krishna et al., 2016).

The high percentage of stages III and IV in the present study may be attributed to the lack of awareness by general practitioners of the probability of cancer and later presented with a more advanced stage in the tertiary hospital (Cunningham et al., 2020). Also, this late presentation may be the cause of the high mortality rate of our patients (about 60%). As regard to tumor characteristics, the tumor size mean was 10.67 cm.
### Table 2. Relation between p53 and ki67 expression and clinicopathological data of the studied cases.

<table>
<thead>
<tr>
<th>Variables</th>
<th>P53</th>
<th>P53 negative</th>
<th>p-value</th>
<th>Ki67</th>
<th>Severe</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>P53 positive (n=19)</td>
<td>3.00 (1-10)</td>
<td>0.609</td>
<td>3.00 (1-13)</td>
<td>3.00 (1-11)</td>
<td>3.00 (1-10)</td>
</tr>
<tr>
<td></td>
<td>P53 negative (n=15)</td>
<td>3.00 (1-13)</td>
<td></td>
<td>3.00 (1-13)</td>
<td>3.00 (1-11)</td>
<td>3.00 (1-10)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>Male</td>
<td>6(31.6)</td>
<td>4(40)</td>
<td>3(25)</td>
<td>5(41.7)</td>
<td>3(25)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>13(68.4)</td>
<td>6(60)</td>
<td>6(60)</td>
<td>7(58.3)</td>
<td>9(75)</td>
</tr>
<tr>
<td>Clinical presentation, n (%)</td>
<td>Abdominal mass</td>
<td>13(68.4)</td>
<td>12(80)</td>
<td>9(75)</td>
<td>7(58.3)</td>
<td>9(75)</td>
</tr>
<tr>
<td></td>
<td>Mild (n=10)</td>
<td>1(6.7)</td>
<td>0.104</td>
<td>1(10)</td>
<td>2(16.7)</td>
<td>4(33.3)</td>
</tr>
<tr>
<td></td>
<td>Moderate (n=12)</td>
<td>12(63.2)</td>
<td>10(66.7)</td>
<td>6(50)</td>
<td>6(50)</td>
<td>4(33.3)</td>
</tr>
<tr>
<td></td>
<td>Severe (n=12)</td>
<td>7(36.8)</td>
<td>5(33.3)</td>
<td>6(50)</td>
<td>9(75)</td>
<td>3(25)</td>
</tr>
<tr>
<td>Tumor stage, n (%)</td>
<td>Stage 1 &amp; 2</td>
<td>3(15.8)</td>
<td>10(66.7)</td>
<td>6(50)</td>
<td>4(33.3)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Stage 3 &amp; 4</td>
<td>16(84.2)</td>
<td>5(33.3)</td>
<td>6(50)</td>
<td>12(100)</td>
<td>0.002</td>
</tr>
<tr>
<td>Laterality, n (%)</td>
<td>Left</td>
<td>12(63.2)</td>
<td>7(46.7)</td>
<td>4(40)</td>
<td>6(50)</td>
<td>9(75)</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>7(36.8)</td>
<td>8(53.3)</td>
<td>6(50)</td>
<td>3(25)</td>
<td>0.226</td>
</tr>
<tr>
<td>Tumor Size, Mean ± SD</td>
<td>10.94±3.56</td>
<td>10.33±3.03</td>
<td>0.599</td>
<td>10.60±3.27</td>
<td>9.83±3.04</td>
<td>11.58±3.62</td>
</tr>
<tr>
<td>Recurrence, n (%)</td>
<td>6 (31.6)</td>
<td>1(6.7)</td>
<td>1(10)</td>
<td>9(90)</td>
<td>9(75)</td>
<td>4(33.3)</td>
</tr>
<tr>
<td>Histology, n (%)</td>
<td>favorable</td>
<td>7(36.8)</td>
<td>1(10)</td>
<td>6(50)</td>
<td>6(50)</td>
<td>4(33.3)</td>
</tr>
<tr>
<td></td>
<td>unfavorable</td>
<td>12(63.2)</td>
<td>15(100)</td>
<td>9(90)</td>
<td>9(75)</td>
<td>4(33.3)</td>
</tr>
<tr>
<td>Histologic subtype, n (%)</td>
<td>Triphasic</td>
<td>18(94.7)</td>
<td>1(5.3)</td>
<td>1(10)</td>
<td>9(90)</td>
<td>9(75)</td>
</tr>
<tr>
<td></td>
<td>Blastema only</td>
<td>1(5.3)</td>
<td>15(100)</td>
<td>9(90)</td>
<td>9(75)</td>
<td>4(33.3)</td>
</tr>
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<td>Risk group, n (%)</td>
<td>low</td>
<td>0(0)</td>
<td>0.052</td>
<td>1(10)</td>
<td>9(90)</td>
<td>0(0)</td>
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<tr>
<td></td>
<td>intermediate</td>
<td>14(73.7)</td>
<td>14(93.3)</td>
<td>12(100)</td>
<td>7(58.3)</td>
<td>7(58.3)</td>
</tr>
<tr>
<td></td>
<td>high</td>
<td>5(26.3)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>4(33.3)</td>
<td>8(66.7)</td>
</tr>
<tr>
<td>ki67, n (%)</td>
<td>Mild</td>
<td>1(5.3)</td>
<td>9(60)</td>
<td>9(75)</td>
<td>4(33.3)</td>
<td>0.014</td>
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<tr>
<td></td>
<td>Moderate</td>
<td>6(31.6)</td>
<td>6(40)</td>
<td>3(25)</td>
<td>8(66.7)</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>12(63.2)</td>
<td>0(0)</td>
<td>2(16.7)</td>
<td>5(41.7)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Abbreviations: N, number; SD, standard deviation.

Tang et al. reported a predominance of cases with large tumor size > 7 cms, like our finding (Tang et al., 2019). Triphasic histology was the predominant histology (97.1%). About 35% showed anaplasia; either focal or diffuse. Percentages of unfavorable histology were variable among previous studies (Table 4).

A high percentage of anaplasia in our study may be due to the aggressive behavior of our cases that was presented also by a higher percentage of Stage III and IV. This may be due to the late presentation of the studied cases to our oncology center.

In respect to p53 immunostaining, about 56% of cases showed a positive reaction for p53. In the previous studies, p53 positivity among studied cases ranged from 8% to 60.3% (Table 4). Moreover, p53 immunoreactivity in the current study was significantly associated with the presence of abdominal pain, unfavorable tumor histology, higher tumor stage and higher ki67 immunostaining.
Table 3, Cox regression analysis of independent predictors of mortality

<table>
<thead>
<tr>
<th>Independent predictor</th>
<th>β</th>
<th>P - value</th>
<th>HR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1 &amp; 2 (r)</td>
<td>1.727</td>
<td>0.025*</td>
<td>5.6 (1.2-25)</td>
</tr>
<tr>
<td>Stage 3 &amp; 4</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td>-0.662</td>
<td>0.440</td>
<td>0.51 (0.09-2.8)</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (r)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P53</td>
<td>3.06</td>
<td>0.001*</td>
<td>21.3 (3.6-74)</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (r)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ki67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild (r)</td>
<td>1.72</td>
<td>0.08</td>
<td>5.6 (0.8-38)</td>
</tr>
<tr>
<td>Moderate</td>
<td>2.99</td>
<td>0.007*</td>
<td>20 (2.3-75)</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval; HR, Hazard ratio; r, reference.

Table 4. Previous studies that were examined the role of p53 IHC in Wilms tumor cases.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>NO of cases</th>
<th>Age</th>
<th>M:F ratio</th>
<th>Stage I, II (%)</th>
<th>Stage III, IV (%)</th>
<th>Unfavorable histology (%)</th>
<th>P53 positivity (%)</th>
<th>Correlation with unfavorable histology</th>
<th>Correlation with higher stage</th>
<th>Correlation with poor survival</th>
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<tr>
<td>Cheah et al., 1996</td>
<td>38</td>
<td>1m-6y</td>
<td>1.2:1</td>
<td>NA</td>
<td>NA</td>
<td>5.3</td>
<td>36.8</td>
<td>Yes</td>
<td>No</td>
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<td>Lahoti et al., 1996</td>
<td>28</td>
<td>4-120m</td>
<td>2:1</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>60</td>
<td>Yes</td>
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<tr>
<td>Govender et al., 1998</td>
<td>93</td>
<td>4 m-14ys</td>
<td>1:1:1</td>
<td>43</td>
<td>57</td>
<td>7.5</td>
<td>8.6</td>
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<td>Beniers et al., 2001</td>
<td>21</td>
<td>4(2-132)</td>
<td>1:1:1</td>
<td>66.7</td>
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<td>19</td>
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<td>Skotnicka-Klonowicz et al., 2001</td>
<td>61</td>
<td>39m (2d-13ys)</td>
<td>1:1:1</td>
<td>63.9</td>
<td>36.1</td>
<td>18</td>
<td>37.7</td>
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<td>Yes</td>
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<td>Sredni et al., 2001</td>
<td>97</td>
<td>3ys (1m-21 ys)</td>
<td>1:1:1</td>
<td>47.4</td>
<td>52.6</td>
<td>7.2</td>
<td>13.4</td>
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<td>Yes</td>
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<td>D’Angelo et al., 2003</td>
<td>63</td>
<td>3(1-8)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>10</td>
<td>8</td>
<td>Yes</td>
<td>No</td>
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<td>Salama and Kamel, 2011</td>
<td>63</td>
<td>NA</td>
<td>1:1</td>
<td>34.9</td>
<td>65.1</td>
<td>6.4</td>
<td>60.3</td>
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<td>Jadali et al., 2011</td>
<td>44</td>
<td>36(4-96)</td>
<td>1.43:1</td>
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<td>56.8</td>
<td>25</td>
<td>54.7</td>
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<td>Franken et al., 2013</td>
<td>48</td>
<td>3.75 (3-9.1)</td>
<td>1:1:1</td>
<td>79.2</td>
<td>20.8</td>
<td>8.3</td>
<td>NA</td>
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<td>Krishna et al., 2016.</td>
<td>31</td>
<td>3-8</td>
<td>1:1.8</td>
<td>90.3</td>
<td>0.7</td>
<td>29</td>
<td>NA</td>
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</table>

Abbreviations; M, month; NA, not available; ys, years

The positive relation between p53 expression and the presence of abdominal pain may be explained by that increased p53 expression which is caused by mutation of Tp53 gene enables accumulating mutation (Rivlin et al., 2011). This in turn causes increased tumor aggressiveness, tumor size or intratumoral hemorrhage that causes stretching of the renal capsule (Davidoff et al., 1998). This is may be a possible cause of pain. The positive relation between positive p53 expression and unfavorable tumor histology was observed by most of the reviewed previous studies except for (Sredni et al., 2001; Krishna et al., 2016). This finding is logical because Tp53 mutation allows the accumulation of other mutations that give the nuclei more atypical morphology and more frequent atypical mitoses.
Figure 1. Immunohistochemical data (a) A case of Wilms tumor with unfavorable histology (diffuse anaplasia) that shows an increase in the greatest nuclear dimension at least three times that of adjacent nuclei of the same cell type, nuclear hyperchromasia and abnormal multipolar mitotic figures (H&E x400). (b) A case of Wilms tumor with unfavorable histology (diffuse anaplasia) that shows an immunohistochemical nuclear positivity for p53 (p53 x400). (c) A case of Wilms tumor with unfavorable histology (diffuse anaplasia) that shows a high immunohistochemical nuclear positivity for ki67 (ki67 x200). (d) A case of Wilms tumor with favorable histology that shows a favorable histology formed of blastemal, epithelial and mesenchymal elements (triphasic histology) (H&E x400). (e) A case of Wilms tumor with favorable histology that shows an immunohistochemical negativity for p53 (p53 x400). (f) A case of Wilms tumor with favorable histology that shows a moderate positive nuclear reaction for ki67 (ki67 x400).
Figure 2. (a) Kaplan-Meier survival curve for overall survival among patients with Wilms tumor stratified by the positive or negative expression of p53. (b) Kaplan-Meier survival curve for disease-free survival among patients with Wilms tumor stratified by the positive or negative expression of p53. (c) Kaplan-Meier survival curve for overall survival among patients with Wilms tumor stratified by low, moderate and high expression of ki67. (d) Kaplan-Meier survival curve for disease-free survival among patients with Wilms tumor stratified by low, moderate and high expression of ki67.
This is because p53 protein in a normal cell is the
gate keeper of the cell cycle that prevent
mutation or induce apoptosis via caspase
activation (Rivlin et al., 2011). Also, a positive
relation was observed between positive p53
expression and higher tumor stage was
observed by previous studies (Lahoti et al.,
1996; Govender et al., 1998; Skotnicka-Klonowicz et
al., 2002) and not observed by
others (Cheah et al., 1996; D'Angelo et al., 2003;
Krishna et al., 2016). This difference may be due
to the different sample sizes among different
studies. Also, there was a positive correlation
between p53 positivity and shorter disease-free
survival and overall survival. So, by proving this
association; target therapy may be beneficial to
improve the survival of Wilms tumor cases. Like
relation with tumor stage, there was a
discrepancy about the relation between p53
expression and patient survival.

Most of the previous studies regarding the p53
immunoreactivity in WT are shown in (table 4).
This variation in different studies could be
attributed to a) different antibodies and
staining techniques used, b) different
thresholds for calling a sample p53 positive, c)
different degrees of anaplasia in the samples or
selection of areas with known anaplasia to be
stained. It is also positive that p53 is
upregulated in areas of tumor hypoxia or
necrosis.

Over the past decade, the importance of Ki-67
in the prognosis of breast cancer has been
widely studied and established, however fewer
studies and literature are available in the
context of renal cancer which has an increasing
incidence (Beckwith, 1983). Regarding ki67
immunoreactivity in the current study, 29.4%
shows a mild reaction, 35.3% shows moderate
reaction and the same percentage shows a
severe reaction.

Several studies investigated the relationship
between the immunohistochemical expression
of ki67 and clinicopathological parameters of
Wilms tumor cases. Several studies reported
that ki67 positivity is associated with higher
tumor stage: like the results of the present
study (Juszkiewicz, 1997; Das et al., 2012;
Krishna et al., 2016). Moreover, our study
proved a positive relation between increased
ki67 positivity and shorter disease-free survival
and overall survival which is reported also by
other studies (Ghanem et al., 2005; Diniz et al.,
2011). However, Jurić et al. reported that Ki-67 is
a relevant marker for assessing the
proliferative activity and tumor cell dynamics of
nephroblastoma, but it may not be a good
clinical prognostic marker (Jurić et al., 2010).
By applying multivariate Cox regression analysis to
test the prognostic yield of our detected
prognostic factors; higher tumor stage
(p=0.025), p53 positivity (p=0.001) and high ki67
positivity (p=0.007) were independent
predictors of mortality (table 3). The tumor
stage was reported to be an independent
prognostic factor by many studies (Sonn et al.,
2008; Davidoff, 2009; Tang et al., 2019).

CONCLUSION
Ki67 proliferative marker and p53 mutated
protein has an important role in Wilms tumor
prognosis.

Study limitations: small sample size is one of
the present study limitations. The other
limitation was the lack of genetic testing for p53
gene mutation due to financial limitation.

Future prospective or recommendations
include doing more studies regarding this point
of research on a larger sample size including
multiple health care centers in Egypt. The aim
will be to study the possible role of mutant p53
protein in the progression of Wilms tumor. If
this role has been proved, targeting this mutant
protein may improve the prognosis of Wilms
tumor cases that are resistant to conventional
chemotherapy. The same is for ki67.

Abbreviations
DFS Disease free survival
DNA deoxyribonucleic acid
H&E Hematoxylin & Eosin
IHC Immunohistochemical
OS Overall survival
Tp53 p53 tumor suppressor gene
WT Wilms tumor

CONFLICT OF INTEREST
All authors declared no conflicts of interest.

FUNDING
No fund was received for this work.
REFERENCES


Role of p53 & Ki67 in Wilms tumor


Egyptian Association for Cancer Research (EACR)
http://eacr.tanta.edu.eg/

EACR is an NGO society that was declared by the Ministry of Social Solidarity (Egypt) No. 1938 in 19/11/2014 based on the initiative of Prof. Mohamed Labib Salem, the current Chairman of EACR. EACR aims primarily to assist researchers, in particular young researchers in the field of cancer research through workshops, seminars and conferences. Its first international annual conference entitled "Anti-Cancer Drug Discovery" was successfully organized in April 2019 (http://acdd.tanta.edu.eg). Additionally, EACR aims to raise the awareness of the society about the importance of scientific research in the field of cancer research in prediction, early diagnosis and treatment of cancer. EACR is also keen to outreach the scientific community with periodicals and news on cancer research including peer-reviewed scientific journals for the publication of cutting-edge research. The official scientific journal of EACR is "International Journal of Cancer and biomedical Research (IJCBR: https://jcbjr.journals.ekb.eg)" was successfully issued in 2017 and has been sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

EACR Chairman,
Prof. Mohamed Labib Salem, PhD
Professor of Immunology
Faculty of Science, Tanta University, Egypt
GUIDE FOR AUTHORS

Publisher: The International Journal of Cancer and Biomedical Research (IJCBR) is an international and interdisciplinary journal of preclinical and clinical studies in the area of cancer and biomedical research. It is a peer-reviewed journal in English, published quarterly (in March, June, September, and December) by the Egyptian Association for Cancer Research (EACR) in both print and online formats (4 issues making a volume). Special issues or supplements may also be produced from time to time upon agreement with the Editorial Board.

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