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Cyclin A immunohistochemistry in Wilm's tumor

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Abstract:

Introduction: In human malignancies, cyclin A overexpression is related to worse prognosis. We examined the relationships between cyclin A immunohistochemistry expression and the clinicopathological features of Wilms tumor (WT), preoperative chemotherapy (PrOpChTh), overall survival (OS), and other factors.

Aim of the study: We systematically reviewed the literature on the prognostic value of Cyclin A IHC in Wilms tumors.

Subjects and Methods: We searched for English language studies on PubMed from 2018 to 2023 about Cyclin A immunohistochemistry in patients younger than 18 years with Wilms tumors. We found two studies about that article, a retrospective study and a non-concurrent cohort study including patients who underwent nephrectomy for WT from January 1996 to December 2015 in a tertiary referral center.

Results: According to the two reviewed studies, Patients with WTs had 33.4% higher cyclin A expression. Blastemal components were much more overexpressed in stage 3 and stage 4 cancers (77.8% and 66.7%, respectively). Cyclin A was discovered to be overexpressed in 66.7% of metastasizing patients but only 33.3% of non-metastasizing patients. High recurrence rates were also connected to CSM and cyclin A immunopositivity. Risk factors such as advanced stage, UFH, extracapsular extension, tumor rupture, lymphadenopathy, and venous thrombosis were not associated with a poor prognosis. Patients who have had recurrences have a worse likelihood of survival.

Conclusions: Overexpression of Cyclin A in WT may indicate a bad prognosis. More patients should be included in future studies. WT's capacity to spread metastatically was unaffected by cyclin A overexpression. More people would be required to adequately investigate the link between cyclin A overexpression and poor outcomes in WT individuals.

Keywords: Nephroblastoma; relapse; Mortality; Immunohistochemistry.

1. Introduction

Cyclins, cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitors are the three types of proteins that regulate cell cycle control (CKIs). Cyclins activate cyclin-dependent kinases (CDKs), which are serine/threonine kinases, to modify the cell cycle [1]. Cyclin A and cyclin-dependent kinases 1 and 2 are required for mitosis and S phase to occur. Krabbe and colleagues (2016) claim that its worldwide expression increases between late S and G2 [2]. It is now working with CDK1 to cause chromosomal condensation and maybe nuclear envelope collapse [3].

According to Thway et al. (2012), immunohistochemistry (IHC) examination of cell-cycle proteins has diagnostic importance in histopathology because uncontrolled cell proliferation is a hallmark of cancer [4]. Many aggressive tumors have a poor prognosis when cyclin A is overexpressed. A cyclin A index of more than 40% has been shown to increase the risk of developing medulloblastoma. Moshovi et al. (2011) discovered that cyclin A overexpression is associated with a longer lifespan in both healthy ovarian carcinomas and colon mucosa [5].

Wilms tumor (WT) is one of the most common genitourinary malignant solid tumors in children [6]. One of every 10,000 children has WT. WT may afflict children at any age, although it is more common in younger children. Somatic mutations in the imprinting regulatory region (IRA) are responsible for 37% of malignancies, 70% of anaplastic tumors, and 10% - 20% of WT genetic variability. The epithelial component of the kidney parenchyma cannot develop adequately without optimal WT1 expression [7]. Its expression may be altered, promoting the formation of cancerous cells.

Recent research linked several features to a reduced survival rate among WT people. Examples include one q gain, advanced tumor stage, and insufficient histologic response to preoperative therapy (PrOpChTh). When the N-Myc proto-oncogene protein (MYCN) is overexpressed in tumor genomes, certain adolescent malignancies have a poor prognosis. MYCN is related to a poor prognosis and is involved in the molecular biology of WT [8]. The loss of heterozygosity of chromosomes 1p and 16q at the time of the initial therapeutic intervention was one of the few genetic markers linked with a poor outcome in WT individuals [9]. IHC has been employed in several research to examine WT cell cycle regulators.

Numerous studies show that WT blastema has high amounts of cyclin E and CKI 2A. (p16) [10]. Elevated cyclin E levels were also linked to metastases and recurrences in the

WT. Tumor development in the clinic is associated with the expression of WT1, TGF-beta, VEGF, MIB1, and cyclin-dependent kinase inhibitor 1B (p27Kip1) [11].

There has been little study on using immunohistochemistry (IHC) staining to predict WT aggressiveness. The purpose of this research is to see whether the cyclin A IHC test can be used to predict WT survival and relapse. This comprehensive investigation aimed to examine cyclin A IHC expression in WT metastases and original tumors, as well as its relationships with clinicopathological characteristics and overall survival (OS). According to the review, the histology finding of WT and progression may be linked to cyclin A overexpression.

2. Subjects and methods

2.1. Subjects

We searched for English language studies on PubMed from 2018 to 2023 about Cyclin A immunohistochemistry in patients younger than 18 years with Wilms tumors. We found two studies about that article. The exploded search terms were Wilms tumor– Cyclin A IHC.

WT Data for the first non-concurrent cohort research was obtained from a single tertiary referral hospital between January 2000 and December 2015. Patients who had chemotherapy before surgery and were not followed up or died for causes unrelated to WT. Another retrospective study was performed in the Institute of Pathology at the University of Belgrade's School of Medicine focused on WT patients who underwent nephrectomy before 2010. Only 43 of the 59 individuals were allowed to take part in the experiment since 14 had to be excluded owing to limited sample size or insufficient clinical data.

All patients' medical records were evaluated before any data for the clinical-morphological investigation was collected. Based on the information we acquired, we were able to categorize the patient based on gender, age in months, tumor stage, tumor histology, and prognosis. The 52 patients varied in age from 7 to 132 months (mean: 52.4%). Females made up 28 of the patients (65.1%).

Inclusion Criteria

We reviewed Wilms tumor cases who had received preoperative chemotherapy in the related two studies.

Exclusion Criteria

We excluded WT cases who underwent primary nephrectomy in the related two studies.

2.2. Study design

Immunohistochemical assay

The pathology lab employed formalin-fixed, paraffin-embedded, and hematoxylin- and eosin-stained frozen tissue slices for tumor staging and grading. To extract the antigens, an anti-cyclinA1 antibody (clone 28,970,002, rabbit polyclonal, Novus Biologicals, USA) and a peroxidase blocking solution (Power stain 1.0 poly HRP/DAB kit for mouse and rabbit, Genemed, USA) were employed. For 45 minutes, the slides were treated with 2ry antibodies at room temperature and relative humidity. The coverslips were glued with Canada balsam before being colored with streptavidin enzyme labels. A good stain is often brown.

All samples were examined by a board-certified urologist and a histopathologist who was not informed of the clinical outcomes of the patients. Three groups were defined based on the amount of nuclear staining: 10%, 10% to 50%, and >50%. Cyclin A grades 2, 3, and H were all deemed appropriate for further study (+).

2.3. Statistical Methods

The study was performed by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. We applied descriptive statistics and then concluded relative prognostic factors and WT survival rate.

3. Results

Radojevet et al. (2019) chose an initial sample of 81 eligible participants in 2019. Two further patients died from unrelated causes, while four more patients died as a direct result of WT. It is safe to assume that nephrectomy was the only therapy for WT for each of the 75 survey participants. The study used patient questionnaires, medical records, and imaging studies. The average length of time between occurrences was 36 months. Women were involved in around 35 instances (46.7% of the total) whereas men were involved in 40 cases (53.3%). The NWTS-5 report details the following stages of tumor development: 60% of the population (45 persons) had stage I diagnoses, followed by stages II (eight), III (ten), and IV (12). Despite this, 20% of the 75 individuals had UFH, 3% had anaplasia, and 10% had a blastemal component. 80% (60/75) of the patients' histology was positive.

However, the tumors burst in three patients (4% overall) and did not completely exist in two patients (2.6% total). In addition, 13.3% (ten patients) of the assessed children had venous thrombosis. Cyclin A IHC in 46 out of 75 cases (61.3%) was positive. WT patients with and without preoperative chemotherapy exhibited almost equal rates of Cyclin A overexpression (37.5 vs. 34.3, p >0.05). Positive cyclin A demonstrates that staining is related to higher recurrence and death rates (p = 0.01 and 0.02, respectively). 20% of patients in the first two stages and 12.5% of patients in the third and fourth phases had Cyclin A overexpression. Stage 3 and stage 4 blastemal components were much more likely to contain cyclin A overexpression than stage 1 and stage 2 blastemal components (p = 0.009). In addition, there was no noticeable difference between WTs with and without metastases regarding the frequency of cyclin A overexpression in different components. (**Table 1**).

Table 1: Factors that affect the overall survival of individuals with Wilms tumors [6].

Variables		Number	P of log-rank test
Gender	Male	40	0.67
	Female	35	
Cyclin A	-	32	0.01
	+	43	
Tumor side	Right	35	0.47
	Left	40	
Extracapsular extension	No	62	0.26
	Yes	13	
Anaplasia	No	68	0.91
	Yes	7	
Unfavorable histology	No	60	0.13
	Yes	15	
Stage	stage I	45	0.11
	stage II	8	
	stage III	10	
	stage IV	12	

Lymphadenopathy	No	64	0.37
	Yes	11	
Venous thrombus	No	65	0.95

Those who received therapy for PrOpChTh had a one-, three-, and five-year survival rate of 97.1%, compared to 71.4% for those who did not. After five years of observation, the OS for patients with stage 1 and stage 2 tumors was 80% and 62.5 %, respectively, but it was 44.4 % and 0% for patients with stage 3 and stage 4 tumors. At 1, 3, and 5 years, survival rates for those with an IR prognosis were 100%, 84%, and 76%, while survival rates for those with an HR prognosis were 90%, 40%, and 20%, respectively (**Table 2**).

Table 2: Factors impacting the overall survival of Wilms tumors patients [12].

Variables		**Univariate analysis**			
		Coefficient *l*	**HR**	**95% CI**	***p***
Prognostic group	IR		1.0		
	HR	1.581	4.859	1.668–14.152	0.004
Stage	Stage 1		1.0		
	Stage 2	0.796	2.216	0.447–10.995	0.330
	Stage 3	1.371	3.938	0.931–16.655	0.062
	Stage 4	3.182	24.102	3.879–149.742	0.001
B (cyclin A expression)	No/focal		1.0		
	Overexpression	1.107	3.025	1.043–8.777	0.042

4. Discussion

WT is the most prevalent pediatric kidney tumor, and it is heavily influenced by the tumor stage and UFH. Recent research has underlined the IHC test's ability to predict WT behavior, especially in low and middle-income countries. Cyclin A was employed as an immunohistochemistry marker [6].

According to the findings of the Stamatakos et al. (2010), cyclin A overexpression in WT was more prevalent in late stages and tumors with metastasis, indicating that cyclin A overexpression may be connected with tumor development. Because of their involvement in carcinogenesis, cyclin mutations may cause a variety of malignancies [13].

If WT cyclin E is overexpressed, the prognosis may improve [14]. Taran et al. (2011) found that those with greater cyclin E1 levels had worse results [15]. Cyclin A is expressed at the cellular level in normal renal tissue and 34% of WT had overexpressed cyclin A in addition to the normal amounts of cyclin A seen in kidney tissue [6]. According to Cyniak-Magierska et al. (2015), cyclin A overexpression has been associated with a variety of pediatric and adult malignancies, including neuroblastoma [16].

The overexpression of cyclin A should be explored in chemotherapeutic resistance research [17]. There is no link between PrOpChTh use and elevated cyclin A levels. It has been shown that cyclin A overexpression correlates with tumor grade but not tumor size across a broad range of tumor types [6]. Cyclin A expression is higher in stages 3 and 4 than in stages 1 and 2.

According to Miao et al. (2015), increased levels of cyclin A expression correspond with more advanced cancers. Increased cyclin A expression probably contributes to the WT's poor prognosis [18]. Although the risk did not rise between stages 2 and 3, the mortality rate in the Sanja et al. group was about four to twenty-four times higher in stage 4. In contrast to epithelial and blastemal cells, stromal cells seldom overexpress cyclin A [6].

Due to an expanding panel of molecular biology markers and the continuous use of WT stage and histological characteristics as the most reliable prognostic indicators, cancers with high-risk (HR-WT) and low-risk (IR-WT) outcomes may soon be distinguished. Cyclin A overexpression was more prevalent in the HR prognostic group, even though this discovery did not achieve statistical significance (WTs with diffuse anaplasia and WTs with blastemal type after treatment) [6]. Cyclin A overexpression is more common in diffuse anaplastic tumors than in blastemal type WT, which might explain this finding. In a recent SIOP study, blastoma type WT was reclassified as high-risk group WT [19]. PrOpChTh did not influence the percentage of cyclin A expression in the blastemal component [6]. WT with diffuse anaplasia may have more proliferative capability [6].

The cyclin protein family controls and regulates the cell cycle through interacting with and activating the cyclin-dependent kinase (CDK) family. Cyclin A protein checkpoints control DNA replication and cell cycle progression. Despite its limited scope, one research investigated the significance of WT cyclin A IHC expression. Radojevic-Skodric et al. (2019) discovered that cyclin A expression was greater in big tumors (10.5 and 4.06 cm) and stages 3 and 4. (p = 0.01 and 0.004, respectively). However, diffuse anaplasia did not vary significantly from controls [6].

Cyclin A expression was not associated with UFH, tumor size, or advanced stage [12]. Patients with cyclin A immunopositivity had a poor outcome and an elevated risk of recurrence.

According to the findings of the Atwa et al. (2022), cyclin A overexpression is more often related to localized anaplasia. According to our findings, those with an IR prognosis had a fivefold reduced death risk than those with an HR prognosis. Our findings showed that cyclin A was overexpressed in WT tumors that had spread to other tissues. Unfortunately, this result was not statistically significant.

Conclusion

Overexpression of Cyclin A in WT may be utilized to predict poor prognosis. Prospective trials with a greater number of patients are recommended in the future. There was no link between cyclin A overexpression and the WT metastatic variant. A bigger patient group would allow for a more accurate assessment of cyclin A overexpression and poor prognosis in WT individuals. The study's shortcomings include a limited number of patients and a retrospective methodology.

Ethical consideration and patient consent: The study was approved by the Faculty of Medicine, Fayoum University Research Ethical Committee (Approval no. M585).

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Conflicts of Interest: All authors declare they have no conflicts of interest.

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