Immunohistochemical Expression of Ki-67 and Minichromosome Maintinance 2 (MCM2) Protein in Wilm's Tumour

Qurat ul Ain Javaid[1], Nadia Naseem[2], Abdul Hanan Nagi[3]

ABSTRACT

Introduction: To assess the immunohistochemical expression of Ki-67 and MCM2 protein in Wilm's tumour. To assess the correlation of MCM2 and Ki-67 proliferative index with tumour stage.

Material and methods: It was a descriptive study conducted in the Department of Morbid Anatomy and Histopathology UHS Lahore, Pakistan. A total of n=50 nephrectomy specimens and paraffin embedded blocks from patients of Wilm's tumour were taken for study.

Results: Out of 50 patients of Wilm's tumour, 66% were male and 34% were female patients with male to female ratio of 2:1. Mean age of the patient was calculated as 4±2.01 years. The mean labeling index percentage of Ki-67 for blastemal component was 21.6%±14.9, for epithelium it was12.9%± 6.3 and 0.65%±0.1 for stromal. The mean LI percentage of MCM2 for blastemal component was 28.1%±19, for epithelial it was 14.2%±7.2 and for stromal 0.4%±0.21. In cases with features of anaplasia, the mean LI % of Ki-67 in blastemal component was 32.6%±23.4, epithelial 17.7%±11.5, stromal 1.09% ± 0.9. Mean LI % of MCM2 in blastemal component was 43.8% ± 29.5, epithelial 21.9% ±15.8 and in stromal 0.06%±0.04. The mean LI % of MCM2 in different component of Wilm's tumour and in cases with features of anaplasia was found to be higher than the LI % of Ki-67. Statistically a positive correlation between LI of Ki-67 and MCM2 and tumour stage was found (P<0.001).

Conclusion: Ki-67 and MCM-2 positively correlate with stage of the tumour. However, MCM-2 is superior to Ki-67 as a marker of proliferation in Wilm's tumour and gives an objective measure of aggressiveness of the tumour.

Keywords: Wilm's Tumour, MCM2, Ki-67, Labeling Index.

INTRODUCTION

Wilm's tumour or nephroblastoma is cancer of the kidneys that typically occurs in children and rarely in adults. The overall prevalence of Wilm's tumour is found as 1 in 10,000 children [1]. A total of 75% of Wilm's tumour occur in children who are under 5 years of age, with a peak incidence age of 2 to 3 years[2]. In about 95% of the patients, the tumour is unilateral and only 1-2% have a positive family history [3]. The studies show that the presence of bilateral or multifocal disease in a patient has a genetic predisposition for developing Wilm's tumour [4]. Wilm's tumour appears relatively more common in Africa and least common in East Asia. In Pakistan, the accurate incidence of cancers and malignant tumours in children is still unknown, however a report from the Pakistan Medical Research Council Cancer Study group revealed that Wilm's tumour under 15 years of age constituted 4.38% of all diagnosed malignant tumours [5].

Proliferation of any tumour cell is closely linked to their rate of DNA synthesis and this proliferation of a cell provides prognostic information of that particular tumour. A perfect biomarker for assessment of proliferation should be crucial for genomic replication and show a broader range of expression that allows a rapid and objective assessment of an individual case. Proliferative markers function in different phases of cell cycle. The minichromosome maintenance proteins (MCM) take part in S-phase genome constancy. MCM expression is up regulated in proliferating cells, providing a diagnostic marker of malignancies [6].

Ki-67, a proliferative marker was first identified by Gerdes in 1991 [7]. Ki-67 gene is located on the long arm of human chromosome 10 (10q25) [8]. Studies indicate that expression of Ki-67 is low during G-1 and early S-phase of cell cycle and it gradually increase to reach a maximum during the phase of mitosis. A quick decrease in its expression starts during anaphase and telophase [9].

This study was designed to assess the immunohistochemical expression of Ki-67 and MCM2 in Wilm's tumour and the relationship of MCM2 and Ki-67 proliferative index with tumour stage and type.

MATERIAL AND METHODS

It was a descriptive study done in the Department of Morbid Anatomy and Histopathology University of Health Sciences Lahore, Pakistan in eight months (April 2014-November 2014). A total of n= 50 nephrectomy specimens and paraffin embedded blocks from Wilm's tumour patients were taken for study from the Department of Urology, Children Hospital Lahore and Jinnah Hospital Lahore. Patients of both gender up to 11 years of age diagnosed with any Wilm's tumour histological type were included in study. On immunohistochemistry, nuclear staining of any type either diffuse, peripheral or nucleolar was taken as positive. Labeling index was calculated by counting 1000 cells manually [15]. Firstly the highest staining areas were marked

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using low power. Then by using 40X magnification all stained cells in each high power field were counted manually. The number hence attained was divided by the total number of cells in that particular field. A total of 5 fields were studied for staining.

STATISTICAL ANALYSIS

Mean ± S.D was used for quantitative variables like age and labeling indices of MCM-2 and Ki-67. Correlation was evaluated by using pearson's correlation and $p \leq 0.05$ was considered significant. Data was analyzed using SPSS version 20.

RESULTS

Total 50 cases of Wilm's tumour were taken for the study. Mean age of the study population was 4 ±2.01 years with an age range of 1 to 10 years. Out of 50 patients of Wilm's tumour, 66% (n = 33) were males and 34% (n = 17) were females. The male to female ratio was calculated as 2:1. Most frequent site of tumour source was from left kidney (n=34; 68%). Most of the tumours were limited to stage I (n=21; 42%) with n=12 tumours progressing in stage II (24%). However stage III had only n=9 (18%) cases and n=8 in stage IV (16%). There was no case in stage V. Most frequent histological subtype was triphasic (n=28; 56%) which was followed by monophasic (blastema component only) in 38% (n=19) and biphasic in 4% (n=2) of cases. Features of anaplasia were found in 42% (n=21) cases. Focal anaplasia was spotted in 12% (n=6) and diffuse anaplasia in 30% (n=15) cases.

The mean LI percentage of Ki-67 for blastemal component was 21.6%±14.9, for epithelium it was 12.9%±6.3 and for stromal 0.65%±0.1. The mean LI percentage of MCM2 for blastemal component was 28.1%±19, for epithelial it was 14.2%±7.2 and for stromal 0.4%±0.21. The mean LI % of MCM-2 in different component of Wilm's tumour cases was found to be higher than the LI % of Ki-67.

Cases showing features of anaplasia had higher proliferation indices of Ki-67 and MCM2. Higher LI % for both markers

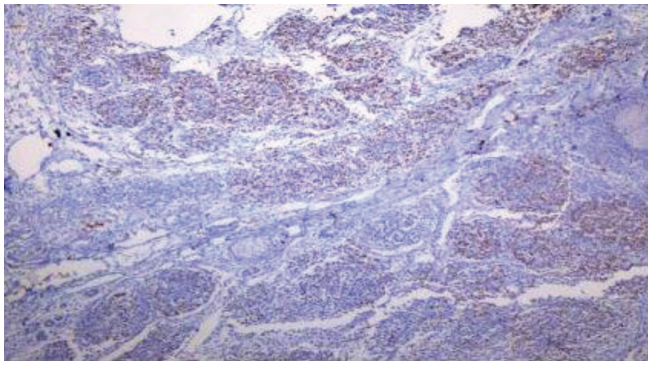

Figure-1: Ki-67 labeling in blastemal component (Stage IV).

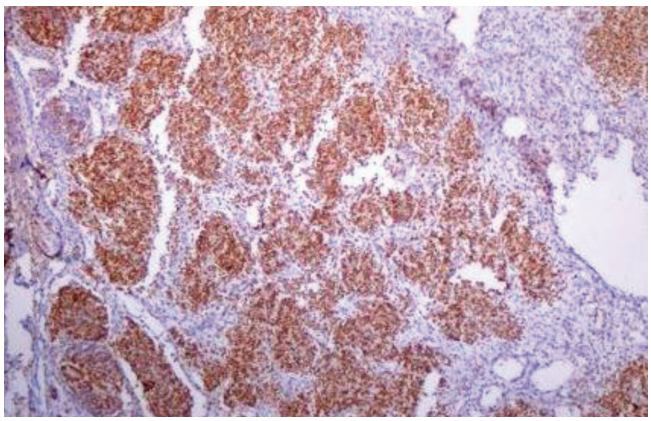

Figure-2: MCM-2 labeling in blastemal component (stage IV)

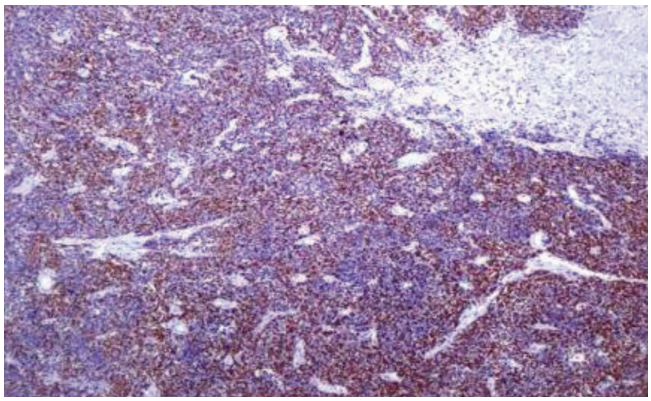

Figure-3: MCM-2 labeling in blastemal component (Stage III, 100X).

Stage		Labeling index % of Ki-67			Labeling index % of MCM2		
		Blastemal component	Epithelial component	Stromal component	Blastemal component	Epithelial component	Stromal component
I	N	21	21	21	21	21	21
	Mean	0.8%	0.78%	0.29%	1.64%	1.68%	0.36%
	SD	0.69	0.48	0.18	1.18	1.05	0.25
II	N	12	12	12	12	12	12
	Mean	1.7%	16.5%	0.11%	6.9%	11.3%	0.05%
	SD	1.72	12.5	0.06	4.25	1.0	0.04
III	N	9	9	9	9	9	9
	Mean	30%	17.4%	1.29%	38.3%	21.3%	0.65%
	SD	12.5	13	0.6	21.9	15.2	0.3
IV	N	8	8	8	8	8	8
	Mean	54%	0%	0%	70%	0%	0%
	SD	21.7	0	0	9.4	0	0
Note: LI of MCM2 is higher than Ki-67.							

Table-1: Labeling indices of Ki-67 and MCM2 in different components with respect to the stage.

was found in blastemal component as compared to rest of the tumour components. The mean LI % of Ki-67 in blastemal component was 32.6% ± 23.4, in epithelial 17.7%±11.5 and in stromal it was 1.09%±0.3. The mean LI % of MCM2 in blastemal component was 43.8% ± 29.5, in epithelial 21.9%±15.8 and in stromal 0.06%±0.04.

LI of Ki-67 and MCM2 increased variably with the stage of the tumour for different components. But stage I and II had increased expression of both markers for epithelial component and in stage III and IV there was increased expression for blastemal component.

In stage I mean LI of ki-67 for blastemal component was 0.8%±0.69. For epithelial component 0.78% ± 0.48 and for stromal 0.29%±0.18. Similarly in stage I mean LI of MCM2 for blastemal component was 1.64% ± 1.18 for epithelial component 1.68% ±1.05 and for stromal 0.36%±0.25.

In stage II mean LI of ki-67 for blastemal component was 1.7% ± 1.72. For epithelial component 16.5%±12.5% and for stromal 0.11% ± 0.06. Similarly in stage II mean LI of MCM2 for blastemal component was 6.9%±4.25. For epithelial component 17.3% ±11.3 and for stromal 0.06% ± 0.05.

In stage III mean LI of ki-67 for blastemal component was 30% ± 12.5. For epithelial component 17% ± 13 and for stromal 1.29% ± 0.6. Similarly in stage III mean LI of MCM2 for blastemal component was 38.3 ± 21.9. For epithelial component 21.3% ±15.2 and for stromal 0.65%±0.30.

In stage IV there was blastemal component only, so the mean LI of ki-67 for blastemal component was 54% ± 12.5. Similarly in stage IV mean LI of MCM2 for blastemal component was 70% ± 9.4 (Table 1). There was no case limited to stage V diagnosed in present study.

Correlation of the stage with labeling index % for both Ki-67 and MCM-2 was made individually for all histological subtypes of Wilm's tumour. A positive correlation was found between labeling index of both Ki-67 and MCM-2 with stage of the Wilm's tumour in epithelial and blastemal component ($p<0.001$; Pearson's correlation).

DISCUSSION

Wilm's tumour is one of the most frequent malignancy of the childhood. The present study is based on the morphological features of Wilm's tumour and the expression of Ki-67 and MCM-2 (new proliferative marker). Total 50 cases of Wilm's tumour were collected for the study. Ki-67 and MCM-2 were the two proliferative markers of our study.

In the present study, the expression of labeling indices of both markers were studied separately in various histological subtypes of Wilm's tumour and the relationship of MCM2 and Ki-67 labeling index with tumour stage and type was also determined. Total 50 cases of Wilm's tumour were collected for the study. The mean age of the patient was calculated as 4 years and age range was 2 to 5 years. Breslow NE, et al 2006 [2] found the similar age group in his study. Concerning gender distribution, male to female ratio was 2:1 in our study. Katarzyna Tarani, et al 2011 also mentioned the male predominance in Wilm's tumour cases [13].

Most frequent site of tumour source was from left kidney (n=16; 32%) [10]. Most frequent clinical presentation was the abdominal mass on examination in all the cases and pain in a few cases. Most frequent histological subtype of Wilm's tumour in our study was triphasic (n=28; 56%) with predominant blastema component and second most common was the monophasic type (n=19; 38%). In monophasic type of Wilm's tumour, only blastemal component was spotted. Katarzyna tarani et al 2011 in his study observed that the most common histological subtype in Wilm's tumour cases is triphasic [13].

The labeling indices of MCM-2 and Ki-67 was studied in various histological components. MCM-2 labeling indices was high in blastemal (mean=19% ± 28.1) and epithelial (mean=7.2% ± 14.2) components of Wilm's tumour as compared to the labeling indices of Ki-67 (blastemal= 14.9% ±21.6, epithelial 6.3% ± 12.9) [12] (Table I).

The overall labeling indices of MCM-2 was found higher than Ki-67 in current study. Dudderidge et.al (2005) studied the labeling indices of both of these markers on renal cell carcinoma and found the LI of MCM2 higher than the Ki-67 in various types of renal cell carcinoma cases [14].

LI of both the markers (Ki-67 and MCM-2) was found higher in patients with the histological feature of anaplasia [11]. LI of both markers were studied separately for each component with anaplasia. It was found high in blastemal component (Ki-67 = 32.6% and MCM-2 = 43.8%) than in epithelial component and statistically significant association of anaplasia was found with LI of both markers (p=0.000).

The LI in epithelial component was higher than blastemal component in stage I (Ki-67=0.78%, MCM-2=1.68%) in stage II (Ki-67=12.5%, MCM-2=11.3%) [12]. LI of both the markers was found to be high in blastemal component in advanced stages III and IV (Ki-67 = 30% and 54%; MCM2 =38% and 70%). Katarzyna et al (2011) mentioned that the high LI is seen in patients limited to the advanced stage and low LI in patients limited to early stage. High LI and predominant blastema component are associated with advanced stage and poor clinical outcome of the patient [13].

In present study a good correlation was found between tumour stage and blastemal component and also between Ki-67 and MCM-2. A good correlation was also found by other investigators regarding tumour stage and blastemal component and also between Ki-67 and MCM-2 [11-13].

CONCLUSION

Ki-67 and MCM-2 positively correlate with stage of the tumour. MCM-2 labels a greater percentage of cells as compare to Ki-67. Therefore, MCM-2 is said to be a superior marker of proliferation in Wilm's tumour and gives an objective measure of aggressiveness of the tumour.

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REFERENCES

1. Owens CM, Brisse HJ, Olsen OE, Begent J, Smets AM. Bilateral disease and new trends in Wilmstumour. Pediatr Radiol 2008;38:30–39.
2. Breslow NE, Beckwith JB, Perlman EJ, Reeve AE. Age distributions, birth weights, nephrogenic rests, and heterogeneity in the pathogenesis of Wilms tumor. Pediatric Blood Cancer 2006;47:260–267.
3. Kenneth H Kraemer, MD and John J Di Giovanna. Xeroderma Pigmentosum. In: Gene Reviews, (Detailed overview of xerorderma pigmentosum). 2003;20:29-268.
4. Hu M, Fletcher J, Mc Cahon E. Bilateral Wilms tumor and early presentation in pediatric patients is associated with the truncation of the Wilms tumor 1 protein. J Pediatric 2013;163: 224-9.
5. Pakistan Medical Research Council Cancer Study Group. Frequency of malignant tumors in seven centres of Pakistan. J. Pak. Med. Assoc.1977;27:335-38.
6. Bailis JM, Forsburg SL. 2004.MCM proteins: DNA damage, muta- genesis and repair Curr Opin Genet Dev 2004;14:17-21.
7. Gerdes J, Li L, Schlueter C. Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. Am J Pathol 1991; 138:867-873.
8. Fonatsch C, Duchrow M, Rieder H. Assignment of the human Ki-67 gene (MK167) to 10q25-qter. Genomics 1991;11:476-477.
9. Lopez F, Belloc F, Lacombe F. Modalities of synthesis of Ki67 antigen during the stimulation of lymphocytes. Cytometry 1991;12:42-49.
10. Soyemi SS, Osuoji RI, Faduyile FA, Sanni DA, Oyewole OO. Morphological Features of Wilms' Tumour in a Tertiary Health Care Institution: Our Findings. J Clin Exp Pathol 2013;3:146.
11. Juric I, Pogrelic Z, Kuzmic prusac, I, Biocic M, Jakov ljevic G, Stepan J, Zupancic B, Culic S, Kruslin B. Expression and prognostic value of the Ki-67 in Wilms' tumor: experience with 48 cases 2010; 26:487-93.
12. Das Ram Narayan, Chatterjee U, Sinha SK, Ray AK, Saha K, Banerjee S. Study of histopathological features and proliferation markers in cases of Wilms' tumor. Indian J Med Paediatr Oncol 2010;33:102-6.
13. Katarzyna tarani, anna stikiewicz, ewa andrzejewska, józef kobos. Minichromosome maintenance 2 (mcm2) is a new prognostic proliferative marker in wilm's tumour 2011;2: 84-88.
14. Dudderidge T, Stoeber K, Loddo M. MCM2, Geminin, and KI67 define proliferative state and are prognostic markers in renal cell carcinoma. Clin Cancer Res, 2005;11: 2510–2517.
15. Rioux Leclercq N, Turlin B, Bansard J. Value of immunohistochemical Ki-67 and p53 determinations as predictive factors of outcome in renal cell carcinoma. Urology, 2000;4295: 0–4.

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