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IMMUNOHISTOCHEMICAL EXPRESSION OF Ki67 AND p53 IN WILMS TUMOUR AND ITS CORRELATION WITH TUMOUR HISTOLOGY AND PATHOLOGICAL STAGING

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INTRODUCTION

Nephroblastoma or Wilms' tumor is a pediatric renal tumour derived from primitive renal epithelial and mesenchymal components. It is the most common abdominal malignant tumour of young children and is known to be associated with chromosomal abnormalities like Beckwith Widemann syndrome and WAGR syndrome,[1]. Overall, Wilms' tumor incidence is 7.8 cases per million children. Peak age of incidence is 2 to 3 years of age, or 99% occurring less than six years of age.

Chemotherapy protocol for Wilms tumour is based on tumour staging and histology. Most patients respond to chemotherapy protocol. However a small fraction relapses or metastasizes. Therefore there is need to identify ideal cost effective prognostic markers for this pediatric tumour. The aim of our study is to evaluate the efficiency of two cost effective Immunohistochemical markers, tumour proliferation marker(Ki67)and tumour suppressor marker(p53) in Wilms tumour. Their expression will be correlated with tumour histology and staging.

Cell kinetic data is an important indicator of the aggressiveness of tumour & clinical response. The old & widely used method for assessing cell proliferation is mitotic count in routinely processed H&E sections. Nowadays, in this IHC era we use Ki67 labelling index to assess the tumour proliferation index. Other nuclear antigens include Ki S1 & PCNA,[2].

MATERIALS AND METHODS

This is a retrospective study which includes 18 cases of wilms tumours, who presented in a tertiary care center in South India from Jan 2009 to Dec 2019. Cases which had received prior chemotherapy were excluded from the study, since the tissue response to chemotherapy such as necrosis and haemorrhage and were not amenable for immunohistochemistry. The records of the patients were retrieved from pathology database and analyzed. The macroscopic examination and microscopic examination were performed in detail to make a pathological staging according to NWTS-5 staging,[3] Formalin fixed 3mm sections were stained by H&E and then by IHC with monoclonal anti Ki67 antibody using streptavidin biotin peroxidase complex. Lymphoid tissue was taken as positive control for Ki-67 immunostaining.

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Ki-67 immunostaining using the monoclonal antibody MIB-1 represents a simple, quantifiable and reproducible method to determine the tumour cell dynamics and proliferation index. The labeling index or growth fraction is calculated by the proportion of positive cells in relation to a total of 1000 cells evaluated. Each slide was evaluated at 40x to find areas with maximum positive cells and then at 200x magnification to count the positive cells. Stromal staining cells and tumour infiltrating lymphocytes are avoided from evaluation. p53 is a 53 kDa nuclear protein product of tumor suppressor gene p53, located on short arm of chromosome 17. Mutations of p53 are detected in mmost of the human malignancies. p53 was semiquantitatively assessed as mild, moderate, or marked.

RESULTS

The study comprised of 18 cases of Wilms tumours reported in a tertiary care center in South India from January 2009 through December 2019. Peak age of incidence was 1-5 years. There was significant male preponderance with a male to female ratio of 3:1. Based on the NWTS-5 classification, of the 18 cases, 13 cases were in stage I, 1 case was in stage II (Fig./Image-1), two cases were in stage III, and two cases were in stage IV and there was no case in stage V (Table 1). 15 cases were triphasic Wilms tumor (Fig./Image-2) and three were biphasic. 14 cases showed favourable histology and remaining cases showed unfavourable histology. Areas of anaplasia were noted in two cases.

Ki67 proliferation index was calculated and correlated with histology and with tumor staging. It ranged from 12-22% in stage I in the epithelial component and 21-36% in the blastemal component. In stage II the Ki 67 labelling index was 21% in epithelial component (Fig./Image-3) and 33% in blastemal component. In stage III, the Ki-67 labelling index was 26% in epithelial component and 35% in blastemal component and in stage IV it was around 30% in epithelial component and 41% in blastemal component(Table 2). In all the stages the blastemal component showed higher Ki67 proliferation index as compared to the epithelial component. There was also a significant increase in Ki-67 labelling index in areas of anaplasia. The expression of p53 was expressed as mild, moderate, or marked (Fig./Image-4,5). No difference was noted in the degree of expression of p53 in epithelial and blastemal components and also in areas of anaplasia. Two cases in stage III and one case in stage IV showed moderate positivity while stages I and II cases were almost negative (Table 3).

Table:1- Distribution of cases according to the staging of the tumour

Stage	Number of cases
Stage I	13
Stage II	1
Stage III	2
Stage IV	2
Stage V	nil

Table:2- Ki-67 labelling index in epithelial and blastemal component

Stage of the tumour	Epithelial component	Blastemal component
Stage I (n=13)	12-22 (mean=16)	21-36 (mean=28)
Stage II (n=1)	21	33
Stage III (n=2)	26	35
Stage IV (n=2)	30	41
Stage V nil	-	-

Table:3 -p53 expression in different stages

Stage of the tumour	P53 IHC expression
Stage I (n=13)	Low
Stage II (n=1)	Low
Stage III (n=2)	Moderate
Stage IV (n=2)	Moderate
Stage V nil	-

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Fig./Image 1: Gross image of Wilms tumour with lesion infiltrating the renal sinus

Fig./Image 2: Triphasic Wilms Tumour (H&E x200x)

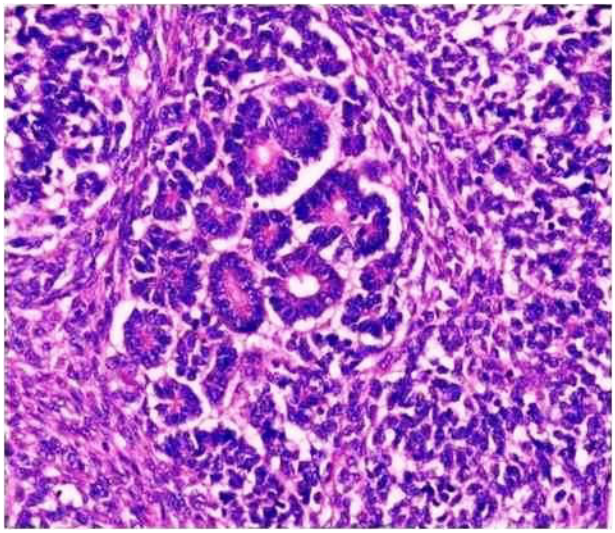

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Fig./Image 3: Ki67 labelling index in Stage II wilms tumour (IHC x200x)

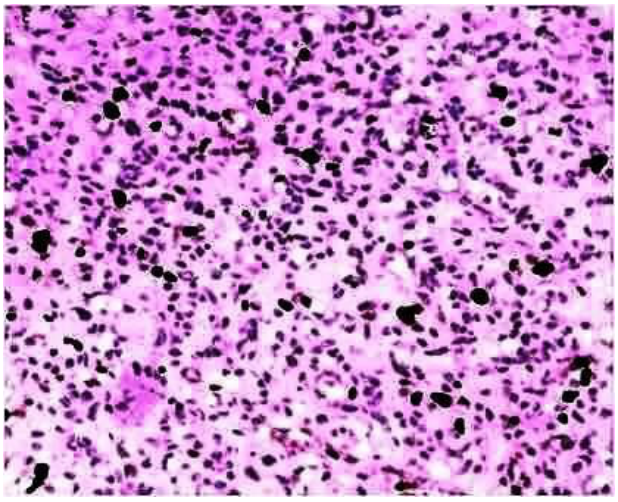

Fig./Image 4: p53 immunostaining in stage I wilms tumour(IHC x400x)

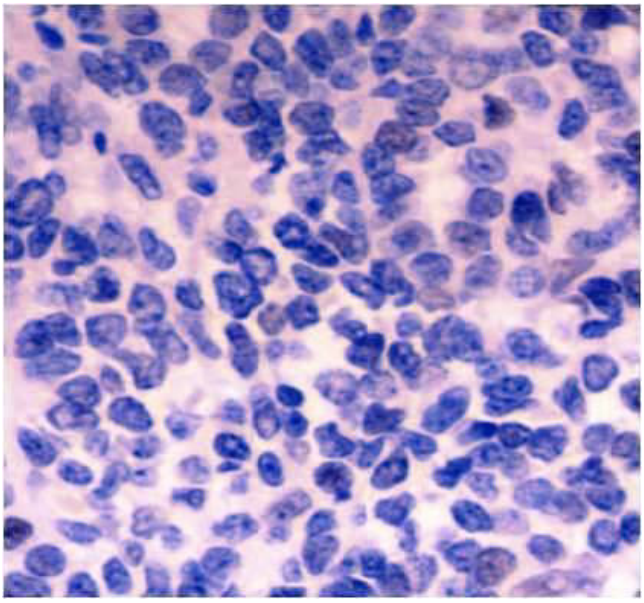

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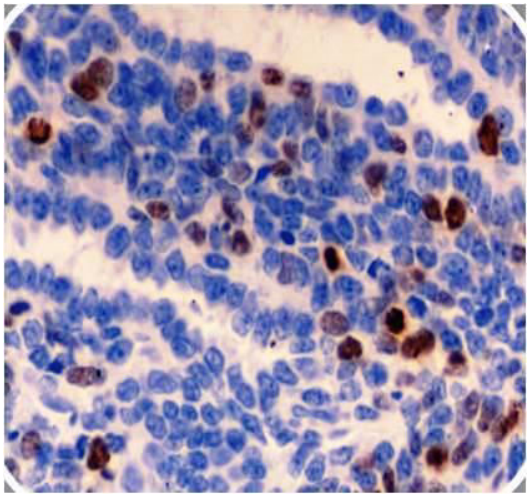
Fig./Image 5: p53 immunostaining in stage III wilms tumour(IHC x400x)



DISCUSSION

Nephroblastoma or Wilms' tumor was first described by Dr.Max Williams. It is an embryonic tumor derived from primitive renal epithelial and mesenchymal components. It is the most common abdominal malignant tumour of young children. It accounted for 3% of all pediatric malignancies,[4]. It almost always occurs in children less than five years of age -90% of cases are diagnosed before the age of three, [4,5] the peak incidence is in the age range of 2-5 years,[6] . Wilms tumor are usually due to sporadic mutations but some are associated with other syndromes, such as WAGR (Wilms-aniridia-genitourinary-mental retardation) syndrome, Denys-Drash syndrome, both with the WT1 mutation, Beckwith-Wiedemann syndrome associated with genetic and epigenetic abnormalities at 11p15, and other congenital anomalies,[7-10] . Thus, lung CT scan, genitourinary US scan, and gene analysis should be performed to rule out the possibility of a syndromic association.

Wilms' tumor arises in any location inside the kidney as embryologic precursors to renal cells. These cells mimic the embryologic development of the kidney and consist of three components: blastemal, epithelium, and stroma components. The blastematous areas are extremely cellular and composed of small round-to-oval primitive cells; the cytoplasm is usually very scanty, but sometimes is more abundant and exhibits an oncocytoid appearance. The epithelial component is characterized by the formation of embryonic tubular structures that closely recapitulate the appearance of normal developing metanephric tubules.

The peak age of incidence was 1-5 years with a median of 2.5 years in our study which correlates with a study by Mishra et al. who also reported a median age incidence of 2.5 years, [11] .Our study showed a female preponderance, which concurs with NWTS study done over a large population group showing a female preponderance, [12] .Most of the cases, 13 out of 18, presented in stage I and 1 case in stage II, 2 cases in stage III, and 2 cases in stage IV. Staging corresponds to other studies, [13] .The histological pattern was triphasic in 15 cases and unfavourable histology was seen in 4 cases which is slightly on the lower side in comparison with a study done by I.Juic et al., [14]

Our study found higher Ki67 proliferative index in higher stages of Wilms tumor and similar findings were recorded in studies, [15-16].The Ki-67 labelling index was higher in areas of anaplasia. The role of Ki67 as a proliferative marker in Wilms tumor stands justified. The role of p53 in the pathogenesis and

progression of Wilms tumors is not very clear.Wilms tumor has been associated with chromosomal abnormalities at the 11p13, 11p15, and 16q regions. A study into the possibility of mutations occurring within p53, the adult tumor suppressor gene, was made. Cheah et al. in their study mentioned that the immunohistochemical expression of p53 protein in Wilms tumor was possibly a result of mutation in the p53 tumor suppressor gene and correlates with histological classification,[17] .However we found nil expression of p53 immunostaining in stages I & II and moderate expression in higher stages. This study does not find immunohistochemical expression of p53 utility in prognostication of Wilms tumor. Skotnicka-Klonowicz et al. in their study found significant correlation between p53 expression and tumor staging, [18] .

CONCLUSION

In, conclusion, our study suggests that Ki-67 has a potential prognostic role in Wilms tumour where it correlates well with staging of the tumour. This biological predictor potentially helps to identify patients with high risk of progression and recurrence and also guides for adjuvant chemotherapy or radiotherapy. However the utility of p53 in the prognostication of Wilms tumour must be validated.

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