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Pathology

A CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY USING WT1 PROTEIN ON WILMS' TUMOUR IN A TERTIARY CARE CENTRE.

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ABSTRACT Wilms' Tumour is the most common primary malignant renal tumour of childhood which accounts for almost 6% of all childhood cancers. WT1 located at chromosome 11p13 was the first Wilms' Tumour gene to be identified. We studied the clinico-pathological profile and expression of WT1 protein in 33 new Wilms' Tumour cases which were received in the Department of Pathology, Gauhati Medical College and Hospital, Guwahati between July 2019 to June 2020 were included in the study. Clinical details of the patients were taken and diagnosis was made by histopathological examination and WT1 staining was done in each cases. In our study maximum cases were seen in 1 year- 6 years age group (78.8%) with mean age at presentation being 42 months and a slight male preponderance with M:F= 1.75:1. Most of the patients (81.8%) presented with abdominal mass. On histopathological examination, triphasic (72.7%) type was the most frequently occurring followed by biphasic type (18.2%). WT1 nuclear positivity was seen in 93.9% of cases of Wilms' Tumour mainly in the blastemal and epithelial components. Early diagnosis of Wilms' tumour with help of WT1 immunostaining may aid in better management and good prognosis.

KEYWORDS : Wilms' Tumour, Nephroblastoma, WT1.

INTRODUCTION:

Paediatric renal tumours account for almost 7% of all childhood cancers reported worldwide. The majority (90%) of cases are Wilms' Tumour (WT or Nephroblastoma), with an annual incidence of approximately 1 in 100,000 children [1]. Among Non Wilms' Tumours important ones are Clear Cell Sarcoma of Kidney (CCSK), Mesoblastic Nephroma (MN), Rhabdoid Tumor of kidney while Angiomyolipoma (AML), Primitive Neuroectodermal Tumour (PNET), Renal Cell Carcinoma (RCC), Rhabdomyosarcoma (RMS) and Lymphoma constitutes some of the rare tumours found in kidney in childhood [2]. Nephroblastoma [Wilms' tumour (WT)] is one of the most common solid tumours found in paediatric age group (age ranging from 6 months to 12 years of age) [19] and accounts for 6-7% of all paediatric malignancies [18]. Nephroblastomas are thought to arise from mesenchymal blastemal cells that fail to differentiate into metanephric structures but continue to proliferate anyways. [3] The first genetic locus was found in patients with the Wilms-Aniridia genital anomaly-retardation syndrome (WAGR). [4] The gene which is located at chromosome 11p13 was cloned in 1990 and was designated as WT1[5]. The WT1 gene encodes a protein with 4 zinc fingers of the Kruppel-type in the terminal region which is required in tissue differentiation and proliferation [6]. The N-terminal half contains a large proline- glutamine-rich domain required for inhibition of transcriptional activation. [7]. Approximately 10-15 per cent of sporadic Wilms' tumours harbor mutations in the Wilms' Tumour-1 protein (WT1) gene. Overexpression of both wild-type and mutant WT1 has also been reported [2, 17]. WT1 protein is expressed during all stages of normal kidney development, but in the mature nephron, WT1 protein expression is mainly restricted to the podocytes. [8] It has also been demonstrated in the mesothelial cells and in stem cells bearing the CD34+ phenotype [9]. The WT1 protein was first classified as a tumour suppressor gene. An activator or oncogenic behavior may be acquired by mutations. The WT1 gene has also been reported in other disorders like hematological malignancies, [10] Mesothelioma, [11] Breast cancer, [12] Genitourinary tumors [13] and Small round blue cell tumors. [14]. There are very few resources in the literature about the utility of WT1 protein in Nephroblastomas which prompted us to investigate the expression of WT1 antibody in wilms' tumour.

METHODS AND MATERIALS:

This is a hospital based cross- sectional study done with 33 diagnosed cases of Wilms' Tumour received in the form of Nephrectomy specimen or biopsy in the Department of Pathology, Gauhati Medical College and Hospital, Guwahati in the period between July 2019 to June 2020. Cases with age below 12 years and all clinicoradiologically suspected and histopathologically diagnosed cases of Wilms' Tumour were included. Information regarding patient's age, sex, duration of illness, clinical data including presenting signs and symptoms and duration, past and family history was taken. General examination to look for any associated features or congenital anomalies such as Genitourinary malformation, Aniridia and comparison of limbs to look for Hemi- hypertrophy was taken. Bio-chemical examination including routine blood examinations, peripheral blood smear, serum creatinine, blood urea, RBS etc. radiological investigations included X-Ray chest, Ultrasonography of abdomen and CECT abdomen. Diagnosis was made by histopathological examination of the formalin fixed specimen that were received in the Department of Pathology, Gauhati Medical College and Hospital, Guwahati after proper gross examination followed by histopathological processing and Hematoxylin and Eosin stainning. Immunohistochemistry using WT1 was done in each case: Nuclear staining in >10 per cent of tumour cells was required to define WT1 positivity.

RESULTS:

The study was performed on 33 cases of Wilms' Tumour. Age ranged from 2 months to 12 years and maximum cases were seen in 1 year- 6 years age group (78.8%), followed by 6 years- 12 years (15.1%). Mean age at presentation was 42months. There was slight male preponderance with Male: Female ratio of 1.75:1. Clinically patients most commonly presented with abdominal mass or lump (81.8%), followed by abdominal pain (12.1%) and hematuria (6.1%). Upon histopathological examination Triphasic (72.7%) type of Wilms' Tumour is the most frequently occurring type followed by Biphasic type (18.2%). No cases of Wilms' Tumour showed presence of any associated feature and anaplasia in our study. Heterologous elements in the form of rhabdoid differentiation were present in 6.1% cases of Wilms' Tumour while 93.9% cases did not show presence of any heterologous elements. WT1 positivity was seen in 31 out of 33 Wilms' Tumour (93.9%) and WT1 was negative in 2 (6.1%) cases of Wilms' Tumour. WT1 nuclear positivity was mainly noted in the blastemal and epithelial components of Wilms' Tumour while stromal components showed fewer or occasional nuclear positivity.

Table 1: Age Distribution In Wilms' Tumour In Present Study.

Age	No. of case	Percentage
0-1 year	2	6.1%
1 year- 6 year	26	78.8%
6 year- 12 year	5	15.1%
TOTAL	33	100%

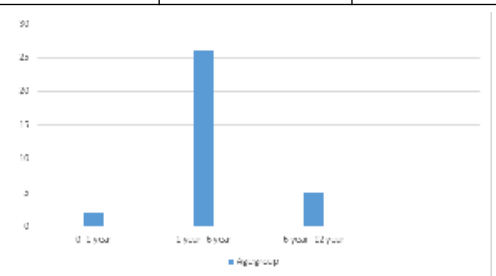


Fig 1: Age distribution in Wilms' Tumour

Observation: Age ranged from 2 months to 12 years and maximum cases were seen in 1 year- 6 years age group (78.8%), followed by 6 years- 12 years (15.1%). The mean age at presentation was 42 months.

Table 2: Gender Distribution In Wilms' Tumour In Present Study.

Gender	No. of cases	Percentage
Male	21	63.6%
Female	12	36.4%
Total	33	100%

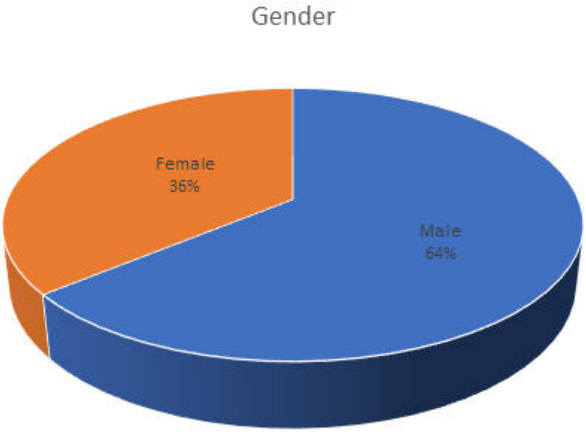


Fig 2: Gender distribution in Wilms' Tumour

Observation: Male preponderance was seen with Male: Female ratio of 1.75%

Table 3: Frequency Of Different Clinical Manifestations Of Patients With Wilms' Tumour.

Clinical manifestation	No. of cases	Percentage
Abdominal mass	27	81.8%
Abdominal pain	4	12.1%
Hematuria	2	6.1%
TOTAL	33	100%

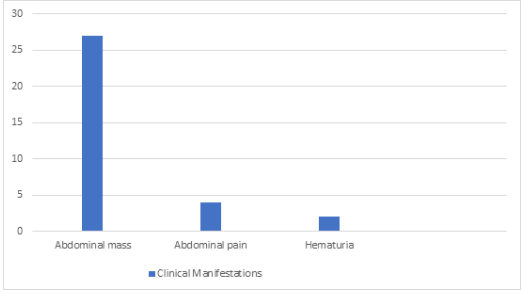


Fig 3: Frequency of different clinical manifestations of patients with Wilms Tumour.

Observation: Patients most commonly presented with abdominal mass or lump (81.8%), followed by abdominal pain (12.1%) and hematuria (6.1%).

Table 4: Distribution Of Different Histological Types Of Wilms' Tumour In Present Study.

Type	No. of cases	Percentage
Triphasic	24	72.7%
Biphasic	6	18.2%
Monophasic	3	9.1%
TOTAL	33	100%

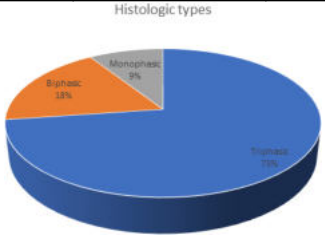


Fig 4: Distribution of different Histological types of Wilms' Tumour

Observation: Triphasic (72.7%) type of Wilms' Tumour is the most frequently occurring type followed by Biphasic type (18.2%).

Table 5: Frequency Of WT1positivity In Wilms' Tumour.

WT1	WILMS' TUMOUR	
	No. of cases	Percentage
Positive	31	93.9%
Negative	2	6.1%
Total	33	100%

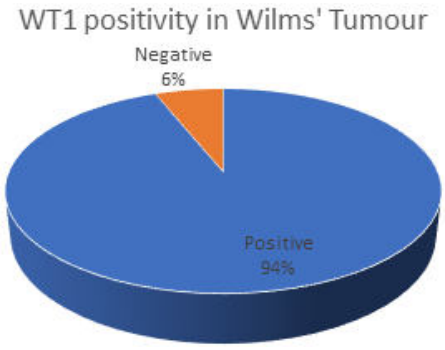
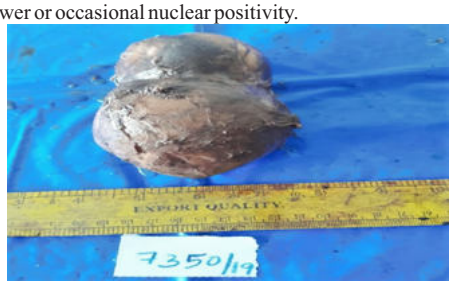
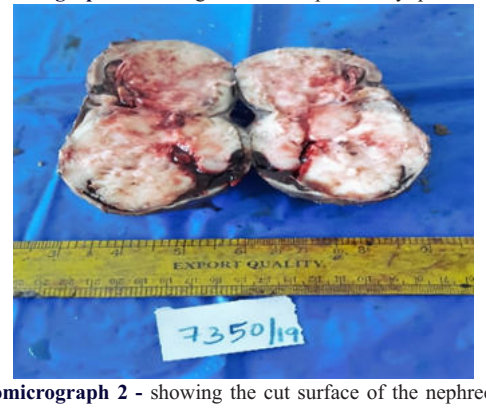


Fig 5: Frequency of WT1 positivity in Wilms' Tumour

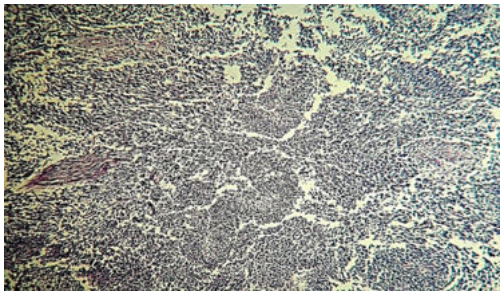
Observation: WT1 positivity was seen in 31 out of 33 Wilms' Tumour (93.9%) and WT1 was negative in 2 (6.1%) cases of Wilms' Tumour. WT1 nuclear positivity was mainly noted in the blastemal and epithelial components of Wilms' Tumour while stromal components show fewer or occasional nuclear positivity.



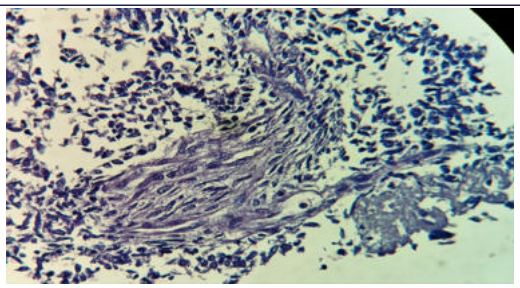
Photomicrograph 1- showing a resected nephrectomy specimen.



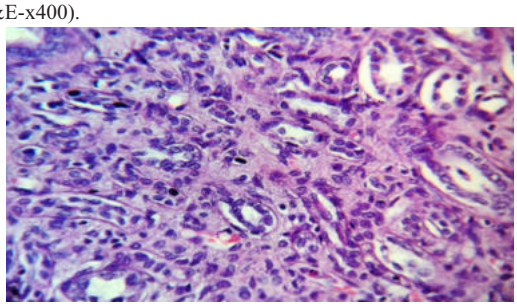
Photomicrograph 2 - showing the cut surface of the nephrectomy specimen which shows the tumour involving almost the whole of the kidney, the cut surface is solid, greyish white with areas of hemorrhage.



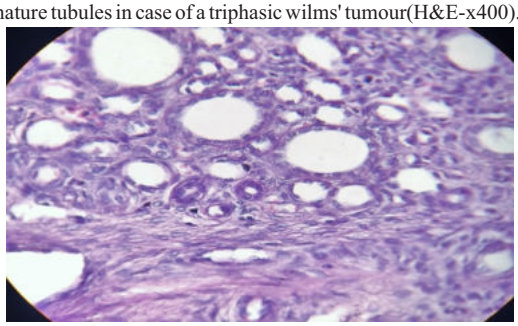
Photomicrograph 3 - showing predominantly blastemal component in case of a biphasic wilms' tumour(H&E-x100).



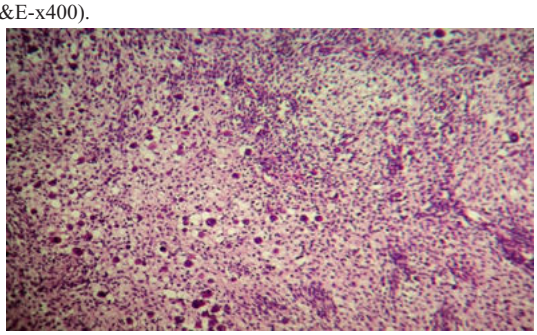
Photomicrograph4 - showing blastemal and stromal component in the form of spindle shaped cells in case of a biphasic wilms' tumour (H&E-x400).



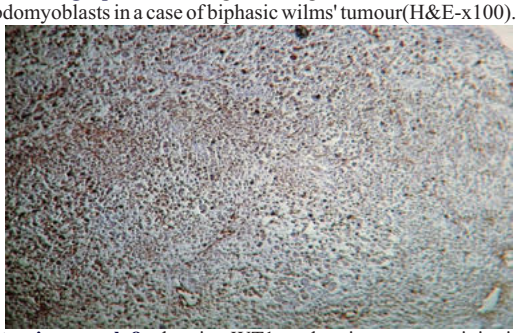
Photomicrograph5- showing epithelial component in the form of immature tubules in case of a triphasic wilms' tumour(H&E-x400).



Photomicrograph 6- showing a combination of blastema, stroma and epithelial tubular formations in a case of triphasic wilms' tumour (H&E-x400).



Photomicrograph 7 - showing heterologous elements in the form of rhabdomyoblasts in a case of biphasic wilms' tumour(H&E-x100).



Photomicrograph 8- showing WT1 nuclear immunoreactivity in the blastemal cells in case of a biphasic wilms' tumour.(x100).

DISCUSSION:

In our study Wilms' Tumour was found to be in the age group ranging from 2 months- 11 years. The result of our study almost corroborate with that of Gerald et al [20] whose study result showed Wilms' Tumour in the range of 1 year- 10 years age group.

The mean age of presentation for Wilms' Tumour in our study was 42months. The studies done by Mazumdar et al [21] and Meher K et al [22] found mean age of presentation of Wilms' Tumour at 49 months and 40 months respectively. Among the 33 cases, 21 (64%) were male and 12 (36%) cases were female with a M:F ratio being 1.75:1. Similar findings were noted in the study results of Paul et al [23] and Mazumdar et al [21] who found male preponderance amongst Wilms' Tumour cases with M:F ratio of 2:1 and 1.8:1 respectively.

Most (81.8%) cases in our study presented with abdominal mass followed by abdominal pain (12.1%) and haematuria (6.1%). None of the patients showed any associated features or conginetal anamoly. In the study of Chen et al [16], the most frequent clinical symptom was abdominal mass (80%), followed by abdominal pain (4%) and haematuria (16%). This study result almost corroborates with that of our observations. Whereas in case of Mazumdar et al [21], the found higher number of cases (55%) presenting with abdominal pain.

In our study we found no associated conditions to be present with any cases of Wilms' Tumour. This finding is in concordance with the findings of Chen et al [16] and Mazumdar et al [21] where they also did not find any associated conditions to be present with Wilms' Tumour. This could be explained by a relatively smaller sample size.

Histopathological results showed triphasic pattern (causes having epithelial, blastemal and stromal component) in n= 24 (72.7%) cases followed by biphasic pattern in n= 6 (18.2%) cases and monophasic pattern in n= 3 (9.1%) cases. Similar results were found in the study of Paul et al [23] and Mazumdar et al [21] who found triphasic type to be the most common, followed by biphasic type but Goyal et al [24] found triphasic type in 65.8% cases, followed by monophasic type in 31.57% cases.

Heterologous elements in the form of rhabdoid differentiation were found in n= 2 (6.1%) cases. Our finding differed slightly with that of Carpentieri et al [15] and Goyal et al [24] who found heterologous elements in 20.83% and 20.05% of their cases respectively.

All (n= 33) cases in our study were of favorable histology with no cases showing presence of anaplasia. Similar findings were seen in the study results of Chen et al [16] and Mazumdar et al [21] but Choi et al [26] found anaplasia in 9.3% of their cases.

Immunohistochemically WT1 nuclear positivity was noted in 31 out of 33 Wilms' Tumour (93.9%) cases. WT1 positivity was mainly noted in the blastemal and epithelial components of Wilms' Tumour while stromal components showing fewer occasional nuclear positivity. Charles et al [25] and Goyal et al [24] in their study found WT1 positivity in 83.3% and 100% cases of Wilms' Tumour. Our study results almost corroborate with these findings.

CONCLUSION:

Wilms' Tumour (Nephroblastoma) is the most common renal tumour of childhood with a relatively good prognosis if detected early. From this study we conclude that the patients presented chiefly with painless abdominal lump in the age group of 1 year- 6 years with a male preponderance. Triphasic type was the most common histologic subtype in Wilms' Tumour. WT1 staining was predominantly noted in the Blastemal and Epithelial components of Wilms' Tumour. Immunohistochemically WT1 immunostaining was demonstrated in 93.8% (n= 31/33) of Wilms' Tumour. Thus we conclude that WT1 immunostaining may be useful in early and correct diagnosis of Wilms' Tumour and thus help in better management and aid further improvement in prognosis of Wilms' Tumour.

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