ORIGINAL ARTICLE

Significance of Immunohistochemistry in Diagnosis of Wilms Tumor and Neuroblastoma in Core Needle Biopsies of Pediatric Population

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ABSTRACT

Background: Neuroblastoma (NBL) is the commonest extra-cranial neoplasm in the pediatric population. Clinically it can present as an abdominal mass, but is usually metastatic at the time of diagnosis so the clinical features can vary. Nephroblastoma, also more commonly known as Wilms tumor (WT), presents as abdominal mass. The early diagnosis and treatment of these tumours is necessary to have a better prognosis.
Aim: To determine the frequency of positivity of Wilms tumor 1 gene (WT1) and neuron specific enolase (NSE) in WT and NBL in order to differentiate between them in the core needle biopsies of paediatric population.
Methods: It was a Cross sectional study and was completed at Histopathology Section, Pathology Department, Children's Hospital & Institute of Child Health, Lahore. Using non-probability consecutive technique, 100 biopsy specimens fulfilling the inclusion criteria were taken. The requisition forms sent from the Oncology, Surgery, Urology and Nephrology departments were collected along with other relevant investigations. The clinical parameters like age, gender and provisional diagnosis made by the clinicians were recorded.
Results: The mean age of children was 3.15 ± 2.14 years with 56% male and 44% female patients. After the confirmation of the diagnosis by immunomarkers, 55% was diagnosed as WT, whereas 45% of the population was labelled as NBL.
Conclusion: In this study 55% children were diagnosed as WT on WT1 and rests of 45% children were diagnosed as NBL on NSE. So in future we can rely on these immunohistochemical markers on core biopsies for undifferentiated abdominal tumors for the definitive diagnosis and early treatment for better prognosis and higher survival rate of these patients.
Keywords: Malignant Tumors, Immunohistochemistry, Round Blue Cell Tumor, Neuroblastoma, Wilms Tumor.

INTRODUCTION

Malignant tumors in children are considered as the second most important cause of death under 15 years of age; However, Childhood cancer accounts for less than 1% of all malignancies[1].

There has been a marked variation in the prevalence of pediatric malignant neoplasms worldwide. In developing countries, these tumors vary from 69.75 cases per million in Indian population to 166 cases per million in Uganda[2].

In Pakistan, the accurate incidence of malignant tumors in children is unknown due to very few cancer registering bodies. However, according to Shaukat Khannum Memorial Hospital Cancer registering data, malignant tumors constitute about 11.44% in children in the last fourteen years[3].

As the name indicates, malignant small round cell tumours (MSRCT) is a group of neoplasms characterized by small, round, relatively undifferentiated primitive cells[4-6]. The primitive morphological features of MSRCT become a diagnostic challenge; thereby requiring immunohisto-chemistry (IHC) and molecular studies[4-8].

NBL and WT represent two of the most common solid tumours of pediatric age group[9].

NBL, being the commonest extracranial malignant tumour of childhood, constitutes 7% of malignant neoplasms in patients under the age of 15 years with a male predominance[10]. NSE, which is significantly specific for neurons and neuroendocrine cells, is an isoenzyme of the glycolytic enzyme, enolase[4] shows positive staining in NBL in 38-95% cases[11].

Renal tumors comprise almost 6% of all malignancies in children less than 15 years of age with a wide spectrum of morphological and histopathological types. WT also known as nephroblastoma or renal embryoma accounts for 95% of all malignant neoplasms of the kidney. The peak age of presentation is between 3-4 years with no sex predilection[12].

WT shows a variety of morphological patterns including blastemal or epithelial-predominant, mixed blastemal, epithelial and sarcomatous types. Blastema-predominant WT mimics NBL clinically as well as cytologically[4]. According to National Wilms Tumor Study group two to three cases of neuroblastoma are registered as blastemal predominant WT every year[13].

The WT1 gene, is a transcriptional regulator, and well known as a tumor suppressor gene located at 11p13, is involved in the development of WT[4,14,15]. In addition, WT-1 is a helpful marker in the differentiating between certain epithelial and mesenchymal tumors[15].

Blastemal component shows positive nuclear staining in 70-100% cases of WT[11].

Among the RBCT, NBL and WT are two important and common malignant neoplasms whose diagnosis on core biopsies is always a challenge to histopathologists as there is marked difference in the treatment as well as the prognosis. There are few studies conducted on childhood RBCT internationally with NBL and WT.[6] In addition it was

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observed that in NBL & WT, there is a wide range of positivity to NSE ranging from 38-95% and WT1 ranging from 70-100% [11]. No such studies have been done in Pakistan. Thus the present study will be done to differentiate between NBL and WT on the basis of immunohistochemistry on core biopsies of undifferentiated abdominal tumors for the definitive diagnosis and rate of positivity of WT1 and NSE in theses tumors in Pakistani pediatric population.

The objective of the study was to determine the frequency of positivity of WT1 and NSE to differentiate between WT and NBL in the paediatric population on needle core biopsies.

MATERIAL AND METHODS

This was a retrospective Cross sectional analysis of abdominal tumors with undifferentiated histology based on H & E received in the duration of two years from 2017 till date at the Histopathology Department of Children's Hospital & Institute of Child Health, Lahore. Data regarding age, size and tumor histology were collected from medical records. Routine H/E staining was performed. Children of less than 9 years were included in the study while biopsies of patients more than 9 years or core biopsy of less than 3mm were excluded from the study.

The surgical biopsies were fixed in 10% buffered formalin and embedded in paraffin according to standard procedures. Serial sections (2-4µm in thickness) were used for H&E and subsequently, WT1 and NSE. The technique was based on PAP (peroxidase antiperoxidase) method by using monoclonal antibodies.

In the present study, nuclear staining for WT1 and cytoplasmic staining for NSE in 10% of tumor cell population was considered as positive staining.

RESULTS

Of the total of 100 children recruited in the study, the mean age of children was 3.15± 2.14 years with minimum and maximum reported ages of almost one month (27 days) and 9 years respectively. 53% were <3 years old, whereas 37% were 3-6 years old and only 10% were 7-9 years old. Moreover, among these 56% were males and 44% were female children.

When diagnosis was made using WT1, 55% children were found having WT (Fig 1, 2), whereas while using NSE, 45% children were found having NBL (Fig 3,4).

Among 53 children under the age of 3 years, 33 (73.3%) had NBL and 20 (36.36%) had WT. Contrarily, among 37 children in age category of 3-6 years, 26 (47.2%) had WT and 11 (24.4%) had NBL. Among 10 children in age category of 7-9 years, 9 (16.36%) had WT and 1 (2.22%) had NBL. There was a highly significant association between age groups and type of tumor found (p-value<0.001) Table 1.

Among 56 male children, 32 (57.1%) had WT and 24 (42.9%) had NBL. Among 44 female children, 23 (52.3%) had WT and 21 (47.7%) had NBL. No association between gender and type of tumor found statistically (p-value<0.627) Table 2.

Fig. 1: Photomicrograph of H&E staining in Wilms Tumor showing predominantly epithelial component X 20

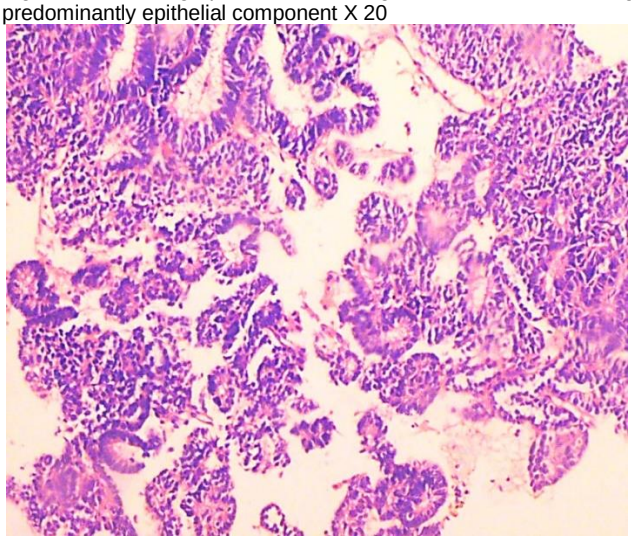

Fig. 2: Photomicrograph of positive nuclear staining by WT1 in epithelial component of Wilms tumor X 20

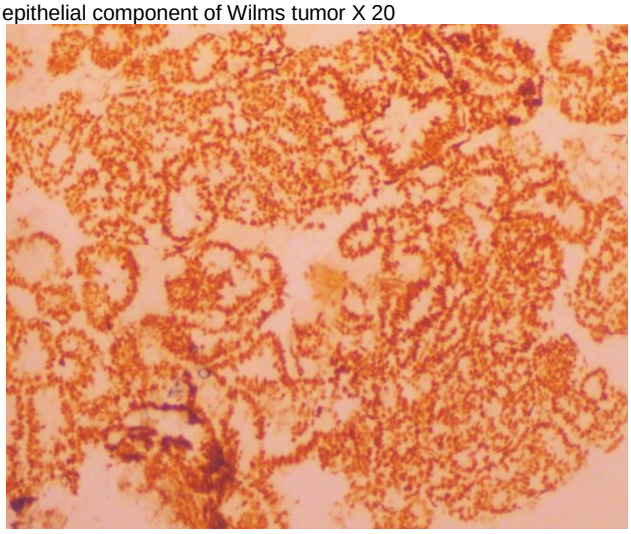

Fig 3: Photomicrograph of H&E staining in Neuroblastoma showing neuropil in addition to round blue cells x 40

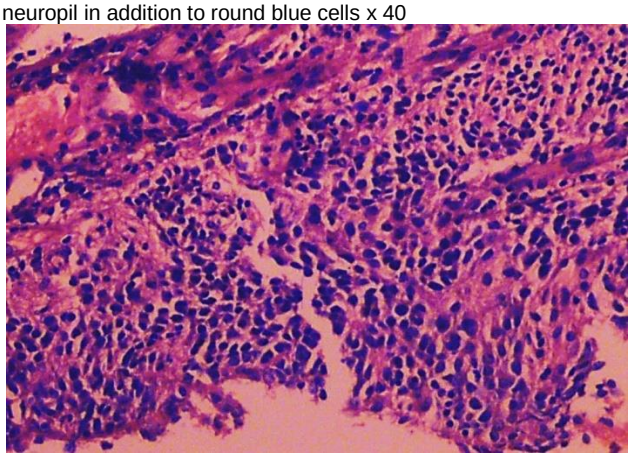

Fig. 4: Photomicrograph of positive staining by NSE in NBL X 10

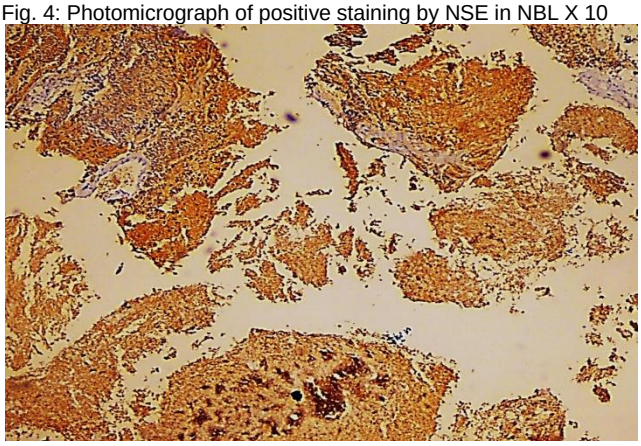

Table 1: Comparison of final diagnosis with respect to age groups

Age group (yrs)	Final Diagnosis		Total
	WT on WT1	NBL on NSE	
<3 years	20(36.36%)	33(73.3%)	53(100%)
3-6	26(47.2%)	11(24.4%)	37(100%)
7-9	9(16.36%)	1(2.22%)	10(100%)
Total	55(55%)	45(45%)	100(100%)

Pvalue < 0.001

Table 2: Final diagnosis with respect to gender

Gender	Final Diagnosis		Total
	WT on WT1	NBL on NSE	
Male	32(57.1%)	24(42.9%)	56(100%)
Female	23(52.3%)	21(47.7%)	44(100%)
Total	55(55%)	45(45%)	100(100%)

Pvalue = 0.627

DISCUSSION

Pediatric solid malignant neoplasms (PSMNs) are a global problem[2]. In the developing world, the prevalence of pediatric neoplasms has been reported from 4.38% to 12.6% of all tumors, while in developed countries, neoplasms in children constitute about 2% of all malignant tumors. The significantly increased number of malignant tumors in children in the developing countries can be attributed to increased overall pediatric population. Approximately 39% of the total population of developing countries comprises of children under the age of 15 years while in western countries the pediatric age group constitutes only 23% of the total population[16].

Malignant tumors remain a great challenge for paediatricians, being a significant cause of childhood mortality. Not only a careful clinical management of those children diagnosed with this problem needs to be conducted, also repetitive epidemiological surveys to see the incidence and prevalence of these cancers must be taken in order to maintain the preventive quality and upgrade where needed[17].

WT is the commonest type of renal tumors with increasing incidence [18] and occurs in approximately 1:10,000 persons, [19] in usually less than 15 years of age.[20] The prevalence is slightly more for the Blacks as compared to Whites, but are only half as great among Asians. [20] Several case-control studies have reached to a conclusion that paternal occupational or maternal hormonal exposures during pregnancy may increase the risk of WT, however small numbers of patients and inconsistencies in the patterns of exposures is an important limiting factor to finalize the eitiology[20] .

In our study, the frequency of WT was seen in 55% children. In Pakistan the incidence of renal tumors is mainly derived from hospital data. Moazam et al, at AKUH showed that WT (28%) was the most common tumor. [21] In our study most of them (47.2%) were in the age group of 3-6 years, whereas 36.36% children of <3years age were diagnosed as WT. Only 16% of the children were in the age bracket of 7-9years, (Table 1). Also, predominantly male children 32 (57.1%) and 23 (52.3%) females had WT in our study (Table 2). The age range in patients with nephroblastoma is reported from under 3 months to 178 months of age. Stones et al noticed in their study, the youngest patient was less than 1 month of age while the oldest was 178 months of age[22].

WT presents at the age of approximately 38 months in the U.S. National Wilms Tumor Study series, with approximately 6 months later presentation in girls than boys. Patients usually present earlier having bilateral tumors, aniridia, cryptorchism/ hypospadias, Beck-with-Wiedemann syndrome, or intralobar nephrogenic rests with a median of 17–27 months. Those having familial disease or multicentric tumors have intermediate age-at-onset, whereas those with perilobar nephrogenic rests are diagnosed at older ages[20].

NBL is one of the other commonest solid tumors in children below the age of five years. The etiology remains unknown, although it has been associated with several other genetic factors. The prevalence of the disease is 1/100,000 children. Clinical features are variable according to the age of patient, the stage of the tumor, metastases and the secretion of several metabolic products by the neolplasm.[23] Stiller et al presented results of NB with help of The International Agency for Research on Cancer that coordinated a worldwide study of childhood cancer incidence, with data from more than 50 countries. They concluded that, the age-standardized rate was 7–12 per million in predominately white Caucasian population, with 6–10% among all childhood malignant neoplasms were, NBLs. Rates were maximum in the first year of life (25–50 per million, 30% of total NBL), and decreased with age to 15–20 per million at age 1–4 years, 2–4 per million at 5–9 years and 1–1.5 per million at 10–14 years[24].

They also observed that in West Asian children, NBL had a relatively lower frequency, suggesting that the prevalence is low. Rates were also low throughout much of southern and eastern Asian pediatric population, including India and China. Although in Japanese children, the prevalence was quite higher, with decreased frequency in Western countries, with the deficit most pronounced in the first year of life. In the United States, white children were more frequently involved (11.5 per million) as compare to the black (8.5 per million). However, black children were older than Whites at the time of diagnosis. In Africa, the highest rate was observed in Bulawayo, Zimbabwe (8.0 per million) whereas the lowest was observed in West Nile, Uganda[24].

In the present study, we observed that 45% children were diagnosed as NBL. Moreover, majority 33(73.37%) of the children were under the age of 3 years, whereas 11(24.4%) were in the age group of 3-6 years. In addition, there was only one (2.22%) child in age category of 7-9 years (Table 1). From the Childhood Cancer Registry in Japan, the prevalence of NBL was 1 among 100,000-150,000 children below the age of 15 years; amongst them, 25% were under 1 year of age. But, mass-screening for this tumor in infancy showed incidence of detection of 1:5,000 babies.[25] An epidemiological study reports about recent birth cohorts compiled in Denmark that show 1 in 7,000 live births have probability of developing NBL before 15 years of age. If 65% of all childhood NBLs could be detected at or before the age of 6 months, then the expected prevalence by screening would be 1/11,000. One can speculate that 1/18,000 live births might possibly benefit from screening at age 6 months, since 59% of these children had tumors in stages III and IV diagnosed after the age of 6 months[26].

CONCLUSION

Accurate diagnosis and classification of MSRCT of children has become critically important for therapeutic purpose. Ancillary diagnostic techniques like immunohistochemistry have emerged as the most valuable adjunct to routine H&E staining and are extremely useful in diagnosing undifferentiated tumors. However, despite advances in immunohistochemistry, cytogenetics and other molecular techniques must also be used to get aid in delineating the entities of small round cell tumors of childhood.

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