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Original Research Article

Predicting outcome of neuroblastoma using N-myc status and Trk-A expression

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ABSTRACT

Background: Neuroblastoma is an embryonal cancer of the postganglionic sympathetic nervous system. It is the third most common pediatric cancer. The aim of the present study was to determine MYCN amplification and Trk-A expression in tissue samples of neuroblastoma cases and to correlate them with clinical status, stage and histopathology of the disease.
Methods: This prospective study was conducted at the Institute of Child Health and hospital for children [ICH & HC], Egmore during the period from June 2011 to March 2012. Ten children of age between 8 months to 12 years diagnosed with neuroblastoma were included in the study. Tissue samples were collected from all patients and sent to evaluate histopathology to confirm the presence of neuroblastoma. Gene expression was studied using TaqMan quantitative RT-PCR. Immunohistochemistry of tissues samples were done to evaluate N-myc amplification and Trk A expression.
Results: The most common presenting symptom was mass in the abdomen (60%) in the patients. In majority, stage 3 neuroblastoma was noticed in 5 (50%) cases. On histopathology, 2 (20%) cases were identified of ganglioneuro-blastoma, and 8 (80%) cases as neuroblastoma. N-myc was amplified in 3 cases (30%). No amplification was noted in all 3 cases (0%) of stage 1. None of the case in this study group showed Trk-A expression.
Conclusions: N-myc amplification was well correlated with the stages of neuroblastoma. It should be considered to be as an important prognostic marker to select appropriate treatment in children with neuroblastomas.

Keywords: Neuroblastoma, Children, N-myc amplification, Trk-A expression

INTRODUCTION

Neuroblastoma is a malignant tumour occur in neuro crest region. It is the common solid tumour (8-10%) seen in children of age group below 5 years.[1] Neuroblastoma is responsible for 10% of chilhood tumours and 15% of all cancer deaths. About 40% of cases are diagnosed by age 1 year, 75% by 7 years, 98% by 10 years.[2] Stage of the tumour and age at the time of diagnosis will predicts the outcome. Prognostically important biomarkers to diagnose the disease are cellular DNA content, N-myc amplification (NMA), deletion of the short arm of chromosome 1 and TRK-A mRNA expression.[3-6] Among them, only N-myc amplification are routinely determined at diagnosis.

N-myc amplification is usually determined by Southern blot or dot blot analysis.[4,7] These methods require large amounts of DNA and radiolabeled plasmid probes and are time consuming. Recently, polymerase chain reaction (PCR) has been introduced for the determination of N-myc amplification.[8] This procedure requires less DNA,

which is of specific concern in small tumor samples obtained by fine-needle aspiration or from bone marrow, and does not require 32P-radiolabeled probes.

The current study was done to evaluate MYCN amplification and Trk-A expression in tissue samples of neuroblastoma cases and to correlate them with clinical status, stage and histopathology of the disease.

METHODS

This was a prospective study conducted on children diagnosed with neuroblastoma at the Institute of Child Health and hospital for children [ICH & HC], Egmore during the period from June 2011 to March 2012. Only new cases of neuroblastoma who have never received chemotherapy before were included in the study. Cases of neuroblastoma who are on chemotherapy or completed chemotherapy were excluded.

After getting approval from Institutional ethics committee and informed consent form the patients or from parents, both boys and girls between the age group 8 months to 12 years were included in the study. Patient's clinical data with any family history of cancer were collected. All base line investigations were carried out. Special investigations like 24 hours urine VMA and bone marrow aspiration cytology were done. Ultra sound and CT scan of abdomen were done to document the size and primary status of the disease. Tissue samples were taken at the time of open biopsy for histopathological diagnosis. Tissue samples were stored in special preservative solution. All the tumour samples were transported on ice to Genetics Department for the isolation of DNA and RNA. Gene expression was studied using TaqMan quantitative RT-PCR. Once neuroblastoma was confirmed by histopathology or immunohistochemistry tissues samples were sent to genetics laboratory to evaluate N-myc amplification and Trk A expression. All the patients received cisplatin (100 mg/m.sq. in divided doses), vincristine (1.5 mg/m.sq. stat), and cyclophosphamide (1000 mg/m.sq. in divided doses D1-D3).

All the observations were expressed in number and percentages by using Microsoft excel.

RESULTS

This study conducted at ICH & HC is a prospective pilot study done between June 2011 and March 2012 and it encompasses a total of 14 children who were diagnosed to have neuroblastoma, of which 4 cases were not included in the study. Of them, 2 cases were diagnosed only based on immunohistochemistry (Synaptophysin, S-100 positive) from bone marrow aspirate which showed small round cells and no tissue was available for genetic study. One of which had meningeal deposits and bone marrow positive, from which tissue sample could not be taken. In the other 2 cases, one was initially diagnosed as rhabdomyosarcoma prostate and chemotherapy was started and the other case tissue sample was inadequate.

In this study group total number of boys was 5 (50%) and the total number of girls was 5 (50%). The youngest age at presentation was 8 months and the oldest child was 12 years. The most common presenting symptom was mass in the abdomen (60%) noted by the parents. Abdominal distension was the next common complaint (20%). Nonspecific complaints like fever, loss of appetite and weight were noted in 2 cases (20%).

Table 1: Clinical characteristics of the study population (n=10).

Characteristics	Number (N)	Percentage (%)
Age in years	1	10
<1	9	90
>1		
Sex		
Boys	5	50
Girls	5	50
Symptoms		
Mass in abdomen	6	60
Abdominal distension	2	20
Non-specific	2	20
Stage		
Stage 1	3	30
Satge 2	0	0
Stage 3	5	50
Stage 4	2	20
Site		
Adrenal	8	80
Paranasal sinus	2	20

Complete abdominal imaging was done in all patients. CT scan showed tumour calcification in 6 cases (60%). The scan helped in 2 cases to distinguish kidney and liver from adrenal mass. It also showed vascular encasement in 7 cases. Repeat CT was done after 4 cycles of chemotherapy to assess tumour in 3 cases and in 2 cases tumour was grossly excised.

The staging was done using the (INSS) International neuroblastoma staging system. In most of the patients, the neuroblastoma was in stage 3 (50%) and the remaining 30% and 20% cases were stage 1 and stage 4 respectively.

On histopathology, 2 (20%) cases were identified of ganglioneuroblastoma, and 8 (80%) cases as neuroblastoma. Among 8 cases of neuroblastoma, 1 (12.5%) showed undifferentiated type.

In this study group of 10 cases, N-myc was amplified in 3 cases (30%) (Table 2). All the 3 cases were above the age group of one year. Two (40%) cases belong to stage 3

and 1 (50%) case belongs to stage 4. Adrenal masses in two cases (25%) and paranasal sinus mass in 1 case (50%) showed N-myc amplification. No amplification was noted in all 3 cases (0%) of stage 1. None of the case in this study group showed Trk A expression.

Table 2: Correlation of N-myc with clinical characteristics of study population (n=10).

Characteristics	Amplified	Unamplified	Total
	N (%)	N (%)	N (%)
Age in years			
<1	0 (0)	1 (100)	1 (10)
>1	3 (33.3)	6 (66.6)	9 (90)
Sex			
Boys	2 (40)	3 (60)	5 (50)
Girls	1 (20)	4 (80)	5 (50)
Stage			
Stage 3	2 (40)	3 (60)	5 (50)
Stage 4	1 (50)	1 (50)	2 (20)
Site			
Adrenal	2 (25)	6 (75)	8 (80)
Paranasal sinus	1 (50)	1 (50)	2 (20)

In this study all the 3 cases of stage 1 tumours were excised completely. Only for one case 6 cycles of chemotherapy was given. All 3 cases are on follow up with no residual mass. Among the 5 cases of stage 3 tumours one had completed surgery and chemotherapy, another one was poor chemo responsive (progressive disease) and the rest responding well to chemotherapy. Among 2 cases of stage 4 tumours one had poor response to chemotherapy and poor general condition. Partial excision was done and now on palliative chemotherapy. The other child is having good response to chemotherapy.

DISCUSSION

ICH & HC is a government run institution and caters to the children belonging to the lower socio economic strata. Majority of parents of children with this disease are illiterate and ignorant of even the common paediatric problems. This is one of the reasons why a good number of our patients in the study presented to us in the advanced stage of the disease.

Most of our children presented with a mass in the abdomen or with abdominal distension, which was either noticed by the parents or by the referring physician. In this study a total of 14 children were diagnosed to have neuroblastoma of which 4 cases were excluded. In this study group of 10 cases the ration of boys and girls was 1:1. The youngest age at presentation was 8 months and the oldest child was 12 years.

In our series 80% of cases were diagnosed with neuroblastoma of adrenal glands and 20% of cases ganglioneuroblastoma. This was in agreement with the studies of Grosfeld et al.[9] In their study, 50% of the cases had adrenal neuroblastoma, 25% cases were diagnosed with paraspinal, 20% with mediastinal and 5% cases with pelvic and neck masses respectively.

The method for N-myc amplification used is the Southern blot which is quantitative, but requires a large amount of tumor DNA that can be only obtained by surgical excision or open biopsy. In the current study PCR techniques was employed for N-myc amplification which requires only small amount of DNA.[1]

In this study group of 10 cases, N-myc were amplified in 3 cases (30%), of which 2 cases belong to stage 3 and 1 case belongs to stage 4. No amplification was noted in all 3 cases (0%) of stage 1. Similarly in a study done by Pratap et al, out of 10 patients, N-myc amplification was in 2 cases belong to stage 3 and in 2 cases out of 16 patients which belongs to stage 4.[10]

CONCLUSION

The findings of the study reinforce the importance of N-myc amplification on prognosis and tumor progression. N-myc amplification technique by PCR helps in the appropriate selection of treatment for neuroblastomas in children.

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