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Comparison of the Initial Treatment Results of N-myc Positive and N-myc Negative Neuroblastoma Patients

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Abstract

Background: Neuroblastoma is the most common extra cranial malignant solid tumor of childhood. Various molecular and cytogenetic factors have been implicated in the pathogenesis of neuroblastoma, some of which have proven useful in predicting clinical behavior. Over expression of the oncogen N-myc, is an important indicator of prognosis.

Materials and Methods: Our study was performed from 2004 to 2008 in Mofid Children's Hospital in Tehran, Iran. In our case control study patients who were diagnosed as neuroblastoma were enrolled. They were checked for N-myc by fluorescence in situ hybridization (FISH) method in tumor tissue. Initially all patients were treated with conventional chemotherapy then were accessed to define their responses.

Results: In our study 18 patients were diagnosed as neuroblastoma. Twelve of them were female and six of them were male. They were 6 month to 6 years old. Eight patients were N-myc positive (case) and ten patients were N-myc negative (control). None of N-myc positive patients responded to conventional chemotherapy, but eight N-myc negative patients responded to conventional chemotherapy. N-myc in neuroblastoma had a significant correlation with the prognosis (PV =0.028).

Conclusion: We suggest conventional chemotherapy for N-myc negative patients and intensive chemotherapy for N-myc positive patients to obtain the best results.

Key words: Neuroblastoma, solid tumor, cancer, childhood, genetics

Introduction

Neuroblastoma is the most common extra cranial malignant solid tumor of childhood [1].The median age at diagnosis is 17.3 months and 40 percent of patients are diagnosed before one year of age [2]. Various molecular and cytogenetic factors have been implicated in the pathogenesis of neuroblastoma [3-4]. N-myc amplification, deletions of 1p chromosome and deletions of 11q chromosome have an important role in choosing treatment protocol [5-7]. N-myc amplification is found in approximately 25 percent of neuroblastomas[8-10]. Patients with diploid tumors characterized by an amplified N-myc locus represent a particularly unfavorable risk group that may benefit from innovative new therapies [4, 11]. Members of the myc gene family, including N-myc, myc, and L-myc, have been found amplified and expressed at high levels in various human cancers [12-14].The complexities in transcription of N-myc expand the means by which expression of the gene might be controlled [15]. Sequences of the short arm of chromosome 2 containing the N-myc oncogen at 2p23-p24 are often involved in DNA amplification [16]. A high expression of N-myc on mRNA level is correlated to the N-myc gene-amplification[17]. Neuroblastoma cells in bone marrow are detected by Southern Blot and in situ hybridization using a N-myc DNA probe [18-19]. N-myc amplified Neuroblastoma cell lines do not express CD44 at all or express a nonfunctional receptor, whereas nonamplified cells constitutively express an active receptor [20]. Fluorescence in situ hybridization (FISH), Southern Blot analysis and Light Cycler monoplex polymerase chain reaction (PCR) are used to assess N-myc[21]. Monoclonal antibodies

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have been developed against the putative N-myc gene product made in Escherichia coli as a fusion protein. Treatment of patients with high risk neuroblastoma (N-myc+) with new protocol have been associated with good results[22-23]. The effect of gamma interferon treatment and distribution of the N-myc protein in the nucleus have also been studied [24].There is an antisense peptide nucleic acid (PNA) targeted against a unique sequence in the terminus of the 5'-UTR of N-myc, designed for selective inhibition of N-myc in neuroblastoma cells[25].We aimed to evaluate the initial treatment results of N-myc positive and N-myc negative neuroblastoma patients undergoing surgery followed by multi-agent moderate intensity chemotherapy. We studied this phenomenon using a case control design.

Materials and Methods

Our study was done from 2004 to 2008 in Mofid Children's Hospital in Tehran, Iran. Eighteen patients who had been diagnosed with partially resectable localized neuroblastoma were enrolled. All patients were diagnosed as neuroblastoma based on clinical findings, imaging studies and laboratory data. They initially underwent surgery for resection of tumor as soon as possible, and to confirm the diagnosis and make a staging state. The surgical treatment was followed by multi-agent moderate intensity chemotherapy. We checked tumor tissue for N-myc using FISH method. Initially patients received six courses of oncovin, carboplatin, etoposide and cyclophosphamide (OPEC) without consideration of N-myc amplification. They were assessed by computerized tomography (CT) scanning after tumor resection and right before starting the chemotherapy. After every three months of chemotherapy and at the end of six courses of chemotherapy patients were assessed again by CT scan to detect the degree of tumor shrinkage. We collected 24 hr urine to measure homovanilic acid and Vanilic Mandelic acid. We also measured serum ferritin and serum ceruloplasmin levels. Bone marrow aspiration, bone marrow biopsy and bone scan were performed to rule out bone and bone marrow metastasis at the start and at the end of therapy. Finally using these assessments we defined if the tumor has responded to chemotherapy or not. Statistical comparison was performed using Fisher exact test.

Results

In our study 18 patients were diagnosed as partially resectable localized tumors. They were 6 months to 6 years old and twelve patients were females and six were males. Microscopic study of tissue samples was compatible with neuroblastoma. All of patients were stage II and stage III. They were divided into two groups; group1 (case) were N-myc positive patients and group2 (control) were N-myc negative patients. The mean age in group 1 and in group 2 were 1.37 and 2.39 respectively. There were eight patients (44 percent) N-myc positive and ten patients (55 percent) N-myc negative. Bone marrow aspiration, bone marrow biopsy and bone scan showed no metastasis at the start and the end of therapy. CT scan studies at the end of chemotherapy in group1 did not show complete remission but in group 2 eight patients (80 percent) showed complete remission. That means all ten N-myc positive patients developed recurrent disease after surgery fallowed by multi-agent moderate intensity chemotherapy. N-myc in neuroblastoma had a significant correlation with the prognosis (P=0.028).

Discussion

The observed overall N-myc amplification prevalence of 44% in our study is higher than other reports of 20 to 25% overall prevalence [26]. In our study none of eight N-myc positive patients responded to conventional chemotherapy after partial surgical resection of tumor which differs from the results of other centers [27-28]. But eight

Table 1: Chemotherapy inducing remission in neuroblastoma patients based on their N-myc status\

	Number	Treatment	Resistance	PV
Case (N-myc+)	8	OPEC	8	0.028
Control (N-myc-)	10	OPEC	2	0.028

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of ten N-myc negative patients (80 percent) responded to conventional chemotherapy which is comparable to the results from other centers [10, 29-30]. Although few patients were referred to our medical center thorough a four years period, the correlation of n-myc with chemotherapy inducing tumor shrinkage was significant. N-myc positive patients were very poor and n-myc negative patients were very good responders to multi-agent moderate intensity chemotherapy. It seems that all neuroblastoma tumor tissues should be checked for N-myc using FISH method after surgery and before starting the chemotherapy. Also we suggest continuing conventional chemotherapy for N-myc negative patients and intensive chemotherapy for N-myc positive patients in the first day of treatment to obtain the best results. N-myc positive patients should be considered for autologous stem cell transplantation after myeloablative chemotherapy and Meta Iodo benzyl guanine in 32MIBG avid patients.

Conclusion

We suggest conventional chemotherapy for N-myc negative patients and intensive chemotherapy for N-myc positive patients to obtain the best results.

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