STUDY ON THE RELATIONSHIP BETWEEN *MYCN* STATUS AND CERTAIN PROGNOSTIC FACTORS IN 131 PATIENTS WITH NEUROBLASTOMA

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ABSTRACT

Neuroblastoma (NBL) is the most common extracranial solid cancer of childhood and is characterized by a remarkable biological heterogeneity, resulting in favorable or unfavorable outcomes. There are many prognostic factors like age, stage, histopathology, plasma and urinary markers and the molecular characteristics of tumor cells. Amplification of the MYCN *gene (MYCNA) is established as the most powerful prognostic factor. This study aimed to investigate, in a series of 131 NBL patients ascertained from 2013 to 2015, the relationship between amplification of MYCN and some other prognostic factors: patient's age, histopathology, VMA/HVA ratio and LDH level. MYCNA was identified by FISH and the* MYCN *status was compared with the other clinic-biological factors. As a result, MYCNA was found on 27/131 NBL patients. The number of MYCNA according to the age group was 9/27 cases below12 months, 4/27 cases 12-18 months and 14/27 cases over 18 months. The favorable and unfavorable histology cases showed a different frequency of MYCNA, 11/24 cases and 13/24 cases, respectively. 20/21 patients with MYCNA have a VMA/HVA ratio below 1, and 17/24 cases of them had LDH level above three times than normal level (both associated with worse prognosis). Therefore, the MYCNA is strongly associated with age at diagnosis >18 months, unfavorable histology, VMA/HVA ratio below 1 and high LDH level. The VMA/HVA ratio and LDH level could be valuable markers for diagnosis and monitoring of disease status, however, the MYCN status determined by FISH is one of the most important tools for treatment stratification.*

Key words: *Neuroblastoma,* MYCN, MYCN *amplification, age at diagnosis, histopathology, biochemistry parameters.*

I. INTRODUCTION

Neuroblastoma, an embryonic tumour of the sympathetic nervous system, is responsible for 15% of cancer- related deaths in childhood [1], [2]. Neuroblastoma is characterized by a remarkable heterogeneity of histology and molecular biology that is reflected in its clinical outcome ranging from spontaneous regression to lethal metastatic disease. The prognosis of neuroblastoma patient previously based on the age at diagnosis, stage, the VMA, HVA, LDH levels and the histopathology [3-6].

Age at diagnosis remains the first independent clinical factor, with infants <1 year of age usually having significantly better disease-free survival [3].

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Received: 29/7/2016;
Revised: 9/8/2016 by Tran Kiem Hao
Accepted: 19/8/2016

Like the age, stage of disease, following the International Neuroblastoma Staging System (INSS), is the most important prognostic factor: stages L1, L2, and MS are considered to have a better prognosis, whereas stageM has a worse prognosis [4]. Besides, some biological factors, such as the catecholamine metabolites, vanillylmandelic acid (VMA) and homovanillic acid (HVA), lactate dehydrogenase (LDH)..., are commonly assessed in children suspected to have neuroblastoma (NB), and the levels of these markers are commonly used for differential diagnosis. The high level of LDH (even not specific for neuroblastoma) or the ratio VMA/HVA <1 is associated with the worst prognosis [6], [7]. Other factor routinely taken into consideration for the prognostic evaluation and treatment of these tumors is the Shimada histopathological classification (favorable versus unfavorable) [5].

Many genetic changes in neuroblastoma, including *MYCN* amplification (MYCNA), have discovered and used mainly for prognosis rather than diagnosis [1]. Of these, *MYCN* amplification is the most important genetic markerin prediction of prognosis and subsequent treatment stratification. Located in chromosome 2p24, amplified *MYCN* gene is seen in 40-50% of high stage tumors (stage M) and uncommonly (5%-10%) in low stage tumors (stage L1, L2, or MS), with the frequency about 20-25% of neuroblastoma tumor.Amplification of *MYCN* has been known for about 30 years to be associated with a poor outcome in neuroblastoma, in which the overall 5-year survival drops to 30% [1], [2].

At the Vietnam National Children's Hospital, there are 40-50 new neuroblastoma cases per year. The detection of MYCNA by fluorescent in-situ hybridization (FISH) technique has been established at the Human Genetic Department for several years. However, until now, the correlation between amplified *MYCN* gene with other clinic-biological factorshas not been analysed therefore, in this study, we investigate the relationship between *MYCN* status and certain prognosis factors in 131 patients with neuroblastoma.

II. MATERIAL AND METHOD

2.1. Material

Neuroblastoma has been confirmed on 131 patients at the Vietnam National Children's Hospital from 2013 to 2015. These patients have had tests on urinaryVMA, HVA and plasma LDH, taken the samples by open or needle core (under untrasound) biopsy for evalution of histopathology and *MYCN* gene.

2.2. Method

Sample

The piece(s) of tumor, used in this study, are collected after the biopsy or fixed in the parraffin.

Fluorescent in-situ hybridization (FISH) technique

The tumor slide has been hybridized with two-colors probe LSI N-MYC (2p24) Spectrum Green/ CEP 2 Spectrum Orange (Vysis) to mapped 2 regions in chromosome 2: N-MYC (2p24) labeled with SpectrumGreen and CEP 2 (2p11.1-q11.1) labeled with SpectrumOrange. The result has been analysed by ISIS software (Metasystem) under the M1 microscope system (Zeiss). The amplification of *MYCN* gene have been detected in the case of more than 10 green signals per cell (scattered or localized mass of signals).

Other laboratory tests

The VMA, HVA and LDH have been examined by gas chromatography/mass spectrometry at the Biochemistry laboratory [7]

The pathology and Shimada classification of tumor have been analysed in Histology laboratory.

III. RESULT AND DISCUSSION

The MYCN amplification

131 patients with neuroblastoma have had their *MYCN* status evaluated at Human Genetics Department. There are 27/131 amplified cases, about 21% of the cases. Our proportion have been similar with the publications over the world [1, 2]. Figure 1 illustrate the diffirence in microscopic fluorescent signals between amplified and not-amplified *MYCN* gene.

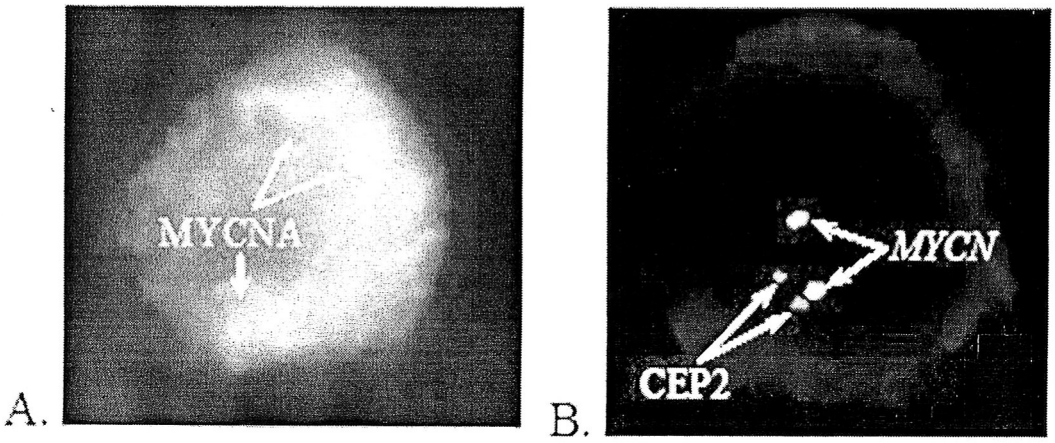

Figure 1. A. MYCN amplification (MYCNA); B. MYCN not-amplification (MYCNN)

The relationship between the MYCNA and the age at diagnosis

We divided our patients in to 3 groups: below 12 months, 12-18 months and over 18 months, correspondent to the diverse outcomes.

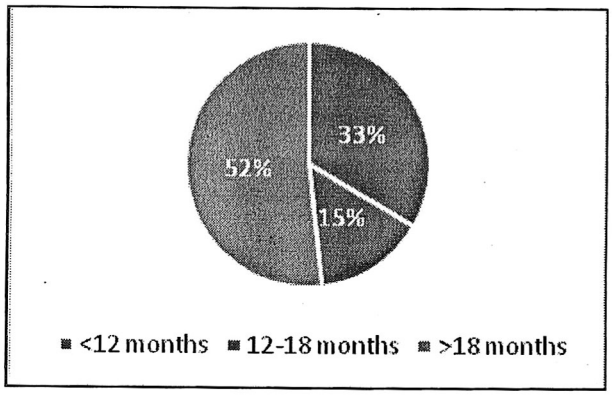

Figure 2. The proportion of MYCN amplification in age groups

The patients of over 18 months groups are often associated with the worse prognosis. In our study, the percentage of MYCNA in this group is 52%, higher than that of other age groups (Figure 2).This result is equivalent to the study of Pedram et al. (2013), which showed the >2,5 years group have the largest number of MYCNA [8].

The relationship between the MYCNA and the histopathology

The pathology result has either diagnosed the neuroblastoma patients or given the prognosis and the stratification information. In 131 patients, 119 cases have been classified following the Shimada histopathological system, favorable histology (FH) versus unfavorable histology (UH).

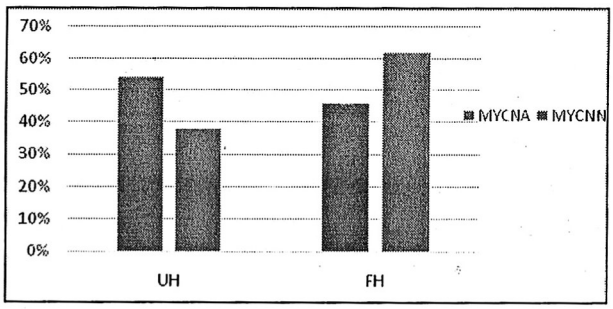

Figure 3. The relationship between the MYCN status and the histopathological classification

Of these, the worse prognosis value belongs to the UH group. Our genetic profile of *MYCN* gene have not only supported for this finding (figure 3), but also equaled the results of George et al. (2001) [9].

The relationship between the MYCNA and the certain biological factors

The clinical doctors usually utilize the certain biochemical factors, like unary HVA, VMA or plasma LDH... as the useful and cost-effective tools for differential diagnosis and following treatment. These factors strongly corelate with the *MYCN* amplification, such as the ratio VMA/HVA below 1 and the high level of plasma LDH found in worse prognosis or/and advanced stage neuroblastoma patients. 114 patients have the unary HVA/VMA examination and 118 patients have the plasma LDH test in our study.

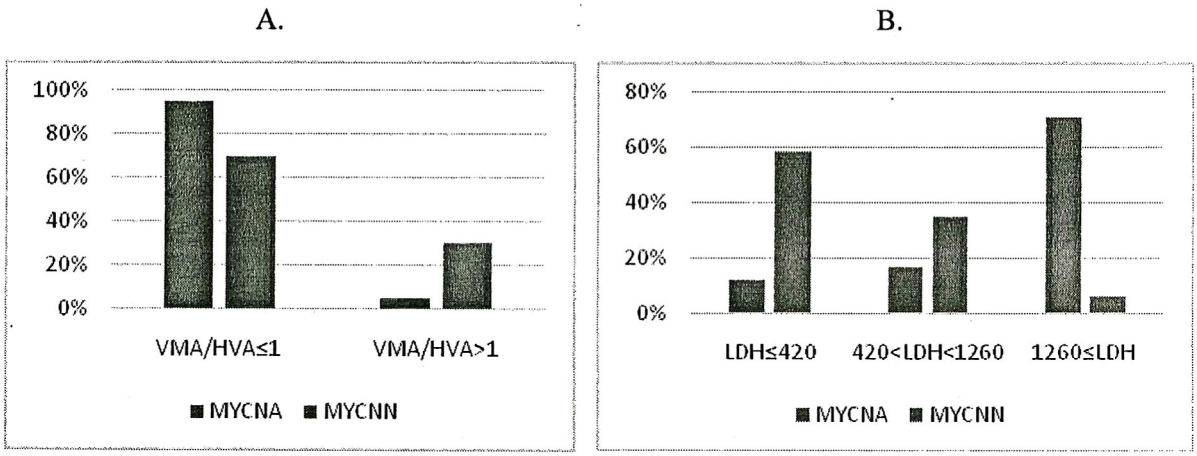

Figure 4. The relationship between the MYCN status and the ration VMA/HVA (A) and LDH (B)

In comparison with *MYCN* status, most of such patients was exposed with the *MYCN* amplification.Specifically, nearly 100% patients with VMA/HVA ratio <1 and approximately 71% oneswith LDH level higher 1260 IU/mL ones are the amplified *MYCN* gene cases (Figure 4). This rate is consistent with the study in 2002 by Eduardo Zambrano [10].

IV. CONCLUSION

The amplification of *MYCN* is strongly associated with worse prognosis factors, like age >18 months, stage M, unfavourable histology, VMA/HVA ratio below 1 and LDH level above 3 times than normal. The other prognosis factors could be easily accessed, however, the *MYCN* status determined by FISH is one of the most important tools for treatment stratification.

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