BIOCHEMICAL PARAMETERS IN NEUROBLASTOMA

S T Bolkar, M S Ghadge and A S Raste

Department of Biochemistry, Tata Memorial Hospital, Parel, Mumbai-400 012.

ABSTRACT

Apart from the biochemical parameters routinely used like Vanillyl Mandelic Acid for the patients with neuroblastoma the parameters like neuron specific enolase, ferritin, lactate dehydrogenase, gamma glutamyl transferase were also studied to assess the utility in diagnosing the patients with neuroblastoma. The study involved 40 healthy ambulatory subjects and 30 untreated cases of histologically proved neuroblastoma referred to the Tata Memorial Hospital for further management and treatment. The urinary Vanillyl Mandelic Acid levels and the serum levels of neuron specific enolase, ferritin, lactate dehydrogenase had increased significantly, $p < 0.001$ whereas the gamma glutamyl transferase had decreased significantly $p < 0.001$, as compared to the normal. Serum neuron specific enolase, ferritin, lactate dehydrogenase alongwith urinary Vanillyl Mandelic Acid could be of help in diagnosing the patients with neuroblastoma.

KEY WORDS

Biochemical Parameters, Neuroblastoma, Vanillyl Mandelic Acid, Neuron Specific Enolase.

INTRODUCTION

Neuroblastoma is the most common solid tumor in children younger than 5 years of age. Because neuroblastoma has a high propensity to rapidly disseminate and may originate in a variety of sites, the mode of presentation is diverse. In 65% of patients the primary tumor arises in the abdomen, with the adrenal gland being the single common site. Infants tend to have more neck and chest primaries. Most patients present with the consequence of metastatic disease, which may involve bone marrow, bone, liver and skin. The prognosis is age and sex dependent, and there is a significant correlation between age and stage at diagnosis (1). Several studies have assessed the prognostic significance of multiple clinical variables in patients with neuroblastoma (2-4). Reflecting its origin within the sympathetic nervous system the tumor usually secretes dopamine and its chief metabolite, homovanillic acid (HVA), in excess. Dopamine, HVA and vanillyl mandelic acid (VMA), measured in both serum and urine, have been used as tumor

markers for neuroblastoma. Levels are typically raised at the diagnosis and can be used to follow disease activity (5).

Various serum markers including neuron specific enolase (NSE), ferritin (FER), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT) along with the urinary estimation of VMA have been studied which may be a valuable tool in diagnosis and monitoring of disease status.

The present study was undertaken to evaluate the utility of urinary VMA and other markers like NSE, Ferritin, LDH and GGT to assist in the diagnosis of neuroblastoma.

MATERIALS AND METHODS

The material for the study comprised of 40 normal healthy subjects and 30 untreated patients with histologically proven Stage III & IV neuroblastoma referred to Tata Memorial Hospital, Mumbai for investigation and management.

Biochemical parameters like vanillyl mandelic acid, neuron specific enolase, ferritin, lactate dehydrogenase, gamma glutamyl transferase were studied to assess the utility in diagnosing the patients with neuroblastoma. VMA was estimated in 24 hours urine sample. Patients were instructed to keep vanillyl free diet for Total five days (i.e. three days prior to collection and two days during the collection). 5ml of

Address for Correspondence :

Dr. A. S. Raste

Department of Biochemistry,
5th Floor, Annexe Bldg.
Tata Memorial Hospital, Parel,
Mumbai 400 012.
E-mail : asharaste@gmail.com

fasting blood was collected in a plain test tube and was allowed to clot at room temperature. The serum was separated by centrifuging the sample at 4000 rpm for 10 minutes. The separated serum was preserved at -20°C until NSE, FER, LDH and GGT were analyzed.

VMA was estimated by a column method using Bio Rad kit, based on ion-exchange chromatography. VMA is retained by an anionic exchange resin, being eluted thereafter once the interfering substances are washed away. The VMA is quantified spectrophotometrically as vanillin after periodate oxidation under alkaline conditions. NSE was estimated using kit from CanAg diagnostic by the EIA. EIA a one step sandwich assay in which two monoclonal antibodies are directed against two separate antigenic determinants on the γ unit of the NSE molecule. The γ unit is found either in the homologous $\gamma\gamma$ or in the heterologous $\gamma\gamma$ isoenzyme. As the monoclonal antibodies used, bind to the γ subunit of the enzyme, the assay detects both the $\gamma\gamma$ and the $\gamma\gamma$ form. FER was estimated using the kit from Abbott, based on the microparticle enzyme immunoassay (MEIA) technology on AxSYM analyzer. LDH was estimated using kit from Boehringer Mannheim by the enzymatic method using pyruvate which is reduced to lactate (6). GGT was also estimated by the method of Gabor Szasz (7) using L- γ -glutamyl-3-carboxy-4-nitroanilide as substrate. LDH and GGT were estimated on a fully automated random access analyzer Hitachi 717.

RESULTS

The results of the study are tabulated in Table 1. Table 1 gives the levels of VMA, NSE, Ferritin, GGT and LDH in 40 healthy normals and 30 untreated patients with neuroblastoma.

In normals, NSE had a mean of $6.2 \pm 3.5 \mu\text{g/l}$ and range 0-13 $\mu\text{g/l}$, Ferritin had a mean of $89.0 \pm 10.95 \text{ ng/ml}$ and range 10-225 ng/ml , LDH mean levels were $320.27 \pm 70.87 \text{ U/L}$ and range 195-470 U/L , GGT had a mean level of $27.2 \pm 10.8 \text{ U/L}$ and range 5-48 U/L . The mean VMA levels were $3.87 \pm 1.9 \text{ mg/g}$ of creatinine and range 1-10 mg/g of creatinine.

In patients with neuroblastoma, mean serum levels for NSE was $60.84 \pm 47.28 \mu\text{g/l}$ and range 11-140 $\mu\text{g/l}$, Ferritin $427.44 \pm 359.88 \text{ ng/ml}$ and range 11.27-988.0 ng/ml , LDH $674.24 \pm 568.93 \text{ U/L}$ and range 84-1992 U/L , GGT 29.21 ± 18.72 and range 7.6-89.4 U/L and urinary VMA $51.4 \pm 45.43 \text{ mg/g}$ of creatinine and range 7.9-153.1 mg/g of creatinine.

The levels of NSE, Ferritin were significantly elevated ($p < 0.001$) which is in confirmation with the findings of

Table 1 : Gives the mean, standard deviation and range of VMA, NSE, Ferritin, LDH and GGT in normals and untreated patients with neuroblastoma (values - Ranges & Mean $\pm$ SD)

Group Tests	Normal (n = 40)	Untreated Patients with Neuroblastoma (n = 30)
VMA (mg/g of creatinine)	1-10 3.87 ± 1.9	7.9 -153.1 $51.4 \pm 45.43^*$
NSE ($\mu\text{g/l}$)	0-13 6.2 ± 3.5	11-40 $60.84 \pm 47.28^*$
Ferritin (ng/ml)	10-225 89.0 ± 10.95	11.27-988.0 $427.44 \pm 359.88^*$
LDH (U/L)	195-470 320.27 ± 70.87	84-1992 $674.24 \pm 568.93^*$
GGT (U/L)	5-48 27.2 ± 10.8	7.6-89.4 29.21 ± 18.72

* $p < 0.001$

Schleiermacher (8). NSE has been found in tumor tissue specimens of all neurocrine derived malignancies tested (9), but abnormal serum levels have been associated with only small-cell carcinomas (10) and neuroblastomas (11).

Serum LDH levels correlate with disease activity and pretreatment values of more than 1000 U/L are associated with a worse prognosis (4). Our findings reveal that the LDH levels were elevated significantly as compared to normal ($p < 0.001$).

Urinary catecholamine secretion is increased in neuroblastoma and is a confirmatory diagnostic marker. Our study too reveals that urinary VMA levels are elevated significantly as compared to the normals ($p < 0.001$) in patients with neuroblastoma. However, serum GGT did not show any change as compared to normals.

A variety of serum or urinary markers have been used to assist in the diagnosis of neuroblastoma and to follow response to therapy. The conventional markers for neuroblastoma are urinary catecholamine metabolites VMA and HVA (12). The catecholamines and their metabolites are characteristic, sufficiently increased to be useful and broadly available; however 24 hours urinary collection is tedious and cumbersome. Several studies have assessed the prognostic significance of multiple clinical variables in patients with neuroblastoma (2-4). However, none has assessed all of the afore mentioned clinical and biologic variables in a large group of patients, so it is difficult to determine which are the most powerful. It appears that selected serum factors - ferritin, NSE

and LDH as well as tumor histology and certain genetic features are the most useful and widely accepted diagnostic indicators in children with neuroblastoma at present.

REFERENCES

1. Chauvin F, Mathieu P, Frappaz D, Lasset C, Favrot MC, Greffe J, et al. Screening for neuroblastoma in France: Methodological aspects and preliminary observations. *Med Paed Oncol* 1997; 28: 81-91.
2. Berthold F, Harms D, Lampert F, Niethammer D, Zieschang J. Risk factors in neuroblastoma in infants. *Contrib Oncol* 1990; 41: 101 – 17.
3. Carlsen NLT. Prognostic factors in neuroblastoma of childhood. Current problems. *Paediatr Rev Commun* 1991; 6 : 63 – 77.
4. Berthold F, Trechow R, Utsch S, Zieschang J. Prognostic factors in metastatic neuroblastomas. A multivariate analysis of 182 cases. *Am J Pediatr Hematol Oncol* 1992; 14: 207-15.
5. LaBrosse EH, Com-Nougue C, Zucker JM, Comoy E, Bohuon C, Lemerie J, et al. Urinary excretion of 3-methoxy-4- hydroxyl phenylacetic acid by 288 patients with neuroblastoma and related neural crest tumors. *Cancer Res* 1980; 40: 1995-2001.
6. Anon : Z Klin Chem Biochem (Quoted from the application notes of Boehringer Mannheim for Hitachi 705) 1970; 8: 658.
7. Szasz G. A Kinetic photometric method for serum gamma glutamyl transpeptidase. *Clin Chem* 1976; 15:124-36.
8. Schleiermacher GH, Rubie H, Hartman O, Bergeron C, Chastagner P, Mechinaud F, et al. Treatment of stage 4s neuroblastoma - report of 10 years experience of the French Society of Paediatric Oncology 2003; 89 : 470-76.
9. Tapia FJ, Polak JM, Barbosa AJA, Bloom SR, Marangos PJ, Dermody C, et al. Neuron-specific enolase is produced by neuroendocrine tumors. *Lancet* 1981; i: 808-11.
10. Carney DN, Marangos PJ, Ihde DC, Bunn PA Jr, Cohen MH, Minna JD, et al. Serum neuron-specific enolase a marker for disease extent and response to therapy of small cell lung cancer. *Lancet* 1982; i: 583-86.
11. Ishiguro Y, Katu K, Shinmizu A, Ita T, Nagaya M. High levels of immunoreactive nervous system-specific enolase in sera of patients with neuroblastoma. *Clin Chim Acta* 1982; 121:173-80.
12. Bertani – Dziedzic L, Dziedzic SW, Gitlow SE. Catecholamine metabolism in neuroblastoma in Pochedly C (ed): *Neuroblastoma: Tumor Biology and Therapy* Boca Raton Fl. CRC, 1990; 69-91.