Ischemia-Modified Albumin as a Biochemical Marker in Children with Neuroblastoma and Soft Tissue Sarcomas

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In this study, the levels of ischemia-modified albumin (IMA) in pediatric oncology patients with soft tissue sarcomas (STSs) and neuroblastoma (NB) were analyzed. To date, there have been no studies concerning IMA in these groups of patients. Ninety-nine children with STSs and NB were analyzed from 2006 to 2009, and 30 healthy children were also enrolled in the study. IMA levels were measured throughout treatment in all patients. The levels of IMA in all cancer patients (mean 116.8 ± 39.3 U/ml), in patients with STSs

(mean 119.8 ± 27.5 U/ml), and in patients with NB (mean 114.6 ± 36.6 U/ml) were significantly higher than in the control patients (mean 87.3 ± 38.3 U/ml; $P = 0.0013$, 0.0066 , and 0.0164 , respectively). IMA levels increased before and during the treatment compared with levels in the controls. The determination of IMA levels in pediatric oncology patients with poor prognoses from STSs and NB may play an important role in predicting response to therapy and overall outcome. *J. Clin. Lab. Anal.* 25:255–258, 2011. © 2011 Wiley-Liss, Inc.

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INTRODUCTION

Reactive oxygen species (ROS), which are produced during normal aerobic metabolism and increase the level of oxidative stress, have been of interest to researchers for many years. Free radicals are thought to be necessary for life and to regulate basic functions; however, in high concentrations, they can be cytotoxic (1). ROS may provoke cellular damage, cell death, mutations, and chromosomal aberrations, and may induce carcinogenesis (2–4). Whether oxidative stress associated with cancer is due to increased production of free radicals or is associated with damage to the antioxidant system remains unclear (5).

In recent years, several studies have described the role of ischemia-modified albumin (IMA) as a new marker for diseases linked to inflammation. The generation of ROS can transiently modify the N-terminal region of albumin and produce an increase in the concentration of IMA (6,7). IMA may represent a sensitive biochemical marker, especially for the diagnosis of myocardial ischemia (8). Several reports demonstrate that ischemia

induces a cascade of proinflammatory reactions that lead to the production of ROS (9,10). IMA is therefore considered a marker of ischemia and oxidative stress originating as a consequence of tissue hypoxia (11–14). There have also been reports describing the relationship between cancer and inflammation. This relationship may involve both an extrinsic and an intrinsic pathway. The extrinsic pathway is related to inflammatory conditions, which increase cancer risk, whereas the intrinsic pathway is driven by genetic changes that cause inflammation, which may also induce oncogenesis (15).

Only one study was found in the literature concerning the role of IMA in benign prostatic hypertrophy and prostate cancer (16). There are no reports published on the concentrations of IMA in children diagnosed with

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advanced stages of soft tissue sarcomas (STSs) or neuroblastoma (NB).

The aim of the study was to evaluate the levels of IMA in children with NB or STSs. The determination of IMA may be valuable in STS and NB patients as a reliable prognostic biochemical tumor marker.

MATERIALS AND METHODS

Patients

A total of 99 children with STSs or NB who were diagnosed and treated in the Department of Pediatrics, Hematology, Oncology and Endocrinology, at the Medical University of Gdansk, Poland, from 2006 to 2009 were enrolled in the study. There were 47 patients with STS (F/M: 22/25), ranging in age from 1.5 to 21.4 years (mean age 10.1 years; median age 10.3 years) and 52 patients with NB (F/M: 18/34) ranging in age from 2 months to 17.8 years (mean age 3.8 years; median age 0.93 years). The ages of the 30 healthy children in the control group did not differ from those of the cancer patients.

STS diagnosis was established with open biopsy of the primary tumor. In children with NB, bone marrow aspiration was performed or a sample of the tumor was taken for histopathological examination. Pathological diagnoses were confirmed at two independent histopathological centers.

Patients with unfavorable prognoses (i.e., advanced disease) were qualified for the study. Patients with active infection or receiving vitamin supplementation were excluded from the study.

Patients received multimodal chemotherapy and/or radiotherapy according to the Cooperative Wiechteil-sarkom Studiengruppe (CWS 2002 and 2006 protocols) and the protocols for high-risk NB (HR1 NB ESIOP).

Control Group

30 healthy children from the outpatient clinics were recruited as a control group (18 girls and 12 boys), aged between 1.4 and 17.9 years, mean age 9.75 years, median age 8.05 years. At the time of the study, on the basis of detailed medical history and clinical examination, all children were found to be in complete health with no contemporaneous disease. None of the participants were taking vitamin and/or antioxidant supplements at least 8 weeks before the date of the study.

The study was approved by the Ethical Committee of Medical University of Gdansk, Poland.

Laboratory Analysis

All assays were performed in the laboratory of the Department of Clinical Nutrition, Medical University of Gdansk, Poland.

All the serum samples were frozen and stored at -70°C . In our study, IMA levels were measured by a method previously described by Bar-Or et al. (17). The concentration of ischemia-modified serum albumin can be determined by addition of a known amount of Co(II) to a serum specimen and measurement of the unbound Co(II) by colorimetric assay using dithiothreitol (DTT). There is an inverse ratio between the amount of albumin-bound cobalt and the intensity of the color formation. 1.5 ml Eppendorf tubes at standard room temperature were used. 200 μl of patients serum was added to 50 μl of the solution of 1 g/l cobalt chloride, followed by vigorous mixing, and a 10 min incubation. Then DTT (50 μl of a 1.5 g/l solution) was added and mixed. After 2 min incubation, 1.0 ml of a 9.0 g/l solution of NaCl was added. The absorbance of assay mixtures was read at 470 nm with an LKB Spectrophotometer. The value of absorbance was given in units (U/ml serum) with the use of ACB Roche calibrator of 221 U/ml as a standard.

Statistical Analysis

The results obtained in the study were subject to statistical analysis.

For continuous variables data are presented as mean and standard deviation (SD). ANOVA variance analysis was used to test the hypothesis of equality of means of particular trials. In groups with low number of cases composite variance was assessed by means of a non-parametric Kruskal–Wallis sum of ranks test (Bartlett's test was used to check the homogeneity of variance).

In all cases P values ≤ 0.05 were considered statistically significant. Statistical analysis was performed using EPIINFO Ver.3.4.3 (08.-11-2007) software.

Pearson's statistical test was used for correlation coefficient (r).

RESULTS

The IMA levels for the control group and the oncology patient group (divided into STSs and NB groups) before and during treatment are shown in Figure 1. The levels of IMA in all cancer patients (mean 116.8 ± 39.3 U/ml), in patients with STSs (mean 119.8 ± 27.5 U/ml), and in patients with NB (mean 114.6 ± 36.6 U/ml) were significantly higher than in the control patients (mean 87.3 ± 38.3 U/ml; $P = 0.0013$, 0.0066 , and 0.0164 , respectively). The patients were also divided into two subgroups: pretreatment and during therapy. IMA levels increased before and during the treatment in cancer patients compared with control patients. All patients presented with the level of albumin in a normal range (mean 39.6 ± 4.6 g/dl). The negative correlation between IMA levels and serum albumin concentrations were

Fig. 1. Distribution of IMA results: 1—control group, 2—all oncological patients, 3—STSs patients, 4—NB patients, 5—all oncological patient before treatment, 6—all oncological patient during treatment. Each bar is presented as mean $\pm$ SD.

noticed ($P = 0.126$, $r = -0.30$). The activities of tested biomarker were analyzed with respect to the response to therapy: in all oncology patients and in patients with different histological types (subdivided as responders [patients who achieved clinical remission] and non-responders [patients with progression of disease]). IMA levels were higher both in the group of children in complete remission (CR) and in children with progression of disease (PD) than in healthy control patients ($P = 0.0093$ and $P = 0.0017$, respectively). IMA levels were also significantly higher in patients with STSs in CR and in patients with NB in CR and PD ($P = 0.0479$, $P = 0.0222$ and $P = 0.015$, respectively).

DISCUSSION

In recent years, IMA has been identified as a biochemical marker for ischemic disorders (6,7). The majority of studies based on the measurement of IMA were performed in adults. No studies in the literature reported the analysis of the albumin cobalt binding in children with solid tumors of unfavorable etiology (including STSs and high-risk NB). Mastella et al. (16) described 102 patients with benign prostate hyperplasia, 84 patients with prostate cancer, and 27 patients with myocardial infarction (MI). They reported that IMA values increased in all diseases, but the highest levels were observed in patients with MI. Their report was the first to evaluate the levels of IMA in prostate cancer.

In our study, a significant increase in IMA levels in all cancer patients was observed compared with healthy control patients. We believe this increase may be linked to oxidative stress and a decrease in function of the antioxidant defense system. Stachowicz et al. (18)

showed statistically significant decreases in erythrocyte glutathione peroxidase and superoxide dismutase activities in children with STSs and NB with PD, which suggests damage to the antioxidant barrier. Additionally, the levels of IMA were much higher before treatment than during therapy and remained elevated compared with levels in the control group. Some authors have suggested that very low or very high serum albumin levels may affect the IMA concentration and contribute to unreliable results (19,20). Lippi et al. (21) and Lee et al. (22) observed a statistically significant inverse correlation between serum albumin and IMA in both healthy adult outpatients and in patients with a suspicion of acute coronary syndrome. Here, we observed the same negative relationship between IMA levels and serum albumin concentrations in cancer patients. Our pilot study demonstrates the potential benefit of measuring IMA levels in predicting outcomes for patients with advanced cancer.

Several studies have been published on the role of IMA in ischemic disorders. The production of IMA may be correlated with the production of free radicals during ischemia and/or reperfusion, reduced oxygen tension, acidosis, and cellular alterations such as the disruption of the sodium and calcium pump function (16). IMA blood levels may represent a biomarker for identification of an acute stroke, as patients with acute ischemic stroke demonstrate elevated levels of IMA (12–14). Other researchers measured IMA levels in cases of intestinal ischemia for risk stratification (23,24) and observed significantly elevated IMA values in patients with ischemia.

There are many potential genetic (e.g., del 1p, amp N-myc, and DDX1) and molecular (e.g., adhesion, angiogenesis, differentiation, and cytokine receptors) prognostic markers for NB; however, researchers are still searching for reliable indicators that predict outcome. Until now, no neoplastic biochemical markers for STSs have been found, and IMA may be particularly valuable in the prognosis of this disease.

In conclusion, the determination of IMA levels in pediatric oncology patients with poor prognoses from STSs or NB may play an important role in predicting response to therapy and overall outcome.

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