have been present in an extremely high titer and simply overwhelmed the capacity of the blocking reagent. Further studies would be necessary to clarify the exact mechanism.

With the ever-increasing use of animal monoclonal antibodies as therapeutic agents (8), immunoassay interference of the type described here is now a real concern for the laboratory.

References

William W.L. Choi1
Sumathi Srivatsa2
James C. Ritchie2*

1 Department of Pathology and Laboratory Medicine
and
2 Division of Endocrinology and Metabolism
Department of Medicine
Emory University School of Medicine
Atlanta, GA

*Address correspondence to this author at: F-147, Emory University Hospital, Department of Pathology and Laboratory Medicine, 1364 Clifton Road NE, Atlanta, GA 30322. Fax 404-712-4780; e-mail jritchi@emory.edu.

DOI: 10.1373/clinchem.2004.045427

Increased Serum Concentrations of Intestinal Alkaline Phosphatase in Peritoneal Dialysis

To the Editor:

Patients suffering from chronic renal dysfunction are monitored by measurement of biochemical markers such as serum total alkaline phosphatase (ALP) to detect increased bone remodeling before the development of advanced renal osteodystrophy. There are at least five different isoforms/isoenzymes of ALP: hepatic, skeletal, intestinal, placental, and tumor-associated (1). These can be separated by electrophoresis and differ in their resistance to heat inactivation, with placentaland tumor-associated ALP being the most resistant. Approximately 95% of the total ALP activity in serum is derived from bone and liver sources; these isoforms occur in an ~1:1 ratio in healthy adults (2).

Bone remodeling leads to release of skeletal ALP from osteoblasts and, hence, to increased total ALP activity per se may stimulate the intestine to secrete more intestinal ALP and intestinal variant ALP, leading to increased ALP activity in serum (3). I wish to report on two patients who were on long-term peritoneal dialysis and had moderately increased serum total ALP activity (2.4 and 4.4 μkat/L, respectively; cutoff value <1.8 μkat/L) without any biochemical signs of liver disease. Serum agarose gel electrophoresis followed by incubation of the gel with a chromogenic ALP substrate revealed a dominant band with electrophoretic mobility identical to that of intestinal ALP (Fig. 1). The band was unaltered by ficin-induced immunoglobulin fragmentation and disappeared after heat inactivation, excluding that it might represent immunoglobulin-bound macro-ALP or placental or tumor-associated ALP.

The two cases support the possibility of an association between peritoneal dialysis and increased serum concentrations of intestinal ALP and caution against interpreting increased total ALP activity in peritoneal dialysis patients as a sign of increased bone remodeling before further investigation by electrophoresis and/or determination of “bone-specific” ALP, for which immunochromel methods are available. It should in this context be noted that macro-ALP interferes with immunochromel tests for skeletal ALP (4). Thus, the presence of macro-ALP should be excluded by electrophoresis or an equivalent method in patients with increased serum concentrations of skeletal ALP.

The molecular mechanism behind the increase in intestinal ALP in peritoneal dialysis is unknown. There

Fig. 1. Agarose gel electrophoresis of ALP isoforms/isoenzymes in serum samples.

Lane 1, reference sample for hepatic ALP; lane 2, reference sample for skeletal ALP; lanes 3 and 6, untreated samples from patients 1 and 2, respectively; lanes 4 and 7, ficin-treated samples from patients 1 and 2, respectively (5 mg of ficin added to 100 μL of serum, followed by incubation at 37 °C for 30 min); lanes 5 and 8, heat-inactivated (65 °C for 5 min) samples from patients 1 and 2, respectively. Note the lipophilic appearance of the intestinal ALP band in lane 3, indicating that it might represent a previously described intestinal variant ALP with its membrane-binding domain retained (6). As expected, this domain is removed by treatment with ficin (lane 4).
Molar DNA in Maternal Serum in a Case of 46,XY Heterozygous Complete Hydatidiform Mole Coexisting with a 46,XX Twin Live Fetus

To the Editor:

Cell-free fetal DNA in maternal circulation is detectable in early pregnancy, increases with gestational age, and decreases rapidly after delivery (1–3). It has been used for noninvasive prenatal diagnoses, such as fetal rhesus status and sex determination (4,5), but cell-free fetal DNA originates from villous tissue or fetal cells circulating in maternal blood has not been determined. Complete hydatidiform mole lacks a fetal component, and DNA originating from a hydatidiform mole (molar DNA) that is detected in the maternal circulation is thought to originate from villous tissue. By analyzing by real-time PCR the amount of sex-determining region Y (SRY) DNA in maternal serum before and after delivery of a 46,XY complete hydatidiform mole coexisting with a normal 46,XX fetus, we have confirmed that the DNA in maternal blood originates from villous tissue.

The patient was a 30-year-old gravida I, para 0. She was examined at our hospital at 20 weeks of gestation. Ultrasound examination showed a normally grown fetus without anomalies with a normal placenta, and a molar placenta. At 28 weeks of gestation, she spontaneously delivered vaginally a 1094-g female infant with a grossly normal placenta, and a molar placenta. Cytogenetic analyses performed on G-bands with trypsin-Giemsa-banded chromosomes showed a 46,XY complete mole and a normal 46,XX fetus.

After obtaining written consent, we sampled the woman’s peripheral blood between 20 and 28 weeks of gestation and after delivery. We centrifuged 6 mL of each maternal venous blood sample twice at 5800 g and collected and stored the serum at −20°C until analysis. We measured the β-human chorionic gonadotropin (β-hCG) concentrations in the maternal serum by enzyme immunoassay (ST AIA-PACK β-hCG), and after extracting DNA from these same serum samples with a QIAamp DNA Mini Kit (Qiagen), we performed real-time quantitative PCR on an ABI PRISM 7700 Sequence Detector (Applied Biosystems) according to the method described previously by Honda et al. (5). The SRY sequence was used as a molecular marker for DNA from the 46,XY hydatidiform mole in the maternal serum.

Serum β-hCG was 367 747.8 IU/L at 20 weeks and gradually decreased as a function of gestational age. The β-hCG concentration was 147 644.2 IU/L at 1 week before delivery and rapidly decreased to 6512.4 IU/L by 1 week after delivery. The β-hCG concentration had decreased to within reference values by 13 weeks after delivery. No increase in β-hCG was observed thereafter. The highest number of SRY copies in maternal serum was observed at 20 weeks of gestation, and the number of copies decreased toward delivery. The concentration of SRY sequence was 124.43 copies/mL at 1 week before delivery and rapidly decreased to 8.24 copies/mL at 1 week post delivery. The number of SRY copies decreased further, and no SRY was detectable at 5 weeks after delivery (Fig. 1).

Hydatidiform mole is a gestational trophoblastic disease and includes complete and partial moles. The presence of Y-chromosome-specific sequences has been demonstrated in the maternal plasma from a woman with a partial hydatidiform molar pregnancy with 69,XXY karyotype (6). In the present study we have shown that the SRY sequence derived from a complete hydatidiform mole exists in maternal serum. Because our case here is a 46,XY heterozygous complete hydatidiform mole coexisting with a normal 46,XX fetus, the SRY sequence in maternal circulation originated entirely from molar placental tissue.

It was recently reported that placental DNA was released into the maternal circulation in three cases of confined placental mosaicism (7). Ohashi et al. (8) suggested that fetal

References

Henrik Zetterberg
Institute of Laboratory Medicine
Department of Clinical Chemistry and Transfusion Medicine
and Institute of Clinical Neuroscience
Department of Experimental Neuroscience
 Sahlgrenska University Hospital
 Göteborg University
 Göteborg, Sweden

Address for correspondence: Center for Neurologic Diseases, Brigham and Women’s Hospital, Harvard Medical School, Harvard Institutes of Medicine, HUM-610, 77 Avenue Louis Pasteur, Boston, MA 02115. Fax 617-525-5305; e-mail hzetterberg@rics.bwh.harvard.edu.

DOI: 10.1373/clinchem.2004.045831