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Letters to the Editor

High immunocontent of S100β protein in amniotic fluid of pregnancies with Down syndrome

S100β protein is a calcium binding protein expressed primarily by astrocytes in both the developing and the mature nervous system[1]. This protein can be secreted by astrocytes, stimulating neuronal differentiation and glial proliferation[2]. Within the cell, S100β modulates cytoskeletal proteins, such as intermediate filament proteins, and regulates the cell cycle[1,3].

The S100β gene has been mapped to 21q22.2-q22.3. This small chromosomal region, when present in an extra copy, is associated with the Down syndrome phenotype which involves a neurodegenerative process similar to Alzheimer's disease. In fact, high levels of S100β protein have been implicated in brain cytoskeletal changes observed both in Alzheimer's disease and Down syndrome[4].

The incidence of Down syndrome increases with maternal age, and advanced maternal age (over 35 years) is presently a common indication for prenatal diagnosis of this chromosome anomaly[5]. However, maternal age by itself is not an efficient screening marker for Down syndrome, since about 80% of such cases occur in pregnancies of women below 35 years of age[6].

It has therefore been suggested that prenatal screening programs to detect pregnancies at increased risk of Down syndrome should be performed during the first and/or second trimester in all pregnant women, irrespective of their age[7,8]. These programs are based on the measurement of several serum markers' concentrations, including alpha-fetoprotein, unconjugated estriol, human chorionic gonadotrophin (hCG), inhibin-A and, as recently proposed[7], pregnancy-associated plasma protein A and the free beta subunit of hCG, with or without first trimester nuchal translucency measurement[7–9].

The aim of screening by serum markers, with or without nuchal translucency, is to identify women who should be offered chorionic villus sampling or amniocentesis because definitive diagnosis of Down syndrome is made by chromosomal studies[8,10]. Although these chromosome studies offer a highly accurate diagnosis, they are expensive and the analysis procedure may be lengthy. We believe that, even if it may not abolish the need for invasive procedures, the development of simpler, faster and less expensive assays is worthwhile.

In this study, we investigated whether amniotic fluid S100β levels could be useful in identification of fetuses with Down syndrome by measuring this protein in normal pregnancies, between 14 and 17 weeks of gestation, and in pregnancies affected with Down syndrome.

Amniotic fluid samples collected from 47 normal and nine Down syndrome fetuses, as diagnosed from karyotype analysis of cultured amniotic fluid cells, were included in this study. Amniocentesis was performed between the 14th and the 17th gestational week, as estimated by ultrasound scan. The amniotic fluid supernatant was kept frozen until the S100β protein measurement was performed.

S100β protein levels were determined using a sensitive commercial luminescence assay (BYK-Sangtec, Sweden). This is a monoclonal two-site immunoassay that uses an antibody covalently bound to isoluminol as a tracer and presents a limit of detection of 0.02 μg/l. After automatic injection of an isoluminol oxidation solution, the luminescence produced was measured in a luminometer (Magic lite™, Chiron Diagnostics, USA). Samples were measured in duplicate and those with a coefficient of variation $> 7\%$ were repeated. The interassay coefficient of variation in our sample was $< 9\%$. Statistical analyses were performed using ANOVA when multiple groups were compared and Student's *t*-test for comparison between two groups.

S100β concentrations in normal pregnancies, as shown in Figure 1, were similar from 14 to 17 weeks of gestation ($P > 0.8$ by ANOVA), with mean values ranging between 0.91 and 1.10 μg/l. As shown in Figure 2, pregnancies affected with Down syndrome presented significantly higher amniotic fluid S100β levels (1.70 ± 0.23 μg/l) than the group of normal pregnancies (1.01 ± 0.07 μg/l; $P < 0.001$ by Student's *t*-test).

Many groups have proposed using S100β measurement as a marker for fetal malformations. Sindic *et al.*[11] reported elevated levels of S100 protein in amniotic fluid of anencephalic pregnancies, but the protein was not detected in normal pregnancies and with other congenital malformations. Anneren *et al.*[12] reported that S100 measured in amniotic fluid alone was not a reliable marker to detect abdominal wall and neural tube defects. In addition only one sample (which was contaminated with blood cells) among eight Down syndrome pregnancies showed high protein levels. It is important to point out that these assay methods were less sensitive and susceptible to cross-immunoreactivity (as suggested by blood interference in the immunoassay)[12], than the luminescence method used in the present study. This technique appears to be more specific and was sensitive enough to enable detection of S100β in all samples.

Recently, a PCR-based technique was described for the prenatal diagnosis of Down syndrome. Although potentially useful, the technique is expensive and laborious[13–14]. A preliminary study showed that a higher concentration of S100β protein is found in the blood of Down syndrome fetuses, but that the normal level in the serum of those women carrying the Down's syndrome pregnancies is normal[15].

Our study shows a statistically significant difference between S100β concentrations in amniotic fluid of normal and of Down syndrome pregnancies. Unlike many commonly measured serum markers there was no variation in S100β levels with gestational age. This indicates that any

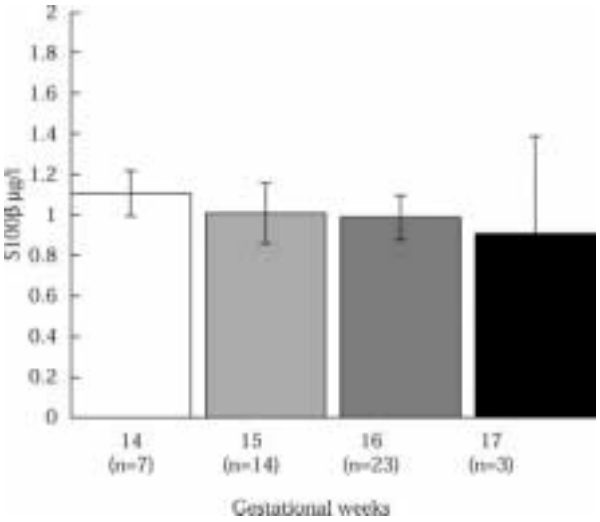

Figure 1. The mean concentrations of S100β protein in amniotic fluid between 14 and 17 weeks of gestation. Data were obtained from 47 normal pregnancies. The bars represent the standard error of the mean in each group. The differences between groups were not statistically significant ($P = 0.83$).

increase of S100β levels could be relevant between 14 and 17 weeks of gestation. However, it should be noted that high concentrations of S100β in amniotic fluid are not specific for Down syndrome[11,12]. We found elevated levels in amniotic fluid of pregnancies affected with trisomy 18 and with familial congenital miocardiopathy (data not shown), suggesting that this marker could have a wider application in the detection of high-risk pregnancies.

Finally, although the potential use of amniotic fluid S100β levels for detection of Down syndrome fetuses should be further evaluated in prospective studies with larger samples, the present data may have implications for the understanding of the neuropathophysiologic aspects of Down syndrome and its correlation with high S100β levels.

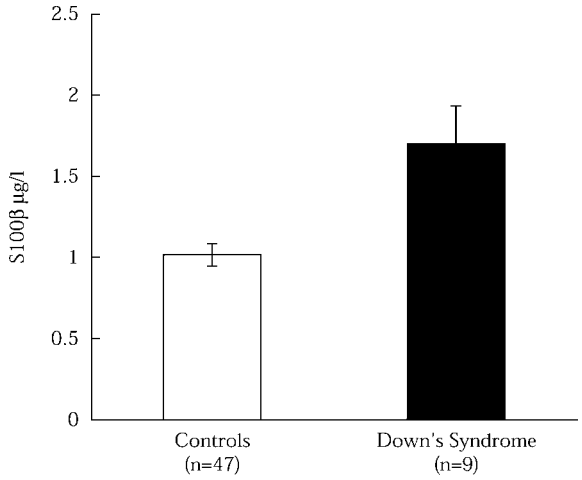

Figure 2. The mean concentrations of S100β protein in amniotic fluid for 47 controls and nine cases of Down syndrome. Control data were obtained between 14 and 17 weeks of gestation. The bars represent the standard error of the mean. Down syndrome cases and controls were significantly different ($P = 0.0009$).

L.C. Portela*¶, A.B.L. Tort*, E.C. Neto‡, R.G. Kessler**, V. Penchaszadeh§, D.O. Souza*, C. A. Gonçalves* and R. Giugliani†**

**Department of Biochemistry and †Department of Genetics, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil, ‡Nobel RIE Laboratory, Porto Alegre, RS, Brazil, §Division of Genetics, Beth Israel Medical Center, New York, NY, USA, ¶Municipal Laboratory of Novo Hamburgo, RS, Brazil and **Medical Genetics Service, Hospital de Clinicas de Porto Alegre, RS, Brazil*

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Pregnancy in a Cesarean scar

We report the case of a 28-year-old multiparous woman who presented with a pregnancy developing in the scar of a previous Cesarean section. An early diagnosis was made by transvaginal ultrasonography which allowed an operative management with preservation of fertility. Sonographic diagnostic criteria and management options are discussed.

Pregnancy developing in a previous cesarean scar is the rarest ectopic pregnancy. An early diagnosis is crucial because of the risk of rupture that can result in severe hemorrhage. This entity has to be distinguished from cervico-isthmic pregnancies which can result in live births[1,2]. To the best of our knowledge (Medline 1966–1999), only nine cases have previously been described[3–11]. We report such an observation diagnosed at 6 weeks of gestation by endovaginal ultrasound and treated through a surgical approach.

The patient, a 28-year-old gravida 3, para 2, at 6 weeks of gestation, presented to our emergency department complaining of slight active vaginal bleeding and lower abdominal pain. The pregnancy test was positive. Her obstetric history revealed two deliveries by low transverse Cesarean sections (first indication, breech presentation; second indication, macrosomia). The patient presented no sign of shock and the gynecologic examination revealed a normal sized uterus, a closed cervix with minor bleeding and minimal pelvic pain. A first transvaginal ultrasound scan was performed in the emergency ward and a live cervical pregnancy with fetal heart tones was suspected. The following morning, the patient was referred to our sonographic unit for a detailed scan.

Transvaginal ultrasound scan demonstrated a decidual reaction in the uterine cavity and a gestational sac of 21 mm diameter within the Cesarean scar containing an embryo of 4.5 mm without cardiac activity. A sagittal plane of the uterus through the gestational sac allowed a precise localization of this pregnancy, embedded at the site of a previous Cesarean scar and protruding into the vesico-uterine space (Figure 1).

A few hours later, an elective laparotomy was performed through a Pfannenstiel incision. The uterus, fallopian tubes and ovaries looked normal and no blood was found in the abdominal cavity. The pregnancy was not visible and the uterine serosa was intact. The ectopic gestational sac was palpable subserosally in the vesico-uterine space. The pregnancy was located in the previous Cesarean scar. An excision of the gestational mass was performed and the previous Cesarean scar was closed by one row of interrupted sutures. Two months later, the patient became spontaneously pregnant. The follow-up of this pregnancy was uneventful and an elective Cesarean section was performed at term leading to the delivery of a healthy girl.

Pregnancy implanted in a previous Cesarean scar is an extremely rare form of ectopic pregnancy. The true incidence cannot be determined accurately because, to the best of our knowledge, only nine cases have previously been reported in the literature[3–11]. The first case was reported in 1990 by Rempen and Albert[3] who diagnosed a 7-week pregnancy implanted at the site of a Cesarean scar by transvaginal ultrasound. A prompt laparotomy with removal of the amniotic sac allowed preservation of the uterus[3]. This first description coincided with the advent of the more popular use of transvaginal ultrasound and it is likely that any cases which occurred before the 1990s were misdiagnosed.

The second case was reported by Herman *et al.* in 1995[5], who did not perform a termination judging that the amniotic sac would coalesce with the uterine cavity. They planned an elective Cesarean delivery at 36 weeks of gestation but, at 35 weeks, the patient was admitted for acute abdominal pain. An emergency Cesarean section was performed but excessive bleeding from the placental site required hysterectomy[5]. Among the other cases, five were treated succesfully through direct injection of either methotrexate, kalium chlorydrate or hypertonic glucose and three others were treated by laparotomy.

The analysis of the previously described cases clearly demonstrate two different type of pregnancies in a Cesarean scar. The first is due to the implantation of the amniotic sac on a scar with progression of the pregnancy in the cervico-isthmic space and in the uterine cavity. Such a situation may allow a viable birth but at an increased risk of massive bleeding from the site of implantation. The second is a deep implantation in a Cesarean scar defect with progression towards rupture and bleeding during the first trimester of pregnancy. To validate this last diagnosis, we propose that three sonographic criteria should be present: (i) the trophoblast must be mainly located between the bladder and the anterior uterine wall; (ii) no fetal parts must be visible in the uterine cavity; and (iii) on a sagittal view of the uterus running through the amniotic sac, a discontinuity in the anterior wall of the uterus should be demonstrated.

Once such a diagnosis is made, a termination of the pregnancy should be considered after explanation of the risks of uterine rupture with life-threatening hemorrhage. Two management options may be considered; the surgical or the medical approach. The medical treatment of cornual and cervical pregnancies has been proven safe by several authors[12,13]. In our case, the presence of a ruptured scar and the fear of heavy bleeding such as described by Lai, 15 days after methotrexate therapy[4] prompted us to elect a surgical approach. Even if a recurrence is unlikely, it seemed that the resection of the old scar and the new closure could minimize this risk.