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Specificity and sensitivity of S100B levels in amniotic fluid for Down syndrome diagnosis

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Abstract

Down syndrome (DS) is the most common chromosomal abnormality and is associated with an extra copy of the chromosome 21. Although several markers are commonly used during pregnancy for the screening of DS, the definitive diagnosis is based on karyotype after amniocentesis, which is an expensive and laborious analysis. S100B is an astrocyte protein which had its gene mapped to the long arm of chromosome 21. Previous preliminary reports have found increased levels of this protein in the amniotic fluid of DS gestations. Aiming to achieve a simpler and cheaper test then karyotype to perform prenatal diagnosis of DS, here we have extended our previous studies and evaluated the real usefulness of amniotic S100B measurement for prenatal DS diagnosis. We have measured S100B in amniotic fluid of 96 pregnancies with DS and of 50 normal pregnancies. Pregnancies with DS presented significantly higher amniotic fluid S100B levels (M = 1.16 ng/mL; IQ = 0.83/1.78) than normal pregnancies (M = 0.51 ng/mL; IQ = 0.38/0.83) ($p < 0.0001$). A receiver operating characteristic (ROC) curve was performed to evaluate the sensitivity and specificity of S100B for DS diagnosis, and presented an area under the curve (AUC) of 0.82, indicating that S100B could be a reliable marker of DS. Moreover, values above 1.67 ng/mL were present only in DS fetuses, representing about 30% of affected pregnancies. However, as an overlap of values was observed between normal and DS gestations, we concluded that amniotic S100B alone is not a good test to discard DS diagnosis.

Keywords: S100B; Prenatal diagnosis; Down syndrome; Amniotic fluid; ROC curve

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Introduction

Down syndrome (DS) is the most common chromosome abnormality, associated with early mental retardation and neurological abnormalities followed by precocious neurodegeneration (Engidawork and Lubec, 2001). The knowledge of the pathological mechanism involved in DS is far from complete understood, although the central point for the DS phenotype is considered to be the overexpression of genes residing in chromosome 21, which is present with an extra copy (trisomy-21) in DS (Engidawork and Lubec, 2001; Fergunson-Smith and Yates, 1984). The incidence of DS increases with maternal age, and advanced maternal age (over 35 years) is presently a common indication for prenatal screening of this chromosome anomaly (Copel and Bahado-Singh, 1999). However, the vast majority of newborns with chromosomal abnormalities are delivered by women younger than 35 years of age, and, not differently in the case of trisomy-21, about 75–80% of all infants born with DS are from young mothers (Copel and Bahado-Singh, 1999).

It has therefore been suggested that prenatal screening for the detection of DS pregnancies should be performed during the first and/or second trimester in all pregnant women, irrespective of their age (Haddow et al., 1998; Wald et al., 1998, 1999a). Most of these programs are based on mother serum biochemical markers, such as alpha-fetoprotein, unconjugated estriol, human chorionic gonadotrophin (hCG), pregnancy associated plasma protein A and the free beta subunit of hCG (Haddow et al., 1998; Wald et al., 1998, 1999a,b). In order to increase the sensitivity of the prenatal screening, these biochemical analyses could be associated with the first trimester nuchal translucency measurement (Haddow et al., 1998; Wald et al., 1998, 1999a,b). Although prenatal screening by serum biochemical analyses and ultrasound provides initial evidences to identify DS fetuses, the definitive diagnosis of DS is currently made by chromosomal studies, which requires amniocentesis or chorionic villus sampling (Haddow et al., 1998; Wald et al., 1998, 1999a,b). In spite of the chromosome analysis offer highly accurate diagnosis, it should be performed in high-specialized centers, being an expensive and laborious procedure, which usually takes a couple of weeks to be completed. Therefore, even without abolishing the need of invasive procedures, the development of simpler, faster, cheaper and more widespread assays to detect DS in amniotic fluid sample is a worthwhile effort.

S100B is a calcium-binding protein expressed and secreted by astrocytes in developing and mature nervous system, involved in neuronal and gliotrophic processes (Donato, 2001). Since S100B gene has been mapped to 21q22.2-q22.3 (Abraha et al., 1999), in a previous work we could observe, probably due to gene dosage effect, increased amniotic levels of this protein in affected pregnancies (Portela et al., 2000, Netto et al., 2004). In the present work, extending our previous analysis, we have focused on the usefulness of amniotic S100B measurement for prenatal diagnosis of DS.

Material and methods

Amniotic fluid samples were collected from 50 normal (from 15th to 18th gestational weeks) and 96 DS fetuses (from 13th to 18th gestational weeks), attended at Centro de Genética Clínica, Universidade do Porto, from 1998 to 2001. The indication for amniocentesis sampling was advanced maternal age in both control and case groups. The DS diagnosis was performed by karyotype analysis of cultured amniotic fluid cells and gestational week was estimated by ultrasound scan. Fetal

development was followed during gestation by ultrasound. The pregnant women were in healthy conditions and had uncomplicated gestations. In control group were included only amniotic fluid of newborns considered normal at birth, according to the clinical parameters used routinely, which are weight, physical examination and Apgar scores. The samples were stored at –70 °C until analyses. S100B protein levels were determined using a sensitive commercial luminescence assay (BYK-Sangtec, 4 Dietzembach, Germany). 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 ng/mL. After automatic injection of an isoluminol oxidation solution, the luminescence produced was measured in a luminometer. All samples were measured in duplicate and were carried out in the same experiment. The coefficient of variation was within 5%. Statistical analysis was performed using Mann-Whitney *U*-two-sided test to compare S100B levels between DS and control groups. Kruskal-Wallis test was performed to compare S100B levels among control subjects with distinct gestational age. Data are expressed as median (M) and interquartile range (IQ). A receiver operating characteristic (ROC) curve was performed to evaluate the sensitivity (detection rate) and specificity (1-false positive rate) of S100B levels in amniotic fluid for DS diagnosis. This study was approved by local Ethic Committee.

Results

Pregnancies with DS presented significantly higher amniotic fluid S100B levels (M = 1.16 ng/mL; IQ = 0.83/1.78) than normal pregnancies (M=0.51 ng/mL; IQ = 0.38/0.83) ($p < 0.0001$), as shown in Fig. 1. Amniotic S100B levels were not statistically different among control subjects with distinct gestational weeks (15th week: M = 0.66 ng/mL; IQ = 0.47/1.18; 16th week: M = 0.59 ng/mL; IQ = 0.30/0.74; 17th week: M = 0.45 ng/mL; IQ = 0.33/0.79; 18th week: M = 0.50 ng/mL; IQ = 0.44/0.79) ($p = 0.51$). Fig. 2

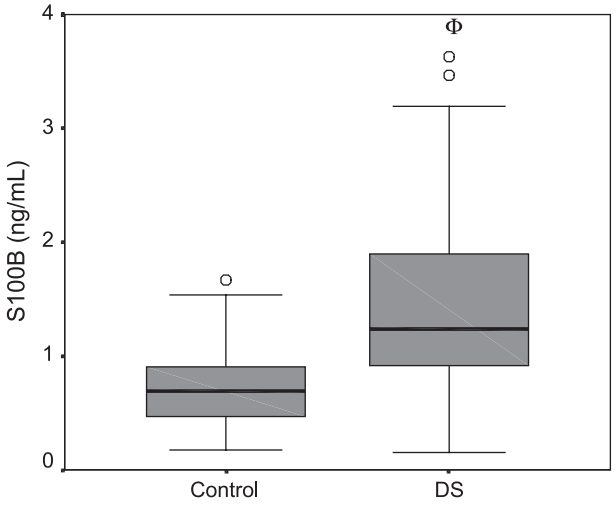

Fig. 1. Boxplot of S100B protein level in amniotic fluid of control and DS fetuses. The difference between groups was statistically significant ($p < 0.0001$). Φ Indicates four outliers with S100B value above 4 ng/mL.

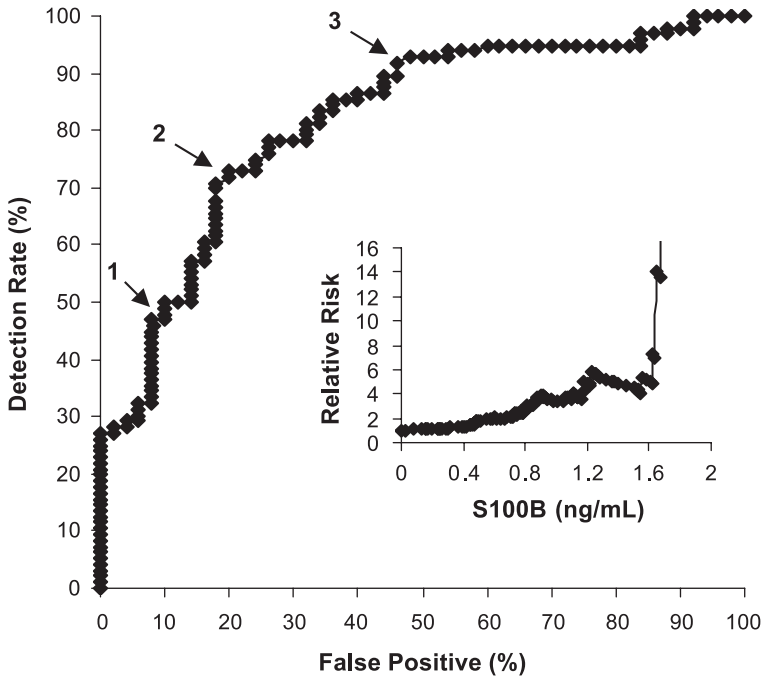

Fig. 2. ROC curve of S100B levels in amniotic fluid and DS diagnosis. AUC = 0.82. Arrow denotes values of: 1 = 1.23 ng/mL; 2 = 0.88 ng/mL; 3 = 0.57 ng/mL. Insert presents data on relative risk and S100B levels. Values above 1.67 ng/mL were present only in DS fetuses (false positive ratio of 0% above this level).

shows the ROC curve obtained for S100B levels in amniotic fluid for DS diagnosis. The area under the curve (AUC) was 0.82. Insert of Fig. 2 presents data on relative risk and S100B levels.

Discussion

Since 80th years the measurement of S100B level in amniotic fluid has been proposed as a prenatal biochemical approach to detect congenital malformations. Accordingly, some authors had reported studies of S100B levels in amniotic fluid of fetuses affected by neural tube defects (anencephaly, open spina biphida) and abdominal wall malformation (Sindic et al., 1984; Anneren et al., 1998). More recently, our group made the first study demonstrating that S100B level in amniotic fluid could be a potential tool for prenatal diagnosis of DS fetuses (Portela et al., 2000). In addition, in this work, the S100B level in control pregnancies presented no variation with gestational age (from14th to 17th), which was also observed in the present study. However, Gazzolo et al. (2001) studying a larger sample could observe a slight positive correlation (r = 0.21) between S100B level and gestational age in amniotic fluid of healthy controls. Importantly, Gazzolo et al. (2003) recently confirmed our findings of increased S100B in amniotic fluid of DS fetuses. Taking together with the present study, it can be assured that an extra copy of chromosome 21 is responsible to increase S100B content in amniotic fluid.

We have thus replicated and extended our previous studies relating S100B and DS by increasing the sample size to 96 patients, which allowed us to perform a ROC curve analysis for S100B levels in

amniotic fluid and DS diagnosis. Accordingly, the ROC curve provides information that S100B levels in amniotic fluid may be a reliable marker to detect DS fetuses (e.g. a S100B level of 0.83 ng/mL is related to 75% of sensitivity and 76% of specificity), which could be considered in future analysis of combined tests (together with FISH and QF-PCR).

The origin of S100B in amniotic fluid is most probably from the fetal nervous tissue, although it is possible that it could also be released, at least in part, from other sites in which it is concentrated, as placental and umbilical tissue (Marinoni et al., 2002; Gazzolo et al., 2000). In spite of the high S100B immunocontent in amniotic levels as well as in brain tissue of DS (Mito and Becker, 1993), no increment was observed in maternal serum level (Abraha et al., 1999).

Conclusion

Although the risks associated to the amniocentesis remains the same, either to perform karyotype or S100B measurement, it is interesting to point that this marker could provide a simpler and faster test, which certainly would be economically attractive and thus widespreadly used, important especially in development countries. However, it should be noted (Fig. 1 and inbox of Fig. 2) that, due to overlapping, S100B in amniotic fluid is not a good marker for discarding the DS diagnosis, making thus its use limited to positive cases, which normally constitute the minority of the samples tested.

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