MODELLING ISOLATED SPINA BIFIDA SCREENING PERFORMANCE USING AXIAL AND SAGITTAL VIEWS OF THE BRAIN AND SPINE ANATOMY AT THE 11-13-WEEK SCAN

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ABSTRACT

Objectives: The aim of this research is to evaluate the modeled predictive value of different features of the fetal anatomy in the detection of isolated open spina bifida, at first-trimester scan. Methods: We retrospectively assessed audited randomized two-dimensional and three-dimensional datasets from 6 cases of open spina bifida and 60 normal fetuses, with no structural or chromosomal associated anomalies, scanned at the same age (12 weeks +4 days). We analyzed direct and indirect signs, using both continuous and categorical variables. Effects were quantified using multiple logistic regression analysis to develop a mathematical model. The performance of screening was determined by Receiver Operating Characteristics (ROC) curve analysis. Results: We found many significant markers’ differences between the two groups. We determined the optimum set of features and their effect on OSB diagnosis. The intracranial subjective markers obtained in axial planes (the dry brain phenomenon, position of the Sylvius aqueduct and the crush sign) had the highest potential on improving OSB diagnostic accuracy. Conclusions: Application of intracranial markers performs better than the direct spine assessment. Application of cranial axial views based screening model (the lateral third-ventricle view and the transthalamic view, used for fetal head biometry) resulted in the highest predicted detection rates, thus a better screening performance than using sagittal views. Following a complete protocol in the FT might be the only efficient way to screen for isolated spina bifida at the 11–13-week scan. The findings should be confirmed in population-based prospective studies.

KEYWORDS: Open spina bifida, first trimester scan, screening.

INTRODUCTION

Open spina bifida (OSB) often leads to severe disability, being considered one of the most devastating nonlethal congenital anomalies. Recent data suggest that affected fetuses may already have an impaired cerebral cortex at 11-15 weeks of gestation.\(^1\)

Most cases of isolated OSB are still detected during the second trimester anomaly ultrasound (US) scan.\(^2\) Although still debated\(^3\), a screening test available in the first trimester (FT) would be desirable. Despite the fact that significant progress has been made in the prevention\(^4\), diagnosis\(^5\) and treatment\(^6,7,8\), and the intellectual potential in affected children is usually preserved, offering an early diagnosis will be a tool enhancing the autonomy of the pregnant women. The standard approach for the FT scan is in many countries transabdominal, due to superior convenience. The direct visualization of the spine defects remained difficult, thus indirect intracranial morphological markers were proposed.

Since 1997, when the lemon sign was found at 10-14 weeks of amenorrhea\(^9\), the US of the fetal head holds promise for the OSB diagnosis at the end of the FT, instead of the fetal spine. The retraction of the–frontal bones (“acorn-shaped head”) was described in the transthalamic axial view, the cerebral peduncles appearing parallel to each other.\(^10\)
More recently the sagittal plane used for the nuchal translucency thickness (NT) measurement was advocated: compression of the fourth ventricle, leading to decrease or disappearance of the normal intracranial translucency (IT)[11,12,13], altered ranges of the brain stem[14], non-visualization of the cisterna magna[15,16,17], decreased frontomaxillary facial (FMF) angle.[8]

In axial views, the third-ventricle plane offers the measurements of the lateral ventricle area, the diameter of the roof of the third ventricle and the transthalamic plane - the diameter of the aqueduct of Sylvius, significantly decreased, all demonstrating the alteration of intracranial collection of cerebrospinal fluid.[19] These alterations were described as the “dried-up brain phenomenon”. Abnormal midbrain position[20] and the posterior-caudal displacement of the mesencephalon (“crash sign”) were described.

Moreover, it was demonstrated that fetuses with OSB have smaller heads[22,23,24], thus having altered intrafetal ratios.[25]

We aimed to evaluate the contribution of each specific fetal anatomical feature to the early OSB detection.

METHODS

The retrospective study was conducted in the University Hospital Craiova, Romania. In the Prenatal Diagnostic Unit (PDU) a transabdominal FT extended protocol[26] at the nuchal scan is used on a daily basis. The institutional ethics committee (Ethical Committee of the University of Medicine and Pharmacy of Craiova) approved to prospectively collect all the FT data. All women scanned in the PDU had provided informed consent for the use of US images for research purposes. The extended protocol includes transverse planes of cranium, for assessing the contour and shape, the choroid plexus and cerebral peduncles planes, and also sagittal planes, with measurement of nuchal translucency thickness - NT and frontomaxillary facial angle - FMF angle, and intracranial translucency - IT. Complete posterior brain morphometry (brainstem- diameter BS and brainstem to brainstem-occipital bone distance ratio-BS/BSOB) is performed in cases with abnormal IT. The spine assessment includes longitudinal planes (preferably using posterior insonation of fetus), confirming the regularity of spine and the continuity of the underlying skin layer.[26] Whenever possible, we acquire and store at least a three-dimensional (3D) static volume of the fetal head (the acquisition plane - the sagittal anterior plane) and a 3D volume of the trunk (the acquisition plane - the sagittal posterior plane). We use a Voluson Expert 750 and an E8 (GE Medical Systems, Zipf, Austria) machine, equipped with 4-8-MHz curvilinear transducer.

From our database, we selected only cases (normal and OSB) that had neither structural associated anomalies nor chromosomal anomalies, and were scanned at the same age. For the purpose of this study, we selected 6 cases of FT diagnosed cases of isolated OSB that were scanned at 12 weeks of amenorrhea (WA) +4 days (crown-rump length CRL = 58.4 – 64.6 mm). We matched them with 60 cases of normal fetuses, scanned at the same gestational age (GA). Inclusion criteria for all cases were: the mentioned CRL value, singleton pregnancy, absence of any (other) structural anomalies, absence of any US markers for chromosomal anomalies, informed consent, audited images, whole extended protocol achieved and stored in the database, known pregnancy outcome.

For each case a number of 7 frozen images or videoclips and two 3D static volumes, stored in the database were reassessed, after anonymization and randomization. The planes were: the sagittal plane used for CRL measurement, the third-ventricle plane, the transthalamic plane, the fourth-ventricle view plane, the transversal abdominal plane, the sagittal plane used for the nuchal NT measurement, the longitudinal plane of the spine in a posterior insonation of fetus. Additionally, the observer (a FMF-certified experienced member of the PDU) had the two 3D static volumes available: the fetal head, acquired from the sagittal anterior plane and the trunk, acquired from the posterior insonation. We used as standard tests second trimester scan, pathological specimens and neonatal features.

For the purposes of this study, the outcomes of interest were to determine the most significant screening marker associated with spina bifida and to evaluate the diagnostic performance (sensitivity and specificity) of examination of each (intracranial and spine) marker for the detection of OSB.

In order to find the most robust screening marker for OSB the following data were retrospectively assessed: continuous variable (CRL, head circumference - HC, occipital-frontal diameter - OFD, biparietal diameter - BPD, the abdominal circumference AC, the ratio BPD/AC, the choroid plexus area, the lateral ventricle - LV area, both measured by the tracing method, the ratio choroid plexus/LV area, the aqueduct of Sylvius to occiput distance (AOD), the IT measurement, BS diameter, BSOB diameter, the BS/BSOB ratio, the FMF angle, the cisterna magna measurement) and categorical variables (the acorn-shaped head, the parallel cerebral peduncles, the dry brain, the crush sign, the subjective caudal displacement of the brainstem, 2D spine assessment, 3D spine assessment). Of note, we decided to interpret the ratio BPD/AC instead of the reported BPD/TAD ratio, because we hypothesized a lower operator dependency and interobserver variability of AC if compared to TAD, and due to the availability of the measurement. The operator was allowed to obtain the data either from the available images and videoclips, or by post processing the 3D volumes. The forms were subsequently filled in.
We chose patients from the two groups so that the CRL measurement was not statistically different. The mean of CRL delta values in the control fetuses group (mean, 63; 95%CI, 62.53-63.46) was not statistically different (p=0.123>0.05) from the ones in the OSB group (mean, 61.70; 95%CI, 59.45-63.95).

Categorical data (presented as %) were compared between the two groups (Normal group vs. Spina bifida group) using Chi-square test. Continuous data (presented as mean (95% Confidence Interval)) were compared between the two groups using Independent-Samples t-Test for normal distribution and the Mann-Whitney test for not normally distributed data. The Kolmogorov-Smirnov test, mean vs. median, the analyses of skewness, kurtosis and histograms for each characteristics demonstrated that for AC, Choroid plexus area, AOD, BS diameter, BSOB diameter and FMF angle, the distribution of values was normal in both Control and Spina bifida groups. So, for these features the Independent-Samples t-Test was used to determine the significance of differences in the mean delta values between the two groups. Significance was assessed at a p-value of less than 0.05.

We subsequently analyzed the correlation between having OSB and these markers. The correlation falls between −1 and +1. Independence between the variables implies that the Pearson coefficient $r$ equals zero. The larger the correlation is in absolute value, the farther the data fall from independence in the linear dimension.

After we established the most significant markers for spina bifida (for p<0.05, noted in Table 2 with $X_1$, $X_2$, ..., $X_n$), we examined the relationship between the dependent discrete variable with two possible outcomes (to have or not to have OSB, noted with $Y$) and these independent variables for Pearson coefficient $|r|>0.5$. Multiple logistic regression$^{[27]}$ is the appropriate regression model to use when the dependent variable is a dichotomous outcome (pass or fail the test of having OSB) and there are multiple explanatory variables. An advantage with logistic regression is that it does not require the assumption of normality or homogeneity of variance (we have normal and not normal distribution of data). The model for the log odds is:

$$\text{logit}(P(\text{Y}=1)) = \alpha + \beta_1X_1 + \beta_2X_2 + ... + \beta_nX_n$$

(Eq.1)

We calculated the regression coefficients $\beta_i$ that refer to the effect of $X_i$ on the log odds when $Y=1$ (the fetus has OSB), where $i$ is from 1 to 9 – the first nine variables from Table 2 for which there is positive or negative predictable relationship and $|r|>0.5$.

Multiple regression analysis was used to determine the power of significant predictors of OSB.

In order to evaluate the performance of screening we determined Receiver Operating Characteristics (ROC) curve analysis for every most significant marker for spina bifida previously obtained.

A p-value of <0.05 was considered statistically significant. The analysis was performed using the statistical software packages SPSS 19.0.

RESULTS

We found significant differences between the two groups for the values of: HC, BPD, BPD/AC, BSOB diameter, BS/BSOB, Cisterna magna, AOD, Choroid plexus area, Ratio Choroid plexus/LV, FMF angle (p<0.05 - see Table 1).

The following subjective markers, used as categorical variables: dry brain(subjective large choroid plexus), the crush sign, caudal displacement of the brainstem, parallel cerebral peduncles and images obtained after the postprocessing 3D static volumes of the spine showed statistically significant differences between the two groups (p<0.05).

Thus, these 15 features should be considered potent markers for the OSB early diagnosis. Correlation analysis shows the most potent features that influence the true positive OSB case diagnosis. The most important features positively influencing the diagnosis are: the dry-up brain phenomenon and the crush sign, the subjective caudal displacement of the brainstem and BS/BSOB (see Table 2). In fetuses with OSB the assessment of BSOB diameter, Cisterna magna (not visible), AOD, BPD/AC, FMF angle were significantly decreased (p<0.01) when compared with normal fetuses.

OSB diagnosis was predicted via a generalized linear model with a log link function. Multiple logistic regression analysis of these characteristics identified a model for the likelihood of OSB occurrence, with 9 most important predictors, as it follows.

Logarithmic

$$\text{Units}=6.37+32.44*X_1+32.44*X_2+12.05*X_3+11.16*X_4-4.71*X_5+18.38*X_6+0.55*X_7+0.11*X_8-0.65*X_9$$

(Eq. 2)

$$\text{Raw Odds}=\exp(\text{Logarithmic Units})$$

(Eq. 3)

$$\text{Probability of OSB}=\frac{\text{Raw Odds}}{1+\text{Raw Odds}}$$

(Eq. 4)

The equation from the mathematical model (Eq. 2) also indicates—the order of significance levels of positive (Pearson coefficient $r>0$) and negative (Pearson coefficient $r<0$) effects of the pregnancy characteristics. The characteristics that influence more the early diagnosis of OSB are the following, listed in descending order: the dry brain, the crush sign, the subjective caudal displacement of the brainstem, the ratio BS/BSOB, BSOB diameter, the cisterna magna, AOD, the ratio BPD/AC, the FMF angle, the BPD, the HC, the ratio
Choroid plexus/LV, the 3D spine post processed images, subjective parallel cerebral peduncles and the choroid plexus area.

The diagnostic accuracy is measured by the Receiver Operating Characteristic (ROC) curve or the sensitivity vs. (1-specificity) plot. An area under the ROC curve of 1 represents a perfect test. The area under the ROC curve was 1 for Dry brain, Crush sign (axial views) and BS/BSOB, BSOB diameter (sagittal views). The area under the ROC curve for other significant marker alone in predicting open spina bifida (>0.9 for an excellent test) was: 0.994 (AOD), 0.992 (FMF angle), 0.986 (BPD/AC), 0.971 (Cisterna magna), 0.917 (Ratio Choroid plexus/LV). The area under the ROC curve for other significant marker alone in predicting open spina bifida (>0.8, <0.9 for a good test) was: 0.888 (BPD), 0.868 (HC), 0.833 (Caudal displacement of the brainstem).

This study reports a very good relationship between the detection rate (DR) and the false-positive rate (FPR) for 7 markers (Dry brain, Crush sign, BS/BSOB ratio, BSOB diameter, AOD, FMF angle, BPD/AC). The calculated screening performance using the subjective Dry brain and Crush sign from axial views and numeric BS/BSOB ratio and BSOB diameter from sagittal views is excellent: 100% DR at any given FPR. The calculated screening performance is also very high using AOD (DR is 98% at a given 5% FPR), FMF angle (DR is 93% at a given 5% FPR) or BPD/AC (DR is 84% at a given 5% FPR).

**Legends**

**Table 1.** Markers’ comparison between the control group and the OSB group. Markers are categorical data (presented as %) and continuous data (presented as mean (95% Confidence Interval)).

**Table 2.** Pearson’s correlation coefficients for significant markers influencing OSB diagnosis.

**Figure 1.** Ultrasound image correlations: first column (a,c,e) - first trimester open spina bifida case, second column (b,d,f) – first trimester normal control case.

First line: the sagittal plane, used for sonographic measurement of nuchal translucency (NT) thickness. Second line: the transthalamic view plane, used for fetal head biometry (measurement of the biparietal diameter and head circumference). Third line: the lateral third-ventricle view plane, used for choroid plexus assessment.

**Figure 2.** Open spina bifida case, 12 weeks +4 days of amenorhoea:

a. sagittal face view abnormal: IT measurement = 2.3 mm, BS diameter = 3.6 mm, BSOB diameter = 3.9 mm, BS/BSOB ratio 0.932, the FMF angle = 68.61, the cisterna magna not visible, subjective caudal displacement of the brainstem present.

b. On-line spine assessment, static 3D surface render mode – negative test.

c. Off-line spine assessment, postprocessing revealed the spine defect – positive test.

d. Medical method used for first trimester termination of pregnancy: pathologic specimen (13+2 WA) – positive postmortem confirmation.

**Figure 3.** Open spina bifida case, with hemivertebrae:

a. Off-line spine assessment, postprocessing failure to demonstrate the overlying skin defect the spine defect – negative test.

b. Medical method used for early second trimester termination of pregnancy: pathologic specimen (16+2 WA) – positive postmortem confirmation.
Comparison between Control group and Spina bifida group was performed using Independent-Samples t-Test or nonparametric Mann-Whitney U-test for continuous variable and chi-square test for categorical variables. p<0.05 statistical significant

Table 2: Pearson's correlation coefficients for significant markers influencing OSB diagnosis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Variables</th>
<th>Pearson Correlation (r)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry brain (subjective large choroid plexux)</td>
<td>$X_1$</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Crush sign</td>
<td>$X_2$</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Subjective caudal displacement of the brainstem</td>
<td>$X_3$</td>
<td>0.803</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BS/BSOB</td>
<td>$X_4$</td>
<td>0.796</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BSOB diameter</td>
<td>$X_5$</td>
<td>-0.791</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cisterna magna</td>
<td>$X_6$</td>
<td>-0.668</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AOD</td>
<td>$X_7$</td>
<td>-0.667</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BPD/AC</td>
<td>$X_8$</td>
<td>-0.647</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FMF angle</td>
<td>$X_9$</td>
<td>-0.576</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BPD</td>
<td>$X_{10}$</td>
<td>-0.469</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HC</td>
<td>$X_{11}$</td>
<td>-0.461</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ratio Choroid plexus/LV</td>
<td>$X_{12}$</td>
<td>0.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3D Spine postprocessing</td>
<td>$X_{13}$</td>
<td>0.454</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parallel cerebral peduncles</td>
<td>$X_{14}$</td>
<td>0.450</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Choroid plexus area</td>
<td>$X_{15}$</td>
<td>0.327</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Correlation is significant at the 0.05 level of p and the Pearson coefficient closer to -1 or 1.
DISCUSSION

Nowadays the FT scan should target all isolated major structural anomalies. When the technical and available expertise issues will be overcome, the offer of an early diagnosis of OSB will provide important information, enhancing the couples’ autonomy. In the second trimester, OSB is an extremely easy US diagnosis, the striking markers being (beside the typical “lemon” and “banana” signs, and usually an easy to see dysraphic defect of the spine) the excess of intracranial fluids: the “hanging” choroid plexuses in the dilated lateral ventricles. On the contrary, in the FT, most US signs are centered on the alteration of intracranial collection, with less cerebrospinal fluid.

The most important advantage of searching markers in the sagittal planes, despite the subtle changes reported, is their widely use in screening for chromosomal anomalies (figure 1a, 1b). The most important advantage of axial planes is the more striking displacement of the structures (figure 1c-f).

In 1997, Sebire et al. reported that in 61 972 pregnancies that had undergone NT screening at 10–14 weeks, all the 29 cases of OSB were missed. A more recent review reported a 16% OSB DR. Still, examples of extremely early diagnosis (9 weeks) are provided, through the transvaginal approach.

Today high DRs are reported, between 92.3% and 100% in the FT. We have also picked up all of the presented cases after extending the FT protocol. The PDU operators stated that the features raising the early suspicion were the intracranial ones in all cases, and moreover, all cases were in fact identified by subjective markers, without any measurements. These observations triggered the present research. The most important finding of this study is the confirmation that, as other study groups reported, OSB can be diagnosed in the FT by visual assessment alone, without using categorical variables.

Our study showed that visualizing the myelomeningocele was impossible in many cases in 2D US, by the transabdominal approach. Moreover, although the direct spinal signs of OSB were missing both in 2D and in 3D online methods, at least a marker showing the brain structure alteration was accessible transabdominal in all cases (figure 2). Thus, in terms of FT OSB screening, despite the growing resolution of modern systems, it seems that we are in the same place where we were at the beginning, around 2002, when we were heading towards indirect signs.

Using both multiple logistic regression analysis and area under the ROC curve, we demonstrate that the subjective Dry brain and Crush sign from axial views and numeric BS/BSOB ratio and BSOb diameter from sagittal views were significantly associated with the fetal risk of having OSB. Yet, subjective markers are crucially dependent on the operator’s experience. Contrary, the measurement of the BS/BSOB ratio at 11–13 weeks is simple and could be easily incorporated into the routine FT scan. As reported before, in our study the IT measurement did not reach significant differences between the two groups, although the following most accurate markers were obtained in the same plane: BS/BSOB ratio and BSOb diameter.

In terms of the spine study, neither 2D US nor volumetric (online and post processed images) US did not reach statistical significance, although in 3 cases the operator was able to obtain suggestive information offline. Although 3D reconstruction surface rendering is usually instrumental in raising the operator’s confidence in spine normality, this approach performed poorer than intracerebral US signs. The area under the ROC curve for 3D spine postprocessing alone in predicting open spina bifida was 0.783 and would be considered a “fair” test, but not good enough. Multiple regression analysis demonstrated, also, that 3D spine postprocessing has a small correlation with detecting OSB.

The ratio choroid plexus/LV area, the numerical correspondent of the subjective marker known as the “dried-up brain” phenomenon, reached statistical significance, yet proved a lower performance when compared with the categorical data.

This research has its limitations, the most important one being the retrospective design and the use of just auditable datasets, highly skilled observers being involved in the acquiring and in interpreting the data. This could have led to the performance overestimation of many markers, especially in regards to the subjective ones. The bias level implied will be clarified by an interobserver variability study, involving less experimented observers.

The strengths of our study include adjustment for GA in both study groups, of pathologic and unaffected pregnancies. We also included only cases of isolated OSB, as the accuracy of the diagnosis is known to be affected by associated structural abnormalities or aneuploidy markers.

Our results suggest that using either axial or sagittal view of the brain will reach, at least in the near future, a better performance as a screening tool than using fetal spine views. Following a complete protocol in the FT might be the only efficient way to screen for isolated spina bifida at the 11–13-week scan. Prospective studies with regards to both axial and sagittal markers are needed for the assessment of these markers’ accuracy in the FT screening for isolated OSB, as the majority of published studies are retrospective. Unless we decide to incorporate the assessment of a specific sign into screening protocols, it is difficult to establish to what extent a specific marker could increase the early DR of OSB.
allowing fewer cases referral to a fetal medicine expert. The present study aims to support in choosing the most valuable marker to be used in such prospective studies. Local conditions and expertise in different centers may affect the accuracy of the FT diagnosis. US being a highly system-dependent and operator-dependent technique. Moreover, it must be highlighted again that the disorder prognosis is dependent mainly on the anatomic level of the lesion, more favorable if the lesion is lower, and that intracerebral signs do not allow the prognosis assessment. Finally, because the FT scan information may radically change the management of the pregnancy, professionals involved in the prenatal diagnosis need to acknowledge and inform the parents about the limits of the FT US scan.

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