Original papers

Med Ultrason 2019, Vol. 21, no. 2, 163-169
DOI: 10.11152/mu-1614

The wide spectrum of ultrasound diagnosis of holoprosencephaly

Cringu Antoniu Ionescu[1], Simona Vladareanu[2], Stefania Tudorache[3], Liana Ples[4], Catalin Gabriel Herghelegiu[5], Adrian Neacsu[4], Dan Navolan[6], Ioana Dragan[5], Daniela-Nuti Oprescu[5]

[1]Carol Davila University of Medicine and Pharmacy, Department of Obstetrics Gynecology Sf Pantelimon Emergency Hospital, Bucharest, [2]Carol Davila University of Medicine and Pharmacy, Department Obstetrics, Gynecology, and Neonatology, Elias Emergency Clinical Hospital, Bucharest, [3]University of Medicine and Pharmacy Craiova, Department of Obstetrics Gynecology, Filantropia Hospital, Craiova, [4]Carol Davila University of Medicine and Pharmacy, Department of Obstetrics Gynecology, Sf Ioan Emergency Hospital, Bucharest, [5]Carol Davila University of Medicine and Pharmacy, Department of Obstetrics and Gynecology, INSMC Polizu Hospital, Bucharest, Romania, [6]Victor Babeş University of Medicine and Pharmacy, Department Obstetrics Gynecology Timisoara

Abstract

Aim: Holoprosencephaly (HPE) is the most common brain malformation. A wide spectrum of anatomical variants are characterized by a lack of midline separation of the cerebral hemispheres. The aim of this study was to assess the ultrasound diagnostic criteria for HPE. **Material and method**: A database of 175 fetuses with central nervous system anomalies identified by ultrasound was collected retrospectively from 2006 to 2016 in this multicenter, retrospective, observational study. Among them 18 cases (10.2%) with HPE were identified. **Results**: The prevalence of HPE was 2.5:10.000 with the sex distribution male:female of 1:1.6. Six cases were alobar subtype, 3 were semilobar, 7 were lobar and 2 were middle interhemispheric variant. In the second trimester, we consider that the abnormal fusion of the lateral ventricles and the absence of the cavum septum pellucidum are the most important landmarks for HPE. Facial abnormalities varied considerably. **Conclusion**: This study illustrates the heterogeneity of HPE with different cerebral and facial appearances.

Keywords: holoprosencephaly; ultrasound; cavum septum pellucidum

Introduction

Holoprosencephaly (HPE) represents a complex malformation of the forebrain, determined by an absence or incomplete cleavage of the prosencephalon (forebrain) during the 4th week of embryogenesis [1,2]. The condition includes a wide spectrum of anatomical variants that are characterized by lack of midline separation of the cerebral hemispheres (telencephalon) and diencephalic structures, extending from the anterior to the posterior depending on the severity.

This malformation has been suggested to be the result of a defect in the ventral induction and patterning of the rostral neural tube by the precordal mesenchyma. Since ventral induction is related to facial development, many cases of HPE have craniofacial abnormalities, leading to a so-called "holoprosencephaly sequence" [1]. The most common facial anomalies include anophthalmia, cyclopia, ethmocephaly, synophthalmia, cebocephaly, proboscis, median cleft lip and palate and hypotelorism present in up to 80% of cases [3,4]. This brain-face correlation was also observed by William DeMyer, who studied a

Received 08.10.2018 Accepted 16.01.2019
Med Ultrason
2019, Vol. 21, No 2, 163-169
Corresponding author: Cringu Antoniu Ionescu
"Carol Davila" University of
Medicine and Pharmacy,
Department of Obstetrics Gynecology,
Dionisie Lupu str, no 37,
020031, Bucharest, Romania
email: antoniuginec@yahoo.com

group of patients with HPE and in the end concluded that *"The face predicts the brain"* [5].

Although HPE is a rare malformation, it is nonetheless the most common malformation of the brain and face in humans, with a prevalence of around 1–1.34 in 10.000 births and, if aborted embryos are included in the estimate, prevalence is much higher at around 1 in 200–250 [4,6–8]. It seems that the lower the gestational age is, the higher the prevalence and this can be explained by the high intrauterine lethality of fetuses with HPE, probably due to the associated genetic and structural defects [9].

The exact cause of HPE is difficult to be identified as this pathology seems to have a multifactorial etiology including teratogen exposure, genetic abnormalities and syndromic association [10]. Maternal diabetes is considered the most important risk factor, potentially increasing the risk by 200-fold. Other risk factors include alcohol, cigarette smoking, salicylates, retinoic acid, and cytomegalovirus infection [11].

HPE is considered by some a *"continuum of forebrain malformations with no clear-cut distinction among the different subcategories"* thus an accurate diagnosis of the exact HPE subtype is often difficult to be established [12]. Since the prognosis of the newborn depends on the HPE subtype, we evaluated the outcome of these fetuses diagnosed with HPE in the second and third trimester.

For the classification of the HPE cases we chose the one proposed by DeMyer et al [5], which consists of three subtypes of HPE: alobar, semilobar, and lobar, depending on the degree of cleavage of the hemispheres. We also included a more recent subtype – middle interhemispheric (MIH) variant, described by Barkovich et al [13].

Materials and methods

Patients selections

From a total of 71.160 births over a 10-year period (2006-2016) in four tertiary university hospitals in Romania ("Elias" Hospital, Bucharest; INSMC "Polizu" Hospital, Bucharest; "Sf. Pantelimon" Hospital, Bucharest; "Filantropia" Hospital, Craiova) we identified 175 anomalies of the central nervous system. From these, 18 cases of HPE (with or without facial anomalies) with relevant cerebral anomalies for each subtype were included. Data were collected retrospectively from medical files and approval from the local Ethics Committee was obtained. We excluded cases with septo-optical dysplasia, ventriculomegaly associated anomalies, isolated absent cavum septum pellucidum or other central nervous system anomalies.

For prenatal ultrasonography GE Voluson 730 Pro, Voluson 730 Expert, and E8 ultrasound machines were used. Postnatal confirmation was made using transfontanellar ultrasound in all cases, magnetic resonance imaging (MRI) in 6 cases or by necropsy. Also, data about clinical evaluation of the newborns that survived was collected. Genetic counseling and testing (amniocentesis or biopsy of the chorionic villi) was offered to all cases, but only in 10 cases the parents opted for these investigations.

Statistical analysis

A descriptive analysis was performed and continuous data are expressed as means and percentages. For statistical analysis and revealing the patterns in our data, we provide univariate and bivariate distributions for our analysed variables in terms of descriptive statistics, using Pivot Tables in Excel 2016, a product of Microsoft Office 365.

Results

The prevalence of HPE was 2.5:10.000 with the sex distribution male:female of 1:1.6. The mean fetal age at diagnosis of the 18 cases with HPE was 23 weeks+5 days (range, 12–37 weeks). Six cases (33.3%) were diagnosed with alobar HPE, 3 (16.6%) with semilobar HPE, 7 (38.8%) with lobar HPE and 2 (11.1%) with middle interhemispheric (MIH) variant HPE. The details about these cases are presented in Table I and examples of HPE cases in figure 1 and 2. The mean age of mothers was 27.5 years (range, 20–34 years) and no relevant medical history or exposure to teratogens was found in any case.

The ultrasound findings found in the study group are presented in Table II. Only 7 babies were born alive between 36-38 weeks and from these only 2 survived until one year old. In 7 cases the parents decided to terminate the pregnancy and there were 4 cases of intrauterine death.

Discussions

Our study confirm the variety of prenatal ultrasound findings that can be encountered in HPE and the importance of ultrasonography in assessing the subtypes of this pathology, especially in relation with the prognostic of the foetuses. The lobar subtype was the most frequently encountered, while the alobar subtype had the worst prognosis.

The alobar subtype is the most severe expression of HPE and is characterized by a complete failure of cleavage of the prosencephalon, resulting in a single midline forebrain with a single forebrain monoventricle [10,11]. In all of our 6 cases of alobar HPE the cerebral hemispheres were completely fused into a holosphere and the

interhemispheric fissure was completely absent, so the resulting brain was smaller than normal. Also a dorsal cyst was observed in 2 cases, these being the pathognomonic characteristics for this subtype of HPE [12]. Three cases were diagnosed as early as the first trimester, due to the absence of the "butterfly sign" formed normally by the two choroid plexuses [14]. Depending on the degree to which the cerebrum surrounds the dorsal membranous roof of the ventricle, the brain was shaped from the sagittal view like a ball where the cortex encircles the monoventricle in 4 cases or a cup where the monoventricle was not completely encircled in 2 cases. We did not encounter the literature reported shape of a pancake where the cortex is flattened at the base of the skull [10,12]. Since in all our cases there was a complete fusion of the two hemispheres, no midline structures were present, includ-

Table I. Clinical, ultrasound and genetic characteristics of the cases with holoprosencephaly

Patient No	HPE Type	Corpus callosum	Face	Karyotype	Age at diagnostic (weeks)	Outcome
1	Alobar	Absent	Synophthalmia absent nose proboscis	NP	18	TOP
2	Alobar	Absent	Cyclopia proboscis	NP	22	ID
3	Alobar	-	Hypotelorism	Triploidy	12	TOP
4	Alobar	-	Hypotelorism	NP	13	TOP
5	Alobar	Absent	Hypotelorism cebocephaly	Mosaicism at chromosome 18	38	Birth 38w
6	Alobar	Absent	Proboscis	T13	14	TOP
7	Semilobar	Absent	Hypotelorism	T13	27	ID
8	Semilobar	Absent	Hypotelorism	T13	37	Birth 37w
9	Semilobar	Absent	Hypotelorism	NP	24	TOP
10	Lobar	Hypoplastic	Hypotelorism	NP	25	Birth 36 w
11	Lobar	Absent	Normal	NP	22	TOP
12	Lobar	Hypoplastic	Normal	T13	27	ID
13	Lobar	Absent	Normal	NP	23	ID
14	Lobar	Hypoplastic	Normal	Normal	28	Birth at 37w
15	Lobar	Hypoplastic	Hypotelorism	Normal	32	Birth at 38w
16	Lobar	-	Hypotelorism	NP	14	TOP
17	MIH	Hypoplastic	Normal	Normal	26	Birth at 39w
18	MIH	Hypoplastic	Normal	Normal	27	Birth at 38w

T13 – trisomy 13; No – number of patients; MIH – middle interhemispheric variant of holoprosencephaly; NP – not performed, TOP – termination of pregnancy; ID – intrauterine death

Table II. Brain ultrasound characteristics encountered for each subtype of HPE

	Alobar (n=6)	Semilobar (n=3)	Lobar (n=7)	MIH (n=2)
Cortical hemispheres fusion	complete	anterior half	basal frontal	posterior frontal and parietal
Interhemispheric fissure and falx cerebri	absent	present posteriorly only	hypoplastic anteriorly and present posteriorly	present in the anterior and posterior poles
Corpus callosum	absent	absent or thin and hypoplastic	thin and hypoplastic	absent or thin and hypoplastic
Cavum septum pelucidum	absent	absent	absent or dysplastic	absent or dysplastic
Lateral ventricles	monoventricle	fusion of the anterior half of the lateral ventricles	hypoplastic and partially fused frontal horns	fused at their middle portion (body)
Third ventricle	absent	absent	visible	visible
Dorsal cyst	present (n=2)	absent	absent	absent
Deep grey nuclei	often completely fussed	incompletely separated	partially fused	normal
Doppler findings	-	-	"rete of vessels" branching from the internal carotids (n=1)	-
Head circumference	macrocephaly (n=1)	microcephaly (n=1)	no	no

n – number of cases

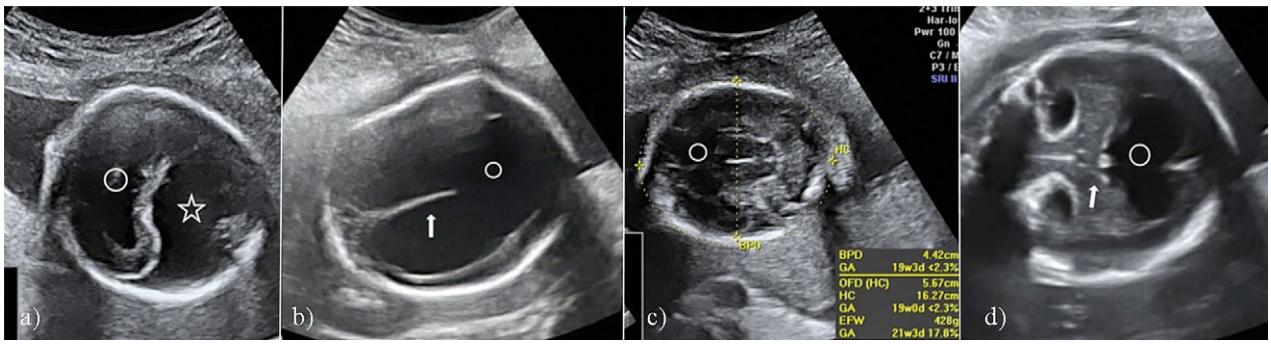

Fig 1. a) 18 weeks old fetus with alobar HPE, the cerebral hemispheres are completely fused with a single midline ventricle in the middle (circle). In the occipital region a large dorsal cyst is present (star), pushing anteriorly the dorsal part of the prosencephalon; b) 27 weeks old fetus with semilobar HPE: severely dilated occipital horns of the lateral ventricles, the rest of the body and frontal horns are fused forming a single "midline ventricle" (circle). The interhemispheric fissure (arrow); c) 23 weeks fetus with semilobar HPE. The cerebral hemispheres are fused in the anterior half (circle) and the BPD and HC are smaller than the 5th percentile; d) 24 weeks old fetus with semilobar HPE: lack of cleavage of the anterior half of the hemispheres, with fused ventricles and absent midline structures (circle), also the deep grey nuclei appear to be incompletely separated (arrow).

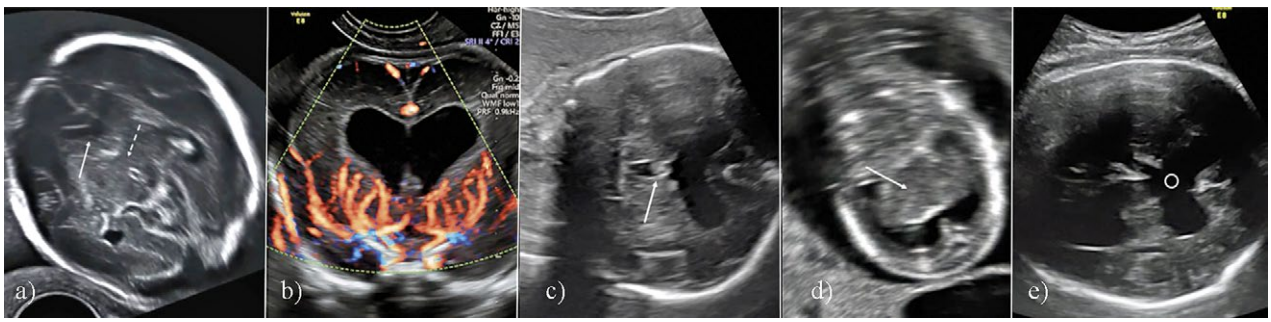

Fig 2. a) 27 weeks fetus with lobar HPE: hypoplastic anterior interhemispheric fissure, fused rudimentary frontal horns, absent cavum septum pellucidum (arrow) and partially fused deep grey nuclei (dotted arrow); b) fetus of 22 weeks with lobar HPE: fused lateral ventricles, the interhemispheric fissure is present but falx cerebri is hypoplastic, abnormal vasculature with a "rete of vessels" branching from the internal carotids; c) 28 weeks fetus with lobar HPE: fussed lateral ventricles, hypoplastic falx cerebri partially separating the cerebral and fussed fornices (arrow); d)fetus of 14 weeks old with hypoplastic interhemispheric fissure and falx cerebri, partially fused lateral ventricles and thalami that appear to be at least partially fused (arrow); e) 38 weeks fetus of middle interhemispheric HPE with both the anterior and posterior poles of the hemispheres well separated, normally defined anterior ventricular horns but with completely fused bodies of the lateral ventricles (circle).

ing the falx cerebri, interhemispheric fissure, cavum septum pellucidum, or corpus callosum as in other published cases [4]. In one case where necropsy was performed, it was clearly visible that the basal ganglia, hypothalamus and thalamus nuclei were fussed in the midline so no third ventricle was visible, a finding that is frequently described in literature [10,15]. In all of our cases the brainstem and the cerebellum were grossly normal, but we could not be sure if there was only a single cerebral peduncle. We suspected this finding because there was only one cerebral holosphere but we could not characterize the corticospinal tracts (hypoplastic or absent) comparing with other studies [4,12].

Despite the fact that in most/many cases of alobar HPE presented in literature, there is an abnormal development of the anterior vasculature, with the anterior and middle cerebral arteries being replaced by a "rete of vessels" arising from the internal carotid and basilar arteries, we could not identify these features in any of our cases [4,12,15].

The semilobar HPE is an intermediate form in which the anterior halves of the hemispheres fail to separate, but there is a degree of separation of the posterior hemispheres [1,15] with a hypoplastic posterior interhemispheric fissure and falx cerebri [4,10]. All of these features were present in our 3 cases of semilobar and also the non-cleaved lobes were smaller than normal, resulting in microcephaly. Another characteristic feature of semilobar HPE that we found was that the frontal horns of the lateral ventricles were fused forming a single "midline ventricle", but the posterior horns and trigones were present.

A dorsal cyst may be present, when the thalami are fused and may lead to macrocephaly [12]; also the hippocampus is usually incompletely formed appearing normal only in the temporal lobes [16]. In our cases only the deep grey nuclei appear to be incompletely separated and no dorsal cyst was identified.

Case reports described that in the most severe cases the vasculature is abnormal (similar to alobar HPE); most of the time an unpaired anterior cerebral artery displaced anteriorly being identified creating the "snake under the skull sign" [10,15]. We did not identify this sign in our series of cases. The lobar HPE is the least severe form and is characterized by a near complete cleavage of the hemispheres, with the interhemispheric fissure present along the entire midline and fused only the most ventral aspects of the frontal neocortex [12,15]. These features were present in all our cases of lobar HPE. Furthermore, the falx cerebri was present anteriorly although it was hypoplastic due to the partial fusion of the frontal lobes, a characteristic finding [16]. The cavum septum pellucidum was absent in all cases, as was the most anterior part of the corpus callosum (usually the rostrum and genu), but most of the posterior body and the splenium were present. Characteristic for the lobar type, the third ventricle was normal and the dorsal cyst was absent [12,15]. Another characteristic sign of lobar HPE, described by Pilu et al [17], is the intraventricular fusion of the fornices, that appears on ultrasound as a hyperechogenic structure, a sign that was also identifiable in our series of cases. In one case we identified abnormal vasculature, forming the characteristic "rete of vessels" that is present in HPE, sometimes seen arising from the internal carotid and basilar arteries or a single azygous anterior cerebral artery present [15].

In the "middle interhemispheric" variant of HPE or syntelencephaly, first described by Barkovich et al, a degree of middle interhemispheric fusion was encountered [13]. Though it was considered at first a subtype of semilobar HPE, close analysis of the few cases reported [18–21] led to the conclusion that the MIH is a different and distinct clinic-neuro-radiologic form of HPE, and it is classified as a new 4th type of HPE, alongside the 3 "classic" types described by DeMyer [2,22]. In both of our cases of middle interhemisheric variant, we observed an abnormal midline continuity in the posterior frontal and anterior parietal regions of the cerebral hemispheres with fusion of the bodies of the lateral ventricles, but with normal interhemispheric separation of the anterior frontal lobes and occipital region as described in literature [12,15]. The cavum septum pellucidum was absent or dysplastic and also the callosal body was absent or at least partially absent, as described in literature [15,23].

Although HPE can be successfully diagnosed in the first trimester [14,24] less than a quarter of our cases were diagnosed before 14 weeks, with a mean age of 23 weeks+5 days. One explanation for this is that an important percentage (50%) of our patients missed the first trimester scan (9 fetuses). Alobar and semilobar types of HPE are easy to diagnose in the first trimester with ultrasound, with reports of cases detected as early as 10 weeks[10]. In our series of cases, 50% (n = 3) of alobar HPE cases were diagnosed in the first trimester, while the rest of the patients missed the first trimester ultrasound. The characteristic finding indicating HPE in the first trimester, when evaluating the fetal head, is the presence of a midline "monoventricle" and the absence of the typical echogenic "butterfly" sign corresponding to the choroid plexuses. Although, there is no consensus concerning the mode of delivery, in all our cases over 35 weeks, the delivery was through cesarean section, but the reason was not fetal macrocephaly in all cases, but because of "defensive medicine" leading to an increase in the incidence of CS in our country [25,26]. As for milder forms of HPE, the correct diagnosis becomes harder and harder to be established as the spectrum of anatomical variants gets closer and closer to normal brain anatomy [27]. For these milder forms identified at the second trimester ultrasound scan, the most valuable clue in our opinion is the absence of the cavum septum pellucidum. This structure is easily identified as it is visualized in the "standard" section for fetal head biometry and is on the "checklist" of most scanning protocols. Most importantly, its absence is a hallmark of all forms of HPE. All cases we reviewed had anterior fusion of the lateral ventricles with a degree of hypoplasia of the frontal ventricular horns, depending on the severity of the case. The rest of the typical brain anomalies in HPE are agenesis/hypoplasia of the corpus callosum, fusion of the deep grey nuclei, and absence of the third ventricle Vascular abnormalities can vary depending on the severity of the malformation and are usually difficult to document by ultrasound (we identified only one case), requiring experienced sonographers and, in some cases, 3D power Doppler ultrasound or MRI evaluation [28].

The outcome in cases with HPE is generally poor, with high rates of mortality, however, some children survive for many years. Higher mortality will correlate with the severity of brain malformations and facial malformations, the presence of genetic abnormalities and the presence of other congenital malformations [29–31]. We also observed this trend in our case series, with intrauterine mortality reaching 37% (4 cases out of 11 in which pregnancy was not terminated). The grim prognosis was confirmed by the low 1 year survival rate below 30% (2 cases of 7 born between 36-38 weeks).

The management of HPE cases is challenging. There are several clinical manifestations commonly observed in children with HPE that survive after birth, including the developmental delay. The degree of delay is variable, correlating with the severity of the brain malformation, but tends to be severe. Seizures are common, and may be difficult to control. Approximately half of the children with HPE in a cohort study had at least one seizure [29].

One limitation of our study is that it is retrospective and with a small number of cases (18 cases). Although the spectrum of anomalies found was very wide, we were able to identify the great majority of structural defects reported as characteristic for each type of HPE in literature. Furthermore, even with these limitations, the key marker for ultrasound diagnosis of HPE is the degree of fusion of the lateral ventricles and the absence of the cavum septum pellucidum in the second trimester. Also, we could not compare ultrasound and MRI according to gestational age, because a MRI was performed in a few cases only. Another limit is linked to the fact that ultrasound evaluation of the fetal brain requires appropriate technical skills to obtain correct diagnostic images and is highly dependent on fetal position, so different expertise in individual centers may affect the accuracy of the specific diagnosis.

Conclusions

This study confirmed the heterogeneity of ultrasound findings in HPE. Although alobar and semilobar HPE can be recognized by ultrasound prenatally during the first and early second trimester, a clear differentiation between the subtypes of this pathology is sometimes difficult and a complete diagnostic may be available only after birth.

Conflict of interest: none

Reference

1. Golden JA. Towards a greater understanding of the pathogenesis of holoprosencephaly. Brain Dev 1999;21:513-521.
2. Lewis AJ, Simon EM, Barkovich AJ, et al. Middle interhemispheric variant of holoprosencephaly: a distinct cliniconeuroradiologic subtype. Neurology 2002;59:1860-1865.
3. Pucciarelli V, Bertoli S, Codari M, Veggiotti P, Battezzati A, Sforza C. Facial Evaluation in Holoprosencephaly. J Craniofac Surg 2017;28:e22-e28.
4. Volpe P, Campobasso G, De Robertis V, Rembouskos G. Disorders of prosencephalic development. Prenat Diagn 2009;29:340-354.
5. DeMyer W, Zeman W, Palmer CG. The face predicts the brain: diagnostic significance of median facial anomalies for Holoprosencephaly (arhinencephaly). Pediatrics 1964;34:256-263.
6. Bulakbasi N, Cancuri O, Kocaoğlu M. The middle interhemispheric variant of holoprosencephaly: magnetic resonance and diffusion tensor imaging findings. Br J Radiol 2016;89:20160115.
7. Matsunaga E, Shiota K. Holoprosencephaly in human embryos: epidemiologic studies of 150 cases. Teratology 1977;16:261-272.
8. Orioli IM, Castilla EE. Epidemiology of holoprosencephaly: Prevalence and risk factors. Am J Med Genet Part C Semin Med Genet 2010;154C:13-21.
9. Kagan KO, Staboulidou I, Syngelaki A, Cruz J, Nicolaides KH. The 11–13-week scan: diagnosis and outcome of holoprosencephaly, exomphalos and megacystis. Ultrasound Obstet Gynecol 2010;36:10-14.
10. Winter TC, Kennedy AM, Woodward PJ. Holoprosencephaly: a survey of the entity, with embryology and fetal imaging. Radiographics 2015;35:275-290.
11. Raam MS, Solomon BD, Muenke M. Holoprosencephaly: a guide to diagnosis and clinical management. Indian Pediatr 2011;48:457-466.
12. Marcorelles P, Laquerriere A. Neuropathology of holoprosencephaly. Am J Med Genet C Semin Med Genet 2010;154C:109-119.
13. Barkovich AJ, Quint DJ. Middle interhemispheric fusion: an unusual variant of holoprosencephaly. AJNR Am J Neuroradiol 1993;14:431-440.
14. Sepulveda W, Dezerega V, Be C. First-trimester sonographic diagnosis of holoprosencephaly: value of the "butterfly" sign. J Ultrasound Med 2004;23:761-765.
15. Hahn JS, Barnes PD. Neuroimaging advances in holoprosencephaly: Refining the spectrum of the midline malformation. Am J Med Genet C Semin Med Genet 2010;154C:120-132.
16. Simon EM, Hevner R, Pinter JD, et al. Assessment of the deep gray nuclei in holoprosencephaly. AJNR Am J Neuroradiol 2000;21:1955-1961.
17. Pilu G, Ambrosetto P, Sandri F, et al. Intraventricular fused fornices: a specific sign of fetal lobar holoprosencephaly. Ultrasound Obstet Gynecol 1994;4:65-67.
18. Fujimoto S, Togari H, Banno T, Wada Y. Syntelencephaly associated with connected transhemispheric cleft of focal cortical dysplasia. Pediatr Neurol 1999;20:387-389.
19. Sener RN. Holoprosencephaly manifesting with fusion of the gyri cinguli. J Neuroradiol 1998;25:52-54.
20. Robin NH, Ko LM, Heeger S, Muise KL, Judge N, Bangert BA. Syntelencephaly in an infant of a diabetic mother. Am J Med Genet 1996;66:433-437.
21. Atalar MH, Icagasioglu D, Sener RN. Middle interhemispheric variant of holoprosencephaly associated with bilateral perisylvian polymicrogyria. Pediatr Int 2008;50:241-244.
22. Simon EM, Barkovich AJ. Holoprosencephaly: new concepts. Magn Reson Imaging Clin N Am 2001;9:149-164.
23. Simon EM, Hevner RF, Pinter JD, et al. The middle interhemispheric variant of holoprosencephaly. AJNR Am J Neuroradiol 2002;23:151-156.
24. Tongsong T, Wanapirak C, Chanprapaph P, Siriangkul S. First trimester sonographic diagnosis of holoprosencephaly. Int J Gynaecol Obstet 1999;66:165-169.

25. Ionescu CA, Dimitriu M, Poenaru E, et al. Defensive caesarean section: A reality and a recommended health care improvement for Romanian obstetrics. J Eval Clin Pract 2019;25:111-116.
26. Mocuta D, Popovici LR, Dumitriu AS, Burlibasa L, Ionescu C, Sfeatcu R. Life Quality - Condition of social welfare. Metal Int 2009;14:62-64.
27. Mercier S, Dubourg C, Belleguic M, et al. Genetic counseling and "molecular" prenatal diagnosis of holoprosencephaly (HPE). Am J Med Genet C Semin Med Genet 2010;154C:191-196.
28. Herghelegiu D, Ionescu CA, Pacu I, Bohiltea R, Herghelegiu C, Vladareanu S. Antenatal diagnosis and prognostic factors of aneurysmal malformation of the vein of Galen: A case report and literature review. Medicine (Baltimore) 2017;96:e7483.
29. Levey EB, Stashinko E, Clegg NJ, Delgado MR. Management of children with holoprosencephaly. Am J Med Genet C Semin Med Genet 2010;154C:183-190.
30. Pleş L, Sima RM, Moisei C, Moga MA, Dracea L. Abnormal ultrasound appearance of the amniotic membranes – diagnostic and significance: a pictorial essay. Med Ultrason 2017;19:211-215.
31. Ionescu CA, Calin D, Navolan D, et al. Alobar holoprosencephaly associated with a rare chromosomal abnormality: Case report and literature review. Medicine (Baltimore) 2018; 97:e11521.