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Prenatal findings and outcomes of the holoprosencephaly spectrum

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Abstract

Objective: To estimate ultrasonographic factors associated with genetic abnormalities in holoprosencephaly cases.

Methods: This study is a retrospective chart review. Study participants were pregnant women who had an ultrasound scan at our center and diagnosed with fetal holoprosencephaly between 2014 and 2024. We retrieved maternal and fetal features, prenatal ultrasonography characteristics, pregnancy and neonatal outcome data from electronic medical records.

Results: Data from 47 cases of holoprosencephaly were analyzed. Genetic results were available in 57.5% (27/47). Of those, 63% (17/27) had a genetic abnormality. There were 11 cases of trisomy 13 (40.7%), 3 cases of trisomy 18 (11.1%), one case of triploidy (3.7%), one case of microarray anomaly and one abnormal exome sequencing result. Lobar (7/47, 14.9%) and semilobar (5/47, 10.6%) variants were less prevalent than alobar holoprosencephaly (35/47, 74.5%). Of 11 trisomy 13 cases, 7 (63.6%) had alobar, 2 (18.2%) semilobar, and 2 (18.2%) lobar type holoprosencephaly. All three trisomy 18 cases had alobar holoprosencephaly. Facial anomalies were the most common group of additional anomalies (31/47, 65.9%), also were associated with genetic abnormalities (75% in those with genetic abnormalities vs. 25%, p=0.03). The majority of cases were terminated (32/47, 68.1%). Only 7 cases were live born, while 5 died postnatally. The 2 children survived both had lobar type holoprosencephaly.

Conclusion: Holoprosencephaly is highly associated with aneuploidies, particularly trisomy 13. Thorough investigation for additional anomalies and genetic etiologies is essential for parental counseling, and have an impact on decision of termination.

Keywords: Holoprosencephaly, trisomy 13, fetal neurosonography, prenatal diagnosis

Introduction

Holoprosencephaly represents a complex congenital malformation of the developing forebrain, characterized by defective midline cleavage during early embryogenesis, leading to varying degrees of incomplete separation of the cerebral hemispheres.[1] It is rather rare with 1/10000 prevelance among live births.[2] This failure of prosencephalic division results in structural anomalies ranging from alobar holoprosencephaly, where there is a single fused ventricular cavity and absence of interhemispheric fissure, to semilobar and lobar forms, characterized by partial cleavage with varying degrees of interhemispheric separation.[3,4]

A wide range of etiologic factors have been reported for holoprosencephaly. Still, chromosomal abnormalities are responsible for the majority of fetuses with HPE, with trisomy 13 accounting for 75%.[5] The etiology is multifactorial, involving complex genetic and environmental factors. Mutations in key developmental genes such as sonic hedgehog (SHH), SIX3, and ZIC2 have been identified in a subset of cases, highlighting the critical role of early forebrain patterning genes in normal

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brain development.[6] SHH is the most common cause of non-chromosomal holoprosencephaly.[6,7] Additionally, environmental factors including maternal diabetes and teratogen exposure contribute to the heterogeneous phenotypic spectrum observed in holoprosencephaly cases.[8]

The most severe form is defined as alobar holoprosencephaly, in which a single, midline forebrain with a primitive monoventricle is characteristic for ultrasonographic evaluation.[9] Most of the time there is a dorsal cyst communicating with the monoventricle.[10] Interhemispheric fissure and falx, corpus callosum, cavum septum pellusidum (CSP) and olfactory bulbus are absent.[6] Semilobar holoprosencephaly is nonseparation of the anterior part of the hemispheres, posterior hemispheres may be separated in different levels and there is partial separation of the ventricles.[11] Lobar holoprosencephaly, consists of less severe anatomical brain defects. More than half of the frontal lobes should be separated to be defined as lobar holoprosencephaly. Interhemispheric fissure and falx can be present, yet hypoplastic.[12]

The vast prevalence of chromosomal abnormalities seen in holoprosencephaly cases highlights the significance of thorough genetic testing and family counseling. The identification of genetic abnormalities in fetuses with holoprosencephaly is made possible by current widespread availability of prenatal diagnostic methods such as chromosomal microarray analysis (CMA) and exome sequencing, which are essential for forecasting the prognosis and letting the families to have a well-informed decision-making process.

The exact processes behind the correlation between chromosomal abnormalities and holoprosencephaly are still not fully understood, Therefore, our aim in this study is to estimate ultrasonographic factors associated with chromosomal abnormality in a large contemporary prenatal cohort.

Methods

This study is designed as a retrospective chart review conducted at a fetal medicine center. Pregnant women who had an ultrasound scan at our center between 2014 and 2024 constituted the study population. Women who received a diagnosis of fetal holoprosencephaly were included to the study. A keyword search of electronic ultrasound database identified cases. Maternal and fetal characteristics, prenatal ultrasound characteristics, pregnancy and neonatal (if applicable) outcome data were extracted from electronic medical records. Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board (Zeynep Kamil Women and Children's Diseases Training and Research Hospital) (Decision-Nr.: 44/2024).

Maternal data (age, parity, presence of diabetes), gestational age (GA) at diagnosis, results of karyotyping and molecular genetic studies were collected. Gestational age was determined based on last menstrual period (LMP) and dating by first trimester crown-lump length (CRL) if there was discrepancy or the date of LMP was unknown. Detailed anatomy scan for additional anomalies has been performed to all fetuses when the diagnosis of holoprosencephaly is established as per routine practice at our center. All cases were offered prenatal genetic testing. Testing consisted of karyotype analysis, chromosomal microarray analysis (CMA) and exome sequencing -but CMA and exome sequencing were available at our institution only after 2021. We analyzed outcome of pregnancy (termination of pregnancy (TOP), GA at TOP, intrauterine fetal demise (IUFD), livebirth) and the neonate (GA at delivery, birthweight, mortality and survival). Neonatal death was defined as death in the first 28 days of life, and infant death as death in the first year.

Holoprosencephaly was classified as alobar, lobar and semilobar. Ultrasonographic diagnosis was made when a single ventricular cavity and absent

midline structures, such as the interhemispheric fissure, falx cerebri, corpus callosum, and CSP was encountered (alobar), when the anterior horns of the lateral ventricles and the septum pellucidum are absent, but the posterior horns of the lateral ventricles are well developed (semilobar), and when the anterior horns of the lateral ventricles are fused with absent septi pellucidi, the roof of the frontal horns is flat, and the fornices are fused in the presence of the interhemispheric fissure and falx cerebri (lobar).[13] All ultrasonographic measurements (biometric and neurosonographic when necessary) were done initially by fetal medicine fellows, and confirmed by a fetal medicine specialist (O.D.), using either a General Electric E6 Voluson ultrasound system (GE Medical Systems, USA) or a Samsung RS85 ultrasound system (Samsung Healthcare, South Korea).

Measures of association for categorical variables were analyzed with Chi-square and Fisher Exact test. Logistic regression analysis was performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs) of binary outcomes. Skewed distributions of continuous variables in groups were compared by Wilcoxon-Rank Sum test. All analyses were performed using STATA software, version 18.0 Basic Edition (Copyright 1985-2021 StataCorp LLC). A p-value of <0.05 was considered statistically significant.

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist was followed to ensure comprehensive reporting.[14]

Results

The keyword search yielded 55 results, examined between January 2014 and January 2024. Five were keyword mismatches (eg. the ultrasound report stated "not holoprosencephaly" or holoprosencephaly was in the differential diagnosis) and three had incomplete outcome data, therefore excluded. A total of 47 holoprosencephaly cases were included and analyzed. Frequency of cases at our center based on the year is demonstrated in Fig 1.

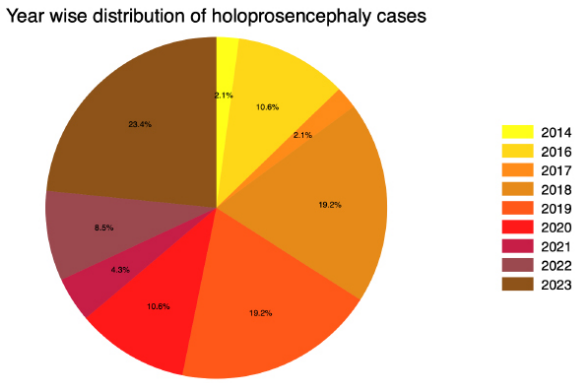

Fig 1. Years wise distribution of holoprosencephaly cases

Table 1. Clinical features and outcomes of cases (n=47)

Gravidity	2 (1-3)
Parity	1 (0-1)
Maternal age (years)	30±7
Gestational age at the time of diagnosis/ referral (weeks)	16 (13-21)
Genetic abnormality[a]	17 (63)
Abnormal karyotype	15 (55.5)
CNV[b]	1 (14.3)
Abnormal exome sequencing result[c]	1 (50)
Outcome of pregnancy	
Termination of pregnancy	32 (68.1)
IUFD	8 (17)
Livebirth	7 (14.9)
Gestational age at the time of delivery (weeks)	35±5
Birthweight (gram)	2105±848
Postnatal death[d]	5 (71.4)
Neonatal death	4 (57.1)
Infant death	1 (14.3)

Data presented as median (interquartile range), mean±standard deviation or n (percentage).
[a] Out of 27 who had genetic testing.
[b] Out of 7 who had chromosomal microarray analysis.
[c] Out of 2 who had exome sequencing.
[d] Out of 7 who were liveborn.
Abbreviations: CNV, copy number variant; IUFD, intrauterine fetal demise.

Clinical characteristics and neonatal outcomes are presented in Table 1. Maternal diabetes was present in 4 (8.5%) cases. From the total of 47 cases (any type of (57.5%) patients had prenatal invasive testing for genetic diagnosis. In all cases, standard

karyotyping were performed. Of the 27 cases with diagnostic procedure performed, in 7 cases CMA was added and in 2 both CMA and exome sequencing were added. Among those who underwent genetic testing, 63% (17/27) had a genetic abnormality. There were 11 cases of trisomy 13 (40.7%), 3 cases of trisomy 18 (11.1%) and one case of triploidy (3.7%). Among 7 cases who underwent CMA, one had a copy number variant (CNV): 98.3 megabase (mb) mosaic gain at 3q.12.1.q29 region, 27.1 mb mosaic gain at 13q12.11q14.2 region and 21.1 mb mosaic gain at 13q21.1q22.2 region. Two cases underwent exome sequencing after normal karyotype and CMA results, an one of those two had an abnormal exome sequencing result: a missense variant in SALL1 gene, this variant was associated with Townes-Brocks syndrome 1 and Townes-Brocks branchiootorenal-like syndrome in the OMIM database.

Table 2. Additional fetal ultrasonographic findings in holoprosencephaly cases with trisomy 13 and 18

Holoprosencephaly and trisomy 13 (n=11)	Central nervous system	1 (9.1)
	Agenesis of corpus callosum	1
	Vermian hypoplasia	1
	Face and neck	8 (72.7)
	Arhinia	3
	Proboscis	2
	Cyclopia	1
	Hypotelorism	3
	Microphtalmia	2
	Exophtalmus	1
	Cleft lip and palate	4
	Persistent hyperplastic primary vitreus	1
	Cystic hygroma	2
	Cardiac	6 (54.5)
	Ventricular septal defect	3
	Atrioventricular septal defect	2
	Hypoplastic left heart sydrome	1
	Gastrointestinal system	2 (18.2)
	Omphalocele	1
	Liver calcifications	1
	Urogenital system	2 (18.2)
	Hyperechogenic kidney	1
	Urinary tract dilatation	1
	Skeletal	2 (18.2)
	Polydactyly	2
	Clinodactyly	1
	Clubfoot	1
Holoprosencephaly and trisomy 18 (n=3)	Central nervous system	1 (33.3)
	Encephalocele	1
	Face and neck	2 (66.7)
	Hypotelorism	1
	Cystic hygroma	1
	Cleft lip and palate	1
	Cardiac	2 (66.7)
	Aortic hypoplasia	1
	Atrioventricular septal defect	1
	Aberrant right subclavian artery	1
	Skeletal	2 (66.7)
	Clenched hand	1
	Clubhand	1
	Clubfoot	1

Data presented as n(percentage). Percentages represent the frequency of that group of anomaly, some cases have more than one anomaly from the group, therefore sum of individual anomalies might not add up to the sum of anomaly as a group.

Table 3. The association of genetic abnormalities with additional ultrasonographic anomalies

	Any genetic abnormality (n=17)	No genetic abnormality (n=10)	p
Central nervous system	3 (50) *2 cases of alobar, 1 lobar	3 (50) *3 cases of lobar	0.46
Facial	15 (75) *11 cases of alobar, 2 lobar, 2 semilobar	5 (25) *4 cases of alobar, 1 semilobar	0.03
Cardiac	13 (100) *9 cases of alobar, 2 lobar, 2 semilobar	0	<0.001
Gastrointestinal	4 (100) *3 cases of alobar, 1 lobar	0	0.09
Renal	2 (100) *1 case of alobar, 1 lobar	0	0.26
Skeletal	4 (66.7) *2 cases of alobar, 1 lobar, 1 semilobar	2 (33.3) *1 case of alobar, 1 semilobar	0.83

Data presented as n (percentage).

Genetic outcomes based on type of HPE are demonstrated in Fig 2. Overall, 4 cases of HPE were isolated. Alobar holoprosencephaly was the predominant type (35/47, 74.5%) in the cohort, with lobar (7/47, 14.9%) and semilobar (5/47, 10.6%) types being less common. Among 11 cases with trisomy 13, 7 (63.6%) had alobar, 2 (18.2%) had semilobar and 2 (18.2%) had lobar type holoprosencephaly. All three of trisomy 18 cases had alobar holoprosencephaly. Overall, 14 (29.8%) had additional central nervous system anomalies, 31 (65.9%) facial anomalies, 20 (42.6%) cardiac anomalies, 8 (17%) gastrointestinal anomalies, 5 (10.6%) renal anomalies, and 11 (23.4%) skeletal malformations, with facial anomalies being the most common group accompanying holoprosencephaly. Univariate logistic regression analysis have shown a 7.5-fold increase in any genetic abnormality if an additional facial anomaly were present (OR= 7.5, 95% CI 1.1-51.5). Table 2 represents accompanying anomalies to HPE in cases of trisomy 13 and 18. Eleven (23.4%) of cases had increased nuchal translucency (>95th percentile). Table 3 demonstrates the comparison of frequencies of each group of additional anomalies based on presence of any genetic abnormality.

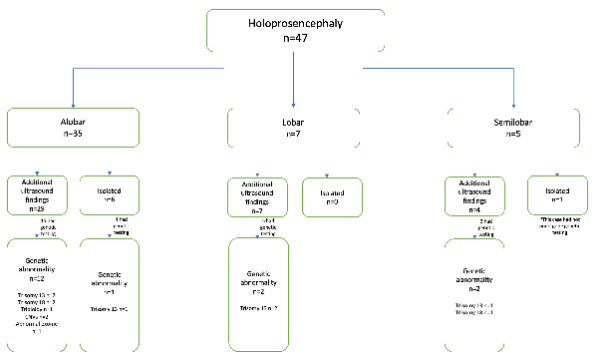

Fig 2. Flow chart of the cohort based on holoprosencephaly type

Thirty-two (68.1%) of families opted for termination of pregnancy. Seventeen (53.1%) of them had prenatal genetic testing and had an abnormal result. Eight (25%) had prenatal genetic testing and did not have an abnormal result. Seven (21.9%) did not undergo prenatal genetic testing. Parents were more likely to choose termination of pregnancy if a genetic abnormality was present (68% vs. 32%, p=0.05) or the diagnosis was made at the first trimester (56.3% vs. 43.7%, p=0.05). Also, presence of an additional ultrasonographic facial finding was more prevalent among terminated cases (78.1% vs. 21.9%, p=0.01). None of the 8 cases resulted in IUFD had prenatal diagnostic testing.

Only 7 of the cases were live born, and 5 of them died in the postnatal period. However, two of the live born cases were still alive by the time this study was finalized. Both had lobar holoprosencephaly and accompanying dysgenetic corpus callosum. Both underwent genetic testing, karyotypes were normal and CMA did not yield any pathologic result. Exome sequencing was not performed. Postnatal MRi confirmed lobar holoprosencephaly in both cases. The infants were one and two years old by the time this study was finalized.

Discussion

Our results once again show that holoprosencephaly is highly associated with genetic abnormalities, mostly aneuploidies and particularly trisomy 13. Facial anomalies commonly accompany holoprosencephaly, and are associated with genetic abnormalities, therefore a high termination rate.

Nearly half of the cases with holoprosencephaly have some kind of chromosome abnormality, with trisomy 13 accounting for 75%, triploidy for 20% and trisomy 18 for 2%.[5,15] Our results confirm the existing numbers, with a 55.5% rate of aneuploidies among the cohort. Trisomy 13 and holoprosencephaly are undeniably interconnected, with trisomy 13 constitiutes 75% of holoprosencephaly cases.[5] Our prevalence of trisomy 13 among the cohort (40.7%) was lower than the existing literature, This might be attributed to the fact that some of the cases with multiple anomalies accompanying holoprosencephaly were terminated already due to the probable adverse prognosis, before any genetic investigation per family preference. If the decision of termination is definite for the parents, some may choose not to prolong the process any further by

diagnostic procedures and waiting for the results. With 3 cases among the 27 who had prenatal genetic testing, trisomy 18 was the second most common genetic abnormality in the cohort, and one case of triploidy follows, as generally reported.[16,17] The specific mechanism through which aneuploidies can lead to holoprosencephaly has not been clearly defined. However, it is interesting to note that ZIC2 and TGIF genes are located on chromosomes 13q32 and 18p11.2, respectively.[18,19]

Alobar type was the most common among trisomy 13 with a 64% frequency. The reported survival rate during the first year of life may reach 54% for cases of solitary HPE without severe facial deformities, even in the alobar type.[20] One can speculate that these isolated cases without facial anomalies would be less likely to have trisomy 13. Three of our cases with alobar holoprosencephaly was live born, yet died in the first year of life- the longest survival was 210 days. None of those cases had genetic testing and all had multiple craniofacial anomalies, therefore it might be safe to say that the neonates probably had a genetic abnormality. None of the fetuses with known trisomy 13 and alobar holoprosencephaly were live born, so we are unable to draw conclusions on survival. However, both of the two surviving cases had lobar holoprosencephaly, the type associated with better survival and neurodevelopmental outcomes.[16] Besides the anatomic type, survival rates have been found to be associated with genetic results, non-syndromic, euploid holoprosencephaly cases generally have better overall outcomes. [21] In line with these reports, both of our surviving lobar holoprosencephaly cases were euploid and had no CNVs.

Facial anomalies, though varying greatly, were usually seen concomitantly with holoprosencephaly (65.9%), and also was significantly associated with genetic abnormalities. Our cohort encompassed a great spectrum of facial anomalies, including arhinia, proboscis, cyclopia, hypotelorism, microphtalmy, exophtalmus and facial clefts (Table 2). Nevertheless, we should be aware of confirmation bias regarding subtle anomalies which may be impacted by subjective evaluation. For example, isolated hypotelorism is almost never diagnosed in-utero.[22] Yet, as fetal medicine specialists, we specifically prioritize the identification of hypotelorism when diagnosing fetal holoprosencephaly due to our extensive understanding of the frequent association between facial defects and this condition. Severe abnormalities such as facial clefts or proboscis are easily identifiable and not open to debate. But we should be cautious not to overstate minor variations and instead rely on established diagnostic criteria.

Montaguti et al. reported that 4 (66.7%) of the 6 alobar holoprosencephaly cases with known aneuplodies had accompanying cardiac anomalies.[23] We have reported that accompanying cardiac anomalies were associated with an abnormal genetic testing result, with 13 of 17 cases with a chrosomal abnormality, CNV or a gene variant detected in exome sequencing had a cardiac abnormality. Overall, nearly 43% of our cohort had additional cardiac anomalies, which is greater than the reported frequencies of 4-8%.[24,25] This could be attributed to the practice of comprehensive ultrasonographic examinations of the fetal heart, including during the first trimester, at our institution. This leads to the detection of subtle findings like ventricular septal defects and therefore increase the number of patients with cardiac anomalies.

Autopsy confirmation is lacking in our study, which is a limitation, due to societal apprehensions and general religious beliefs of parents regarding autopsy. This may introduce a bias into our analysis, potentially leading to an inaccurate estimation of the detection rate, by overestimating. However, the majority (approximately 75%) of the cohort consisted of alobar cases, which is the type rarely confused with other cranial anomalies. Another limitation is the lack of of exome sequencing in most of the cases, exome sequencing was available only after 2022 at our instituition. Knowing the strong relationship of holoprosencephaly with variants in SHH, SIX3 and ZIC2 genes, widespread use of exome sequencing

would yield more results, therefore lead us to a better understanding of etiologic factors.

Conclusion

Holoprosencephaly is highly associated with aneuploidies, particularly trisomy 13. Accompanying facial and cardiac anomalies to holoprosencephaly are associated with genetic abnormalities. Thorough investigation for additional anomalies and genetic etiologies is essential for parental counseling, and could have an impact on decision of termination.

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