Table 4 Monogenic syndromes in which CDH occurs and chromosomal aberrations are detected

Syndrome	Band	Gene	OMIM	Number of deletions in patients[a]
Cornelia de Lange syndrome	5p13.2	*NIPBL*	608667	0/28
	10q25.2	*SMC3*	606062	0/28
Denys–Drash syndrome	11p13	*WT1*	607102	0/28
Donnai–Barrow syndrome	2q31.1	*LRP2*	600073	0/28
Matthew–Wood syndrome	15q24.1	*STRA6*	610745	0/28
Spondylocostal dysostosis	19q13.2	*DLL3*	602768	0/28
	15q26.1	*MESP2*	605195	0/28
	7p22.2	*LFNG*	602576	0/28
	17p13.1	*HES7*	608059	0/28

[a] Number of patients in whom deletions for each gene were calculated by the CNstream program

Subsequently, we compared the location of these segments with loci at which chromosomal abnormalities have been frequently reported in non-isolated CDH (Table 5) [6, 7, 9, 18–30]. Although several segments overlapped with these loci, most of the overlapping regions were considered to be experimental artifacts after examination of the logR ratio and B allele frequency using BeadStudio platform. However, one region on 6q27 between cnvi0107052 and rs7767980; Chr6:169,866,304–169,884,311 (NCBI36/hg18) was obtained as deleted segment in two non-isolated CDH patients. One of these patients was clinically diagnosed with Fryns syndrome, and the other exhibited a dysmorphic face. Both of these patients died from having severe hypoplastic lungs. No common CNV in this region was found in the Database of Genomic Variants (DGV) (http://www.projects.tcag.ca/variation/) [31]. This 18-kb deleted region includes exon 4 of the *t-complex-associated-testis-expressed 3* (*TCTE3*) gene.

Discussion

Although there is a great deal of evidence implicating a genetic etiology in CDH and several genes have been shown to cause abnormal diaphragm development in mice, few CDH-related mutations have been identified in humans. Identifying chromosomal abnormalities in patients with non-isolated CDH has helped to map the candidate regions that might harbor CDH-related genes. Recently, array-based techniques have been widely used to detect micro-chromosomal changes that were previously undetectable karyotypically and to delimit the candidate regions.

Table 5 Frequently reported loci in which CDH occurs and chromosomal aberrations are detected

Locus[a]	dup, del	Study author	Number of deletions in patients[b]		
			370K	550K	660K
1q41–q42.12	del	Slavotinek et al. [7]	0/14	0/3	0/11
		Kantarci et al. [6]			
		Youssoufian et al. [18]			
3q21–q23	del	Wolstenholme et al. [19]	0/14	0/3	0/11
4q31.1–q32.3	del	Young et al. [20]	0/14	0/3	0/11
	del, dup	Celle et al. [21]			
6pter–p25.1	del	Batanian et al. [22]	0/14	0/3	0/11
		Baruch et al. [23]			
6q23–qter	del	Krassikoff et al. [24]	0/14	0/3	2/11[a]
		Shen-Schwarz et al. [25]			
8p23.1	del	Shimokawa et al. [26]	0/14	0/3	0/11
		Slavotinek et al. [7, 27]			
8q22.1–q24.13	del	Temple et al. [28]	0/14	0/3	0/11
15q23–q24.3	del	Sharp et al. [29]	0/14	0/3	0/11
15q26.1–q26.2	del	Scott et al. [9]	0/14	0/3	0/11
		Slavotinek et al. [7, 27]			
		Klaassens et al. [30]			

del deletion, *dup* duplication

[a] Frequently reported locus

[b] Number of patients in whom deletions within each locus were calculated by the CNstream programs

In the present study, we performed high-resolution CNV analysis using SNP arrays and a CNstream program specifically designed for Illumina microarrays. We detected the deletion of an 18-kb segment on 6q27 in two non-isolated patients but not in 100 normal controls. This small genomic region is not listed on the DGV. This region contains the *TCTE3* gene, which belongs to the dynein light chain family. The mouse homolog *TCTE3* gene is located in the *T/t*-complex of mouse chromosome 17. The *T/t*-complex is a genomic variant in mouse populations, and the t haplotype contains recessive lethal mutations that affect embryonic development [32]. *TCTE3*(−/−) mice have been generated, but they showed no apparent anomalies except for reduced flagellar motility [33, 34]. Therefore, a direct link between *TCTE3* and CDH was not evident in the mouse. However, *TCTE3* encodes a putative light chain of the outer dynein arm of cilia. The fact that human diseases caused by ciliary dysfunction show various phenotypes, including skeletal defects [35], suggests that this gene could be a candidate genetic factor responsible for some cases of CDH.

The length of the detected region was only 18 kb, and it is difficult to detect such a short segment using low-resolution analyses. Analyses with 660K or higher resolution became available only a few years ago, and the difference in resolution may explain why the deletion was undetected in the 370K and 550K arrays.

Many reports suggest that the retinoid signaling pathway plays an important role in the development of CDH. Of the genes involved in this pathway, *COUP-TFII* has been considered a particularly strong candidate because this gene was within the minimal region delimited by Klassenes et al. [30]. Slavotinek et al. [7] and Scott et al. [9] have performed sequencing analysis of *COUP-TFII* in a large number of CDH patients, but no mutations in this gene were found. On the other hand, a mutation and variants of *FOG2* and *HLX* genes were reported in isolated CDH cases [15–17]. In the present study, microdeletions in the genomic regions containing these genes were not identified. It is possible that sequence changes, but not segmental deletions of these genes, are responsible for the development of CDH. Another possibility is that the sensitivity of the array used in this study was limited by array's probe density. Combinatory analyses using re-sequencing and higher resolution array technologies will be required to better understand the genetic architecture involved in the pathogenesis of CDH.

Conclusion

A focal deletion of an 18-kb genomic region containing a part of the *TCTE3* gene on 6q27 was identified in two non-isolated CDH patients. Because the *TCTE3* belongs to the dynein light chain family and human diseases caused by ciliary dysfunction show various phenotypes (including skeletal defects), this gene could be a candidate genetic factor responsible for some cases of CDH.

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Conflict of interest The authors have nothing to disclose.

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ORIGINAL ARTICLE

Effect of insulin-like growth factors on lung development in a nitrofen-induced CDH rat model

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Abstract
Purpose Both the mortality and morbidity associated with congenital diaphragmatic hernia (CDH) are mainly caused by pulmonary hypoplasia and persistent pulmonary hypertension. A previous study revealed that insulin-like growth factors (IGFs) play important roles in fetal lung development. The aim of this study was to investigate the effect of IGF-1 and IGF-2 on tissue cultures of fetal hypoplastic lungs obtained from nitrofen-induced CDH model rats.
Methods Pregnant rats were exposed to nitrofen on day 9 of gestation (D9). Fetuses were harvested on D18 by caesarian section. Lung specimens of the CDH (+) fetus were divided into three groups; control, IGF-1, and IGF-2. The specimens from the control group were cultured in culture medium without IGFs. The IGF-1 group specimens were cultured with IGF-1 (500 ng/ml), and those in the IGF-2 group were cultured with IGF-2 (500 ng/ml). The mRNA expression of TTF-1, T1α and α-SMA were analyzed in each group using real-time RT-PCR after 24 and 48 h of incubation. Immunohistochemical staining of these markers was also assessed for each of the cultured specimens.
Results There was a significant increase in the expression of both TTF-1 and T1α mRNA in the IGF-2 group, in comparison to the control group after 48 h of culture. Immunohistochemical staining revealed that the cell morphology was changed from cuboidal to squamous type in the IGF-2 group.
Conclusions An increased mRNA expression of the markers related to type 1 and 2 alveolar epithelial cells, and morphological changes in the epithelial cells were observed in the IGF-2 group. The administration of IGF-2 to nitrofen-induced hypoplastic lungs might lead to alveolar maturation, which thus results in their improved development.

Keywords Insulin-like growth factor · Lung development · Pulmonary hypoplasia · Congenital diaphragmatic hernia · Nitrofen

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Introduction

Congenital diaphragmatic hernia (CDH) still remains a challenge for neonatal surgeons. According to reports about the current postnatal therapies, the overall survival rate is now over 70% at some institutions [1, 2]. Nevertheless, CDH patients with severe pulmonary hypoplasia still have high mortality and morbidity rates [3]. To improve the outcome in such CDH patients with severe pulmonary hypoplasia, fetal treatment in order to accelerate the maturation process of hypoplastic lungs, especially in the later stages of pregnancy, is thought to be essential.

Various growth factors are considered to play important roles during the process of pulmonary organogenesis [4]. Among such growth factors, the effect of insulin-like growth factors (IGFs) on organ development has been

indicated throughout the perinatal period [5–11]. In addition, IGFs are known to be endocrine hormones that affect somatic changes, and have also been shown to affect cell proliferation and differentiation in some tissues via either autocrine or paracrine mechanisms [6]. In our previous study, the administration of either IGF-1 or IGF-2 to mouse lung explants demonstrated an increased in the number of type 2 alveolar epithelial cells (AECs-2) [12]. The biological actions of IGFs are transduced through specific surface receptors, insulin-like growth factors I receptor (IGF-1R), insulin-like growth factors-2 receptor (IGF-2R) and the insulin receptor (IR) [13]. Recent studies have revealed that these three receptors are all down-regulated in the hypoplastic lungs of the nitrofen-induced CDH model rat [14, 15]. Therefore, the IGFs are thought to be involved in the proliferation and differentiation of alveolar cells in fetal rat lungs.

We designed this study to investigate the hypothesis that the administration of IGFs improves the proliferation and differentiation during the lung development of the nitrofen-induced CDH model in the later stages of gestation. We investigated the mRNA expression of the markers of type 1 alveolar epithelial cells (AECs-1) and AECs-2 by real-time reverse transcription polymerase chain reaction (RT-qPCR). In addition, the localization of these marker proteins was also examined by immunohistochemical staining.

Materials and methods

Experimental animals

Pregnant Sprague-Dawley rats were purchased from a commercial breeder (Japan SLC, Inc., Shizuoka, Japan). The day when the vaginal plug was confirmed was designated as day 0 of gestation (D0). On D9 (term = D22), 100 mg nitrofen (Wako Pure Chemical Industries, Ltd., Osaka, Japan), which was dissolved in 1 ml of olive oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan), was given intragastrically. Fetuses were harvested on D18 by caesarian section. Lung specimens were dissected out from the CDH (+) fetuses, and were incubated in various media using an organ culture system.

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Kyushu University (approval No. A21-115-0).

Total RNA extraction and real-time RT-PCR

Total RNA was extracted from each specimen using the TRIzol® Plus RNA Purification Kit (Invitrogen, CA, USA) and PureLink™ DNase Set (Invitrogen, CA, USA) according to the manufacturer's instructions. First-strand cDNA was synthesized using the SuperScript™ III First-strand Super Mix (Invitrogen, CA, USA). All samples were subjected to PCR amplification and were normalized to β-actin. All PCR reactions were performed using the LightCycler® Fast Start DNA Master SYBR Green I kit (Roche Applied Science, Mannheim, Germany). The accession numbers, primer sequences and product sizes for thyroid transcription factor-1 (TTF-1), podoplanin (T1α), α-smooth muscle actin (α-SMA) and β-actin are all listed in Table 1.

Tissue culture

Left lungs were dissected from D18 CDH (+) fetuses and were sliced into several specimens. Each specimen was placed on a polyethylene terephthalate filter (Falcon 353093 cell-culture insert, 8 μm pore size, on 353502 companion plate: BD, NJ, USA) and then was assigned to either the control, IGF-1, or IGF-2 group. The control group ($n = 6$) was cultured in DMEM/F-12 medium (Invitrogen, CA, USA) containing penicillin–streptomycin (100 U/ml and 100 mg/ml: Invitrogen, CA, USA), and L-ascorbic acid (150 μg/ml: Nacalai Tesque, Inc., Kyoto,

Table 1 Real-time RT-PCR primers

Gene	Accession number	Sequence (5′–3′)	Product size
TTF-1			
Forward	NM_013093.1	AAATTTGGGGGTCTTTCTGG	128
Reverse		AGAGTGCATCCACAGGGAAG	
T1α			
Forward	NM_019358.1	AAGCCTCAGATGACTCAATGACC	164
Reverse		ACTTTCTGAGCATCTGGGATGAG	
α-SMA			
Forward	NM_031004.2	GCTTCCTCTTCTTCCCTGGAG	105
Reverse		AGATGGCTGGAAGAGGGTCTC	
β-Actin			
Forward	NM_031144.2	TTGCTGACAGGATGCAGAAG	108
Reverse		TAGAGCCACCAATCCACACA	

Japan). The IGF-I group ($n = 6$) was cultured in media supplemented with 500 ng/ml of recombinant human IGF-1 (291-G1, R&D Systems, Inc., MN, USA). The IGF-2 group ($n = 6$) was cultured in media with recombinant human IGF-2 (292-G2, R&D Systems, Inc., MN, USA). These cultures were incubated for 24 or 48 h. The mRNA expression of TTF-1, as a marker of AECs-2, T1α, as a marker of AECs-1, and α-SMA, as a marker of smooth muscle, were analyzed in each group and were compared.

Immunohistochemistry

The cultured specimens were fixed in 4% paraformaldehyde phosphate buffer solution for 24 h. Thereafter, they were embedded in paraffin. Paraffin sections (5 μm) were dehydrated in xylene, rehydrated through alcohol, and incubated in methanol with 3% H_2O_2 to block endogenous peroxidase. All specimens were pretreated in 10 mM citrate buffer (pH 6.0) for 15 min using a microwave. The sections were then incubated with 10% normal goat serum and incubated overnight at 4°C with the primary antibodies. The lung specimens were stained with mouse monoclonal antibody to TTF-1 (sc-56606: Santa Cruz Biotechnology, Inc., CA, USA: dilution 1:50) to detect the AECs-2. T1α (11-035, anti-rat podoplanin monoclonal antibody: AngioBio Co., CA, USA: dilution 1:100) was stained to clarify the expression patterns of the AECs-1. In addition, an α-SMA antibody (monoclonal anti-α-SMA (1A4) antibody, Sigma, MO, USA: dilution 1:400) was also used to determine the degree of vascular development in the fetal lung (Table 2). These sections were then washed in phosphate-buffered saline (PBS) and incubated with biotinylated goat anti-mouse immunoglobulin G antisera. Then samples were washed in PBS and incubated with peroxidase-conjugated streptavidin. Immunohistochemical signals were visualized using 3,3′-diaminobenzidine substrate. Slides were counterstained with hematoxylin and examined by standard microscopy.

Statistical analysis

The mRNA expression of TTF-1, T1α and α-SMA from each of the groups at each culture time was analyzed and compared using a Student's t test. A value of $P < 0.05$ was considered to be statistically significant.

Results

Relative mRNA expression levels of TTF-1, T1α and α-SMA

There was a significant increase in the expression of both TTF-1 and T1α mRNA in the IGF-2 group at 48 h of culture compared to the controls ($P = 0.018$ and 0.016) (Fig. 1a, b). The mRNA expression of α-SMA also increased in the IGF-2 group, but it did not reach statistical significance, compared to the control ($P = 0.06$) (Fig. 1c). At 48 h of culture, a significant difference in TTF-1 and T1α mRNA expression levels was also observed between the IGF-1 and IGF-2 groups ($P = 0.018$ and 0.008). However, no significant difference was observed between the IGF-1 group and the controls at any of the time points examined (Fig. 1a–c).

Table 2 Antibodies for immunohisitochemical staining

Marker	Clone	Working dilutions	Antigen retrieval	Source
TTF-1	SPM150	1:50	Citrate	Santa Cruz (CA, USA)
T1α	(11-035)	1:100	Citrate	AngioBio (CA, USA)
α-SMA	1A4	1:400	Citrate	Sigma (MO, USA)

Immunohistochemical analysis of the lungs after 48 h of culture

The TTF-1 protein, as a marker of AECs-2, was localized in the nuclei of the main bronchial and respiratory epithelial cells at all time points (Fig. 2a–c). The cuboidal TTF-1 positive cells on the distal lung epithelia decreased in number in the IGF-2 group. There were morphological changes in the TTF-1 positive cells, from a cuboidal to flattened form, only in the IGF-2 group (Fig. 2c, arrow).

The T1α protein, as a marker of AECs-1, was located on the surface of the epithelia in distal lung buds (Fig. 2d–f). There were no significant differences in the distribution among these groups with regard to the T1α staining.

The α-SMA was mainly distributed in the muscle layers of both arteries and bronchi. The protein was also located in the mesenchymal cells of the alveolar septa (Fig. 2g–i). There was no apparent difference in the α-SMA distribution among the three groups.

Discussion

The high mortality rate of human severe CDH patients is caused by hypoplastic lungs and the associated persistent pulmonary hypertension [3]. In hypoplastic CDH lungs, the maturation of epithelial cells is delayed, which is thought to be due to arrested development during the canalicular or saccular stage of the lung development [16, 17]. A reduction in bronchial divisions occurs in the hypoplastic lungs, resulting in the inhibition of a secondary increase in respiratory bronchi and the number of alveoli [18]. The alveoli in the hypoplastic lung were shown to be an

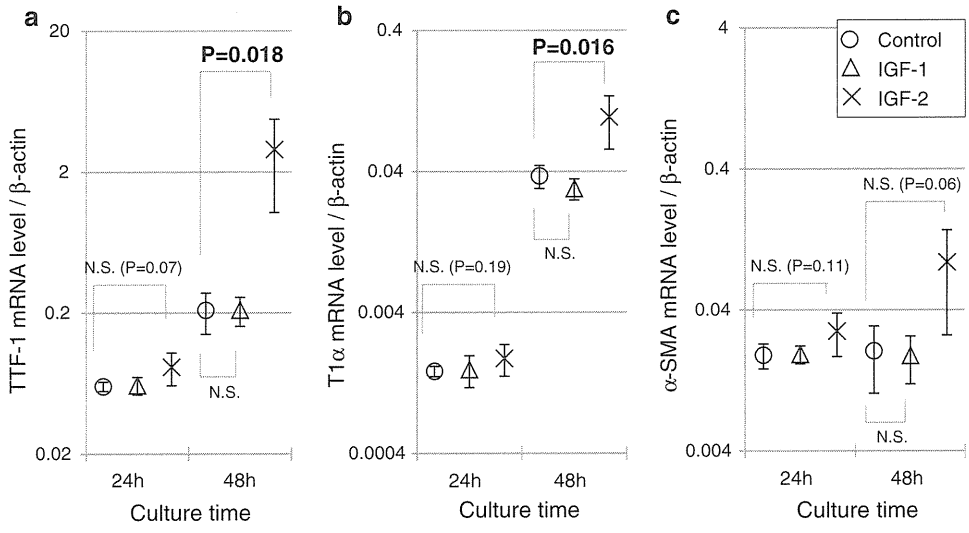

Fig. 1 Relative mRNA expression levels of TTF-1, T1α and α-SMA at 24 and 48 h of culture. The messenger RNA expression levels of TTF-1 (**a**), T1α (**b**), and α-SMA (**c**) are shown as averages (*open circle*, *open triangle*, *multi symbol*, respectively) and 95% confidence intervals (*bars*). Each chart has two time points (24, 48 h) and three groups (control, IGF-1, IGF-2). The expression levels of TTF-1 and T1α were significantly increased ($P = 0.018$ and 0.016) in the IGF-2 group at 48 h of culture, compared to the controls. The mRNA expression of α-SMA also was increased in the same group, although the increase did not reach statistical significance

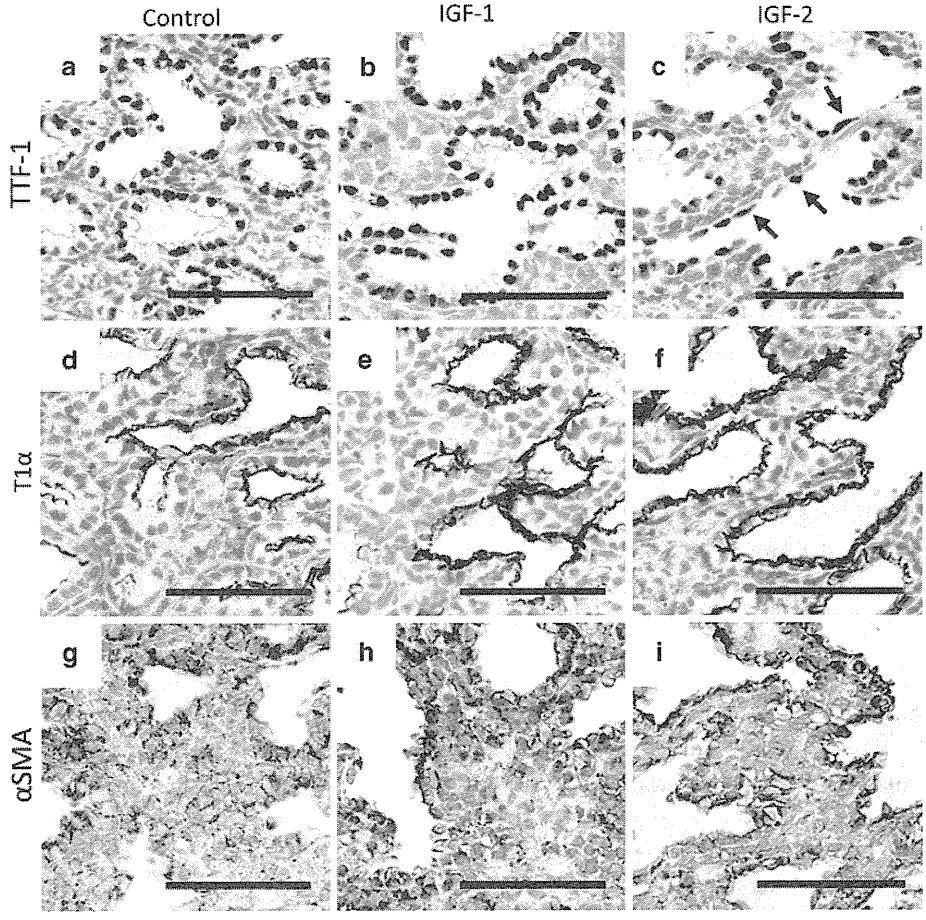

Fig. 2 An immunohistochemical analysis of the effect of IGFs at 48 h of culture. Immunohistochemical staining of TTF-1, T1α and αSMA was performed on the cultured specimens. In the TTF-1 staining (**a**–**c**), the number of cuboidal TTF-1 positive cells decreased in the IGF-2 group, and were replaced with the squamous type cells (**c**, *arrows*) after 48 h of culture. Such an effect was not observed in the other groups (**a**, **b**). There were no significant differences in either T1α (**d**–**f**) or α-SMA (**g**–**i**) staining among the groups. *Scale bars* 50 μm

immature form with thickened intraalveolar septa. The thickened intraalveolar septa cause a reduced capillary–air interface, which is essential for gas exchange [19]. Improvements in these factors are essential for improving the fetal outcome.

The differentiation from AECs-2 into AECs-1 is one of the key processes that occurs during the late gestational stage of lung development [20]. In addition, this differentiation has been reported to be impaired in the nitrofen-induced CDH model rat on D21 [21]. Retinoic acid has been demonstrated to be a promoter for this differentiation and also promotes pulmonary alveologenesis [22, 23]. In our previous study, the administration of either IGF-1 or IGF-2 to the cultured lungs of normal mouse fetuses at late gestation also demonstrated increased numbers of AECs-2 [12]. Therefore, we thought that the IGFs might induce the differentiation of alveolar epithelial cells during the late fetal period.

IGF-1 and IGF-2 are small peptide hormones which share 62% of their sequences, and they are homologous to proinsulin. IGF-1 and IGF-2, their receptors, and binding proteins, are all expressed in the fetal lungs of humans, rodents, and other species [8]. In addition, previous studies have shown that IGFs play important roles, especially in the late stage of lung development [7–10]. The deletion of either IGFs or IGFRs in transgenic mice causes the pulmonary hypoplasia or delayed lung maturation, respectively, thus resulting in fetal respiratory failure and perinatal death in both transgenic types [7–11]. In the targeted disruption of IGF-1, the phenotype of the mice included the following morphological findings: a lower airway volume, retarded epithelial growth, and a failure of capillary remodeling. Therefore, about 60% of IGF-1 knockout mice die because of respiratory failure during the perinatal period [7]. On the other hand, IGF-2 knockout mice demonstrate immature lungs with thicker alveolar septa and poorly organized alveoli, in comparison to the lungs of wild type mice [8]. Such mice, which had a null mutation of IGF-1R, tend to die just after birth due to respiratory failure [9]. In addition, IGF-2R knockout mice also die during the neonatal period due to the multiple organ malformations, including lung abnormalities [11]. These reports seem to support the hypothesis that addition of IGFs or an increase in the IGFs signaling in the lung might improve the outcome of CDH patients with severe pulmonary hypoplasia.

In this study, we investigated the effect of IGF-1 and IGF-2 on the fetal hypoplastic lungs taken from CDH model rat fetuses in the later stages of gestation using a tissue culture technique. Upon the administration of either IGF-1 or IGF-2 in the culture medium including the hypoplastic lung samples, only administration of IGF-2 demonstrated a significant increase in the mRNA expression of the markers related to both AECs-1 and AECs-2. Furthermore, in the immunohistochemical examination, the morphological changes of TTF-1 positive cells, from a cuboidal to squamous form, were also observed after 48 h of incubation with IGF-2. AECs-2 have been thought to be a progenitor of AECs-1, and the morphological changes of AECs are necessary for their maturation [20]. Therefore, the observed changes in cell form and the increase in the mRNA expression levels of markers of both AECs-1 and AECs-2 in the IGF-2 group are thought to reflect the maturation process of AECs. In contrast, IGF-1 did not demonstrate such an effect.

The biological functions of IGF-1 and IGF-2 are thought to be mediated through three receptors: IR, IGF-1R and IGF-2R. IGF-1R has been shown to have strong affinity for both IGF-1 and IGF-2. On the other hand, IGF-2R mainly binds IGF-2 with high affinity, while it interacts minimally with IGF-1 and insulin [24]. In the present study, only the IGF-2 group showed a positive effect on the rat CDH lung, suggesting that the effect was mediated by not only IGF-1R, but also IGF-2R. In fact, in the D18 CDH (+) rat lung, both IGF-1R and IGF-2R are shown to be distributed in both the alveolar epithelium and the mesenchymal cells [15]. Therefore, the administration of IGF-2 via amniotic fluid or an intratracheal space may be applicable to induce the improvement of lung maturation in the nitrofen-induced CDH model rat. In addition, IGF-1R and IGF-2R were both observed at term in normal rats (D21) [15]. Therefore, during the perinatal period, the rat lung might respond to the administration of IGFs.

In conclusion, our data showed that the administration of IGF-2 to nitrofen-induced hypoplastic lungs might lead to alveolar maturation in the later stages of gestation. Therefore, the administration of IGF-2 might result in improvements in lung development in premature fetuses in this stage. Based on our results, it is possible that fetal treatment with IGFs, especially IGF-2, may induce the lung maturation of hypoplastic CDH lungs. Therefore, perinatal treatment using IGF-2 might improve the outcome of severe CDH patients by improving the maturity of the hypoplastic lungs during the late fetal period.

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ORIGINAL ARTICLE

The Japanese experience with prenatally diagnosed congenital diaphragmatic hernia based on a multi-institutional review

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Abstract
Purpose To review the recent Japanese experience with prenatally diagnosed congenital diaphragmatic hernia (CDH) based on a multi-institutional survey.
Methods A multicenter, retrospective cohort study was conducted on 117 patients born between 2002 and 2007 with isolated prenatally diagnosed CDH. All patients were managed by maternal transport, planned delivery, immediate resuscitation and gentle ventilation. The primary outcome measurements were the 90-day survival and intact discharge. The examined prenatal factors included gestational age (GA) at diagnosis, lung-to-head ratio (LHR), lung-to-thorax transverse area ratio (L/T) and liver position. Physical growth and motor/speech development were evaluated at 1.5 and 3 years of age. Data were expressed as the median (range).
Results The mean GA at diagnosis was 29 (17–40) weeks. The LHR and L/T were 1.56 (0.37–4.23) and 0.11 (0.04–0.25), respectively. There were 48 patients with liver up. The mean GA at birth was 38 (28–42) weeks. The 90-day survival rate and intact discharge rate were 79 and 63%, respectively. Twelve patients had major morbidity at discharge, and 71% of these patients had physical growth or developmental retardation at 3 years of age.
Conclusion This multicenter study demonstrated that the 90-day survival rate of isolated prenatally diagnosed CDH was 79%, and that subsequent morbidity remained high.

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A new treatment strategy is needed to reduce the mortality and morbidity of severe CDH.

Keywords Congenital diaphragmatic hernia · Gentle ventilation · Prenatal diagnosis · Fetus · Multicenter study

Introduction

Congenital diaphragmatic hernia (CDH) is one of the most challenging anomalies faced by pediatric surgeons and neonatologists. During the past few decades, many innovative techniques, including high-frequency oscillation (HFO), inhaled nitric oxide (NO), extracorporeal membrane oxygenation (ECMO) and gentle ventilation (GV), have been introduced for the treatment of CDH [1, 2]. Additionally, prenatal diagnosis has also made a contribution to the improvement of the outcome of CDH [3, 4]. In many high-volume centers, immediate start of gentle ventilation following planned delivery has become the standard strategy for the treatment of prenatally diagnosed CDH. Despite these advances in fetal and neonatal care, mortality and morbidity remain high in a subset of severe CDH. To offer appropriate information to the family before birth, and to develop a multi-institutional consensus on selection criteria for fetal intervention, it is necessary to analyze the most recent outcomes of prenatally diagnosed CDH. This study was conducted to review the modern experience of prenatally diagnosed CDH treated in five Japanese centers dedicated to this condition.

Materials and methods

A multicenter retrospective study was conducted on 117 patients born between 2002 and 2007 with isolated prenatally diagnosed CDH. Patients with associated life-threatening or chromosomal anomalies were excluded. The participating centers included three children's medical centers and two university hospitals. All patients were managed by maternal transport, planned delivery, immediate resuscitation and gentle ventilation. To achieve GV, the goals of the blood gas parameters were set at $PaCO_2$ < 60–70 mmHg and pre-ductal SpO_2 > 90%. Once these gas data were obtained, ventilator settings, including FiO_2 and mean airway pressure (MAP), were decreased promptly. The upper limit of MAP was set at 18–20 cmH_2O. In each center, HFO, NO and ECMO were available from the entry criteria of each patient. Diaphragmatic repair was performed when respiratory and circulatory stabilization was achieved. The goal of the preoperative stabilization was appropriate blood pressure to keep diuresis and appropriate blood gas data (PaCO2 < 60–70 mmHg, pre-ductal SpO2 > 90%).

This study was approved by the institutional review board of the participating centers (the approved number of subjects was 314).

We reviewed the charts of all patients and their mothers to collect the following data.

Prenatal data

The prenatal data examined included gestational age (GA) at diagnosis, the presence of polyhydramnios, initial lung-to-head ratio (LHR), initial lung-to-thorax transverse area ratio (L/T) and liver position (liver up/liver down). When LHR or L/T was measured several times, the earliest data were analyzed as the initial data.

Postnatal data

Data abstracted postnatally included: GA at birth; birth weight; sex; side of defect; mode of delivery; Apgar score at 1 min; use of NO, HFO and ECMO; highest MAP; duration of mechanical ventilation; duration of oxygen supplementation; date of surgery; need for patching; date of discharge; and significant morbidity at discharge. Significant morbidity included the need for respiratory support (supplemental oxygen, mechanical ventilation), nutritional support (tube feeding, parenteral nutrition) or circulatory support (use of vasodilators).

Physical growth (height and body weight) and motor/speech development were evaluated at 1.5 and 3 years of age. Height or body weight less than −2SD was defined as physical growth retardation. The inability to walk alone was defined as motor developmental retardation. The inability to speak more than 3 words at 1.5 years or to talk normally at 3 years was defined as speech developmental retardation.

Outcome measures

The primary outcomes of the study were 90-day survival and intact discharge. Intact discharge was defined as discharge from the hospital without any of the significant morbidities mentioned above.

Comparisons

To investigate the prognostic factors, comparisons of the prenatal and postnatal data were made between the 90-day survivors and 90-day non-survivors.

Statistical analyses

Data were expressed as the median with the range. The statistical significance of differences was determined by Fisher's exact probability test or the chi-square test for categorical data and the Wilcoxon-test for continuous data. Differences with a P value of <0.05 were considered as significant.

Results

Prenatal data

The GA at diagnosis was 29 (17–40) weeks, and 24 patients had polyhydramnios. The initial LHR was 1.55 (0.37–4.23) and the initial L/T was 0.11 (0.04–0.25), measured at 31 (18–40) weeks. There were 48 patients with liver up and 69 patients with liver down.

Table 1 Postnatal data

Postnatal data	Median (range), *n* (%)
Gestational age at birth (weeks)	38 (28–42)
Birth weight (kg)	2.78 (1.04–4.04)
Sex	
Male	63 (53.9)
Female	54 (46.2)
Mode of delivery	
Vaginal	55 (47.0)
C-section	62 (53.0)
Apgar score at 1 min	4 (1–9)
HFO	
Yes	116 (99.1)
No	1 (0.9)
NO	
Yes	94 (80.3)
No	23 (19.7)
ECMO	
Yes	19 (16.2)
No	98 (83.8)
Highest MAP (cmH_2O)	14 (12–15)[a]
Side of the defect	
Left	109 (93.2)
Right	6 (5.1)
Bilateral	2 (1.7)
Diaphragmatic repair	
Yes	104 (88.9)
No	13 (11.1)
Age at repair (hours)	69 (26–101)[a]
Diaphragmatic closure	
Direct	54 (51.9)
Patch	50 (48.1)
Survivors	
Duration of mechanical ventilation (days)	20 (11–101)[a]
Duration of O_2 supplementation (days)	32 (17–54)[a]

[a] Median (interquartile range)

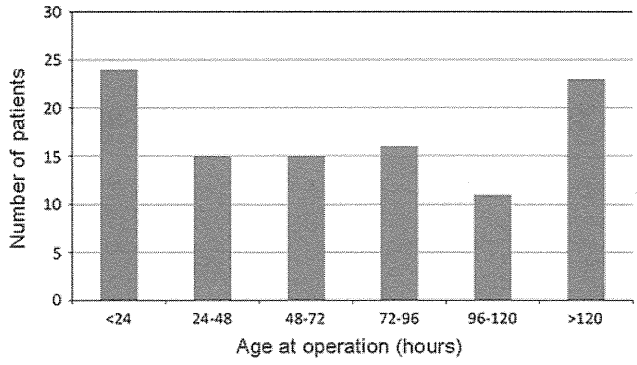

Fig. 1 Age distribution at surgery (hours). Each *bar* indicates the number of patients every 24 h after birth

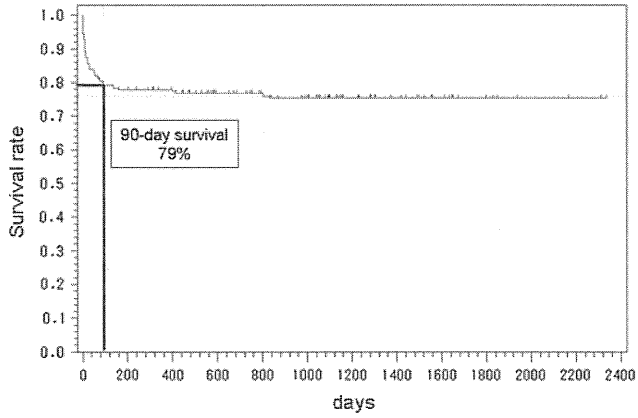

Fig. 2 The survival curve reached a plateau at 90 days. The 90-day survival rate was 79%

Postnatal data

The patients' postnatal characteristics are shown in Table 1. The GA at birth was 38 (28–42) weeks, and the birth weight was 2.78 (1.04–4.04) kg. The mode of delivery was vaginal in 55 patients and cesarean section in 62 patients. HFO was used in 116 patients (99%) and NO in 94 patients (80%). ECMO was used in 19 patients (16%); 7 of these patients survived for 90 days and 2 patients had an intact discharge. The highest MAP was 14 (12–15) cmH_2O. The side of the diaphragmatic defect was left in 109 patients, right in 6 patients, and bilateral in 2 patients. Diaphragmatic repair was performed in 104 patients (direct closure: 54 patients; patch closure: 50 patients); closure was conducted at a median of 69 h after birth. Figure 1 shows the number of patients who underwent diaphragmatic repair every 24 h after birth. The timing of surgery was almost equally distributed up to more than 120 h.

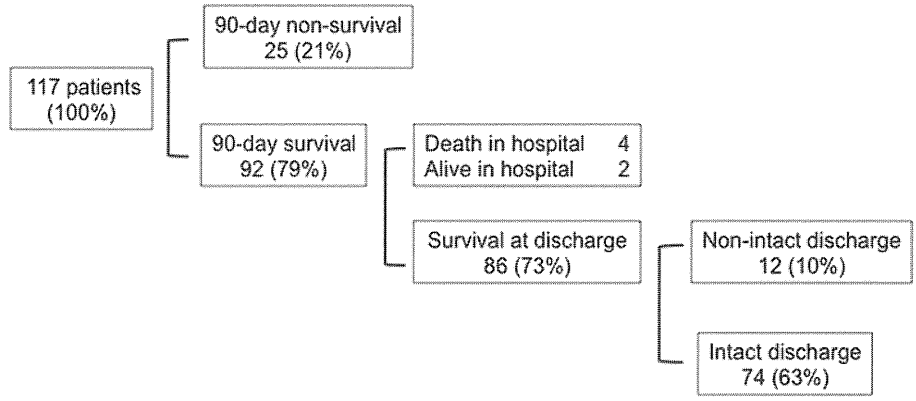

Fig. 3 Summary of the outcomes

Table 2 Comparisons of the incidence of physical growth and motor/speech retardation (intact discharge vs. non-intact discharge)

	Intact discharge ($n = 74$)	Non-intact discharge ($n = 12$)	p
1 year and 6 months			
Any retardation	44% (26/59)	80% (8/10)	0.045
Physical growth	24% (14/59)	60% (6/10)	0.029
Motor/speech	30% (18/59)	70% (7/10)	0.029
3 years			
Any retardation	27% (10/37)	71% (5/7)	0.036
Physical growth	16% (6/37)	57% (4/7)	0.037
Motor/speech	19% (7/37)	43% (3/7)	0.323

Table 3 Comparisons of prenatal data, birth weight and gestational age at birth between 90-day survivors and non-survivors

	90-day survivors ($n = 92$)	90-day non-survivors ($n = 25$)	p
GW at diagnosis (weeks)	29.0 ± 5.8	27.3 ± 5.4	0.249
Polyhydramnios	23% (17/91)	41% (7/24)	0.261
LHR	1.772 ± 0.703	1.273 ± 0.435	0.004
L/T	0.126 ± 0.043	0.096 ± 0.040	0.006
Liver up	28% (26/92)	88% (22/25)	<0.001
Birth weight	2.743 ± 0.526	2.700 ± 0.488	0.404
GA at birth (weeks)	38.0 ± 2.1	37.6 ± 1.7	0.127

Data are expressed as the mean ± SD

Among the survivors, the median duration of mechanical ventilation and O_2 supplementation were 20 and 32 days, respectively.

Outcome measures

Figure 2 shows the overall survival curve, which reached a plateau at 90 days. The 90-day survival was 79% (92/117). Among the survivors, six patients did not qualify for hospital discharge: four patients died in the hospital after 90 days of age, and two patients were still alive in the hospital at the age of 18 and 24 months. Therefore, 86 patients (73%) survived to discharge, including 12 with some major morbidities. Finally, the rate of intact discharge was 63% (74/117). These results are summarized in Fig. 3.

The details of the major morbidities at discharge in 12 patients are as follows:

supplemental O_2: 5;
supplemental O_2 + vasodilator: 2;
supplemental O_2 + tube feeding: 1;
supplemental O_2 + mechanical ventilation + tracheostomy: 1;
tube feeding: 3.

In the 12 non-intact discharge patients, the rate of physical or developmental retardation was 80% at 1.5 years and 71% at 3 years of age. In contrast, in the intact discharge patients, the rate of physical or developmental retardation was significantly lower (Table 2).

With regard to the relations of liver position and outcomes, the 90-day survival rate was 54% (26/48) in liver up and 96% (66/69) in liver down. The intact discharge rate was 29% (14/48) in liver up and 87% (60/69) in liver down. There were significant differences ($p < 0.05$) in the rate of 90-day survival and intact discharge between liver up and liver down patients.

Comparisons

There were no differences in GA at diagnosis, the incidence of polyhydramnios, birth weight and GA at birth between the 90-day survivors and 90-day non-survivors. The initial LHR and L/T were significantly higher in 90-day survivors compared to non-survivors. The incidence of liver up was significantly higher in 90-day non-survivors (Table 3).

Discussion

This is the first Japanese multicenter study of prenatally diagnosed CDH managed by planned delivery and followed by GV. Because five high-volume centers participated in this study, the data from a large series of prenatally diagnosed CDH could be collected in a comparatively short period. As most of the new strategies for CDH treatment, including HFO, NO, ECMO and GV, were introduced in the 1990s, all patients in this study were treated based on these established modern treatments throughout the study period. Therefore, this study should have revealed the most current outcomes for prenatally diagnosed CDH with minimal historical bias.

Our outcomes were somewhat better than the data from the large CDH study group registry in the USA, which noted a 70.5% "survival to discharge" of 1,222 infants born between 1995 and 2006 with prenatal diagnosis [5]. In most of the previous reports, including the CDH study group, "survival to discharge" was taken as the primary outcome. However, the rescue of more severely affected patients resulted in more patients with severe morbidities, including long-term respiratory support, nutritional support and circulatory support. In this study, a total of 12 patients were discharged with major morbidities (9 on respiratory support, 4 with tube feeding and 2 receiving vasodilators). Our results indicate that significant numbers of CDH patients are alive with major morbidities, resulting in poor quality of life. Thus, survival to discharge does not accurately reflect the treatment results if quality of life is taken into account. Because the overall survival curve reached a plateau at 90 days, 90-day survival does seem to be a good index to evaluate the short-term outcomes of CDH.

Our data have also shown that the rate of physical or developmental retardation at 1.5 and 3 years of age in the intact discharge patients was lower compared to the non-intact discharge patients. This suggests that intact discharge is a useful index to predict the long-term outcome of CDH.

This study has also clarified the latest treatment policy. With regard to the timing of delivery, the median gestational weeks at planned delivery was 38 weeks (range 28–42). According to the CDH study group, infants born at 37–38 weeks, compared with those born at 39–41 weeks, had less use of ECMO and a trend toward a higher survival rate was found among infants born through elective cesarean delivery [5]. Because the degree of pulmonary hypoplasia and vascular abnormalities become relatively more severe as gestation progresses [6, 7], there may be a potential benefit from delivering infants with CDH early. Although the best timing of delivery is unclear, 38 weeks is the most common and may be an appropriate timing for delivery of fetuses with CDH.

With regard to the mode of delivery, our data showed that cesarean section was likely to be selected in severe cases. Although the best mode of delivery remains unclear in prenatally diagnosed CDH, recent data have suggested that elective cesarean delivery may be associated with greater rates of survival without ECMO [8]. A prospective randomized trial is needed to determine the best mode of delivery for fetuses with CDH.

The timing of surgery also remains controversial. Some centers delay surgery until physiologic stabilization has occurred, while others prefer early surgery immediately after birth [3]. As a result, the timing of surgery was almost equally distributed to up to 120 h after birth in this study. Our data showed that the timing of surgery was not related to the survival rate. This lack of importance may be due to the progress made in the postoperative medical management of the patients.

With regard to the mode of ventilation, HFO was used immediately after birth in almost all cases. HFO has become the first-line ventilator mode for CDH in Japan. While ECMO was used in 19 patients, only 2 patients who were on ECMO had an intact discharge. Because of the advances in neonatal respiratory care, the role of ECMO has become limited in the treatment of prenatally diagnosed CDH in comparison to the past. A prospective randomized study may be necessary to determine if ECMO can improve the outcome of prenatally diagnosed CDH.

Our data have revealed that the initial LHR and L/T were significantly higher in 90-day survivors compared to 90-day non-survivors. Because of the wide distribution of LHR and L/T in each group, it is difficult to determine a cutoff to distinguish fetuses with expected poor outcome from fetuses with good outcome. Although LHR has been the most common method for lung assessment, there are several reports that have described that LHR is not a reliable predictor of outcome in fetuses with CDH [9–11]. According to our data, liver position was strongly correlated with 90-day survival as well as LHR and L/T. It is important to consider these factors together to predict outcomes of prenatally diagnosed CDH more precisely. In addition to LHR, L/T and liver position, measurement of other prognostic factors, such as total fetal lung volume [12], herniated liver volume [13, 14] and the observed to expected normal mean for gestation (o/e) LHR [15], are also required to establish an entry criteria for fetal intervention.

A major limitation of this study is the late diagnosis. The initial measurement of LHR and L/T were conducted at 31 weeks of gestation. Although L/T is consistent during gestation [16], LHR increases with gestation. It is therefore preferable to use o/e LHR to obtain a gestation-independent prediction of survival [15]. This fact should be considered when using our data as a selection criterion for fetal

intervention, which is currently being performed at 26–28 weeks' gestation.

The present study has demonstrated that a significant number of CDH patients are alive with major morbidities, despite good survival rate. A new treatment strategy, including fetal intervention, is therefore needed to reduce the mortality and morbidity of severe CDH.

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Re-evaluation of stomach position as a simple prognostic factor in fetal left congenital diaphragmatic hernia: a multicenter survey in Japan

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KEYWORDS: congenital diaphragmatic hernia; fetus; gentle ventilation; liver; stomach

ABSTRACT

Objectives *To document outcome and to explore prognostic factors in fetal left congenital diaphragmatic hernia (CDH).*

Methods *This was a multicenter retrospective study of 109 patients with prenatally diagnosed isolated left CDH born between 2002 and 2007. The primary outcome was intact discharge, defined as discharge from hospital without major morbidities, such as a need for respiratory support including oxygen supplementation, tube feeding, parenteral nutrition or vasodilators. All patients were managed at perinatal centers with immediate resuscitation, gentle ventilation (mostly with high-frequency oscillatory ventilation) and surgery after stabilization. Prenatal data collected included liver and stomach position, lung-to-head ratio, gestational age at diagnosis and presence or absence of polyhydramnios. Stomach position was classified into four grades: Grade 0, abdominal; Grade 1, left thoracic; Grade 2, less than half of the stomach herniated into the right chest; and Grade 3, more than half of the stomach herniated into the right chest.*

Results *Overall intact discharge and 90-day survival rates were 65.1% and 79.8%, respectively. Stomach herniation was classified as Grade 0 in 19.3% of cases, Grade 1 in 45.9%, Grade 2 in 13.8% and Grade 3 in 21.1%. Multivariate analysis revealed that liver position was the strongest prognostic variable for intact discharge, followed by stomach position. Based on our results, we divided patients into three groups according to liver (up vs. down) and stomach (Grade 0–2 vs. Grade 3) position. Intact discharge rates declined significantly from liver-down (Group I), to liver-up with stomach Grade 0–2 (Group II), to liver-up with stomach Grade 3 (Group III) (87.0%, 47.4% and 9.5% of cases, respectively).*

Conclusion *Current status and outcomes of prenatally diagnosed left CDH in Japan were surveyed. Stomach herniation into the right chest was not uncommon and its grade correlated with outcome. The combination of liver and stomach positions was useful to stratify patients into three groups (Group I–III) with different prognoses.*

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is one of the most challenging anomalies for pediatric surgeons and neonatologists. The rate of prenatal detection has been increasing over time, and is now over 50%[1–3]. A recent survey by the Japanese Association of Pediatric Surgeons reported that 73.5% of neonatal CDH cases in Japan had been diagnosed prenatally[4]. Prenatal detection allows management at experienced centers and avoidance of inadvertent events such as pneumothorax, distention of

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the gastrointestinal tract or resuscitation failure. This has improved the outcome of patients diagnosed prenatally, but limitations have led to an ongoing debate regarding the role of fetal intervention.

The prognosis of a patient with prenatally diagnosed CDH is estimated from several factors, including liver position and measurement of contralateral lung size (i.e. lung-to-head ratio (LHR) or lung-to-thoracic ratio). Stomach position, whether herniated into the chest or not, was formerly used as a factor for prediction of prognosis[5–7]. We reported previously an observation that stomach herniation into the right chest is an ominous sign in fetal left CDH[8]. In this study, we investigated the prognostic value of stomach position using a new grading system.

METHODS

A retrospective chart review was conducted on all isolated prenatally diagnosed CDH patients born during the period 2002–2007 at the National Center for Child Health and Development, Kanagawa Children's Medical Center, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka University Hospital or Kyushu University Hospital. We included in the study cases with presence of a left-sided CDH without associated life-threatening or chromosomal anomalies. All patients delivered at our centers and neonates were managed by immediate resuscitation followed by neonatal intensive care, including gentle ventilation mostly with high-frequency oscillatory ventilation (HFO) and preoperative stabilization. All institutions had extracorporeal membrane oxygenation (ECMO) and nitric oxide (NO) inhalation capability, which were initiated according to the clinical decisions of each team; indication criteria were not defined prospectively. This study was approved by the institutional review boards of all participating centers.

Prenatal data

The following data were collected for each patient: gestational age at diagnosis, presence or absence of polyhydramnios (maximum vertical pocket ≥ 8 cm), position of fetal liver and stomach, and LHR measured on maternal admission. Only those cases with obvious liver herniation (more than one-third of the left thoracic space occupied by the liver) on prenatal imaging studies were grouped as 'liver-up', eliminating questionable cases. Position of the stomach was categorized as: Grade 0, abdominal; Grade 1, left thoracic; Grade 2, less than half of the stomach herniated into the right chest; and Grade 3, more than half of the stomach herniated into the right chest (Figure 1). The lung area was measured by multiplication of the longest diameter of the lung by its longest perpendicular diameter in the cross-sectional plane at the level of the four-chamber view of the heart.

Postnatal data

Data collected postnatally included sex, gestational age at birth, birth weight, mode of delivery, Apgar score at 1 min, need for HFO, NO inhalation, ECMO and patch repair. Major morbidities at discharge, such as a need for respiratory support including oxygen supplementation, tube feeding, parenteral nutritional support or vasodilators, were recorded.

Outcomes

The primary and secondary outcomes were intact discharge (defined as discharge from hospital without any need for respiratory support including oxygen supplementation, tube feeding, parenteral nutritional support or vasodilators to control pulmonary hypertension) and 90-day survival rate.

Statistical analysis

Data are reported as median (range) or frequency (percentage). Univariate analyses were performed using chi-square, Fisher's exact and Cochran–Armitage tests. Crude odds ratio (OR) and 95% CIs for intact discharge failure, including death, were calculated. Multiple logistic regression analysis was also performed to estimate the OR of the prenatal variables adjusting for correlation among them. We used a stepwise selection method (variable selection criteria, $P < 0.20$) to select the variables correlated with intact discharge failure. All reported P-values are two-sided and not adjusted for multiplicity. $P < 0.05$ was considered statistically significant. Data were analyzed with SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

The characteristics of the 109 patients with isolated left CDH managed by the five participating centers between January 2002 and December 2007 are summarized in Table 1. The distribution of liver and stomach positions is shown in Figure 2. Almost all (67/69) of the liver-down patients had stomach Grades 0–2, while more than half (21/40) of the liver-up patients had stomach Grade 3.

With respect to therapeutic interventions used after birth, all except one patient ($n = 108$, 99.1%) were ventilated with HFO. Inhaled NO was administered in 87 (79.8%) patients. ECMO was used in 16 (14.7%) patients, only four of whom survived to discharge, two with oxygen supplementation. Surgery to repair the diaphragm was performed in 98 (89.9%) patients, of whom 46 (46.9%) required patch repair.

At 90 days of postnatal life, 22 patients had died and 87 (79.8%) were alive. After 90 days, only four patients died (at 92, 136, 403 and 802 days) and only two patients were still in hospital at the time of the survey. Eighty-one patients survived to discharge, including 10 patients

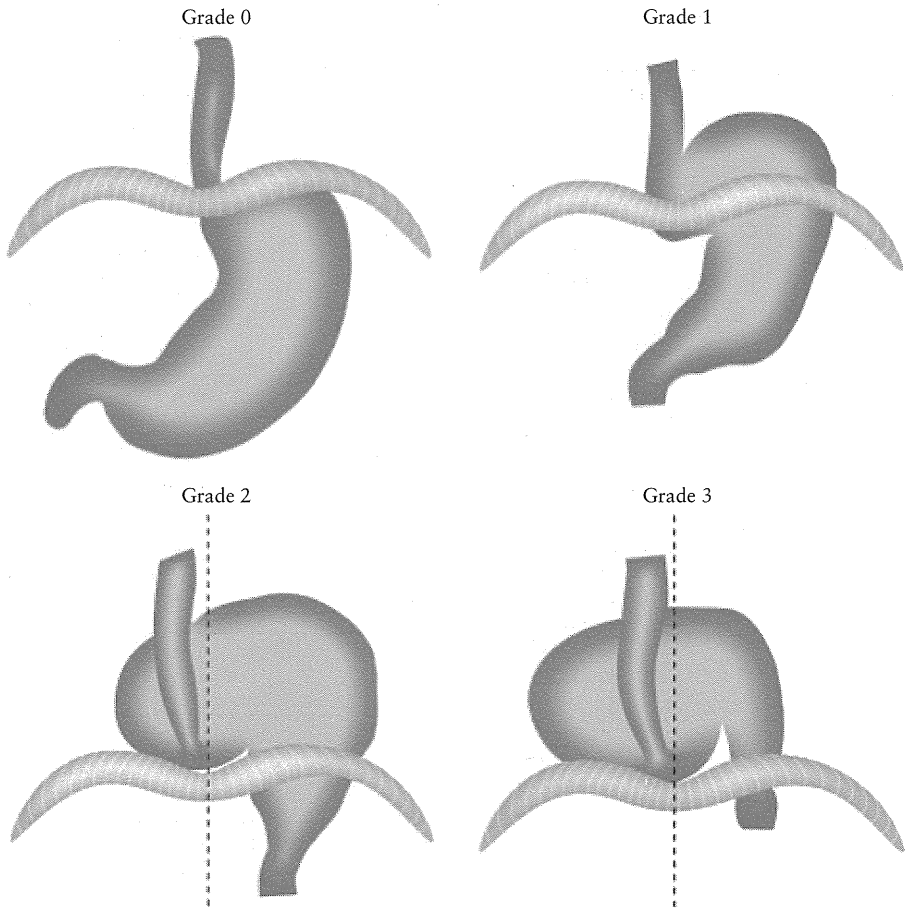

Figure 1 Schematic diagrams showing the four grades of stomach position in patients with left congenital diaphragmatic hernia. Stomach position was categorized as Grade 0, abdominal; Grade 1, left thoracic; Grade 2, less than half of the stomach herniated into the right chest; and Grade 3, more than half of the stomach herniated into the right chest.

with some major morbidities (seven patients required oxygen supplementation, four required tube feeding and two required vasodilators). Thus, the rate of intact discharge was 65.1% (71/109).

The results of univariate analysis are shown in Table 2 and those of multivariate analysis are in Table 3. Adjusted ORs of liver position and stomach position for intact discharge failure were statistically significant. While the OR of LHR was not statistically significant, the magnitude of this risk was not negligible. Adjusted ORs of these three variables became less significant than the crude ORs because they confounded each other.

Stomach position grade was also correlated with the need for patch repair, the need for patch repair being 0% (0/20) for Grade 0, 46% (22/48) for Grade 1, 62% (8/13) for Grade 2 and 94% (16/17) for Grade 3 ($P < 0.001$).

Based on these results, we divided patients into three groups according to liver (up vs. down) and stomach (Grade 0–2 vs. Grade 3) position (Figure 3). Intact discharge rates declined significantly from Group I (liver-down), to Group II (liver-up with stomach Grade 0–2), to Group III (liver-up with stomach Grade 3) (87.0%, 47.4% and 9.5% of cases, respectively).

DISCUSSION

This multicenter study has revealed the outcomes of prenatally diagnosed left CDH managed at perinatal centers with immediate resuscitation and gentle ventilation: a 90-day survival rate of 79.8% and an intact discharge rate of 65.1%. The results compare favorably with reports from leading centers of the world[9,10], considering that patients were all diagnosed prenatally and had relatively low birth weight. Our results reflect the current status in Japan as a whole, compared with previous reports that reflected smaller, single centers[11,12].

A new concept for prognostic evaluation of CDH, intact discharge, was introduced in this study. Intact discharge was defined as discharge from hospital without any respiratory, nutritional or circulatory support. Previously, studies had been focused mainly on therapies that reduce perinatal and neonatal mortality of CDH[13–15]. However, it is well known that to save the lives of the more severely affected patients results in a significant increase in survivor morbidity[16–18]. Intact discharge may serve in counseling the parents and could be an important goal of prenatal intervention. Whether patients with intact discharge have

Table 1 Characteristics of patients with left congenital diaphragmatic hernia

Characteristic	*Median (range) or n (%)*
Prenatal data	
Gestational age at diagnosis (weeks)	28 (17–40)
Liver position	
Up	40 (36.7)
Down	69 (63.3)
Stomach position	
Grade 0	21 (19.3)
Grade 1	50 (45.9)
Grade 2	15 (13.8)
Grade 3	23 (21.1)
Initial LHR	1.59 (0.37–4.23)
Gestational age at initial LHR (weeks)	31 (18–40)
Polyhydramnios	22 (20.2)
Postnatal data	
Gestational age at birth (weeks)	38.3 (28.4–41.0)
Birth weight (kg)	2.79 (1.04–4.03)
Sex	
Male	59 (54.1)
Female	50 (45.9)
Mode of delivery	
Vaginal	51 (46.8)
Cesarean section	58 (53.2)
Apgar score at 1 min	4 (1–9)

LHR, lung-to-head ratio.

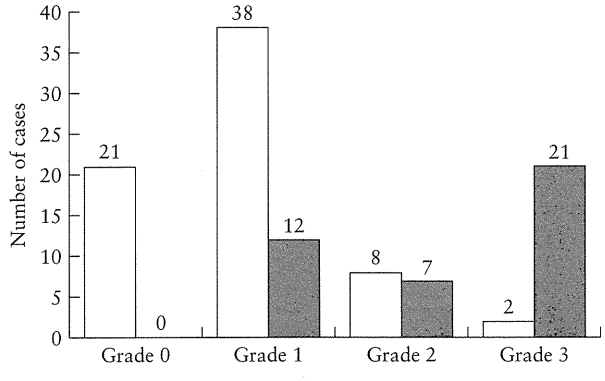

Figure 2 Frequency of each of the four grades of stomach position in liver-down (□) and liver-up (■) patients with left congenital diaphragmatic hernia. Stomach herniation into the right chest was not uncommon, especially in liver-up cases.

better long-term outcomes must be assessed in future studies.

Although LHR is the most commonly used prenatal predictor of survival[19,20], it is sometimes difficult to identify the margin of the hypoplastic lung in the severe form of CDH. Therefore, LHR is not free from interinstitution and interobserver variation. Additionally, unadjusted LHR has been shown to increase with gestational age[21–23]. The prognostic value of LHR has been questioned[24–26], and the observed to expected (o/e) LHR[22] has been developed to overcome this. O/e LHR measurement requires normal control values in each population.

Table 2 Univariate analysis to determine factors significantly associated with intact discharge failure, including death, of patients with left congenital diaphragmatic hernia

Variable	*Crude OR (95% CI)*	P
Liver position (up)	17.58 (6.56–47.12)	< 0.001
Stomach position		
Grade 0	Reference	< 0.001*
Grade 1	6.3 (0.8–52.1)	—
Grade 2	13.3 (1.4–127.6)	—
Grade 3	95.0 (9.7–928.3)	—
LHR (per 0.5)	0.34 (0.20–0.60)	< 0.001
GA at diagnosis (< 30/≥ 30 weeks)	0.99 (0.96–1.02)	0.326
Polyhydramnios	1.43 (0.55–3.75)	0.463

*Cochran–Armitage test for trend. GA, gestational age; LHR, lung-to-head ratio; OR, odds ratio.

Table 3 Multiple logistic regression for intact discharge failure, including death, of patients with left congenital diaphragmatic hernia

Variable	*Adjusted OR (95% CI)*	P
Liver position (up)	6.52 (1.79–23.82)	0.005
Stomach position (per grade)	2.59 (1.21–5.53)	0.014
LHR (per 0.5)	0.58 (0.30–1.11)	0.100

A stepwise selection method (variable selection criteria, $P < 0.20$) was used to select the correlated variables from those in Table 2. LHR, lung-to-head ratio; OR, odds ratio.

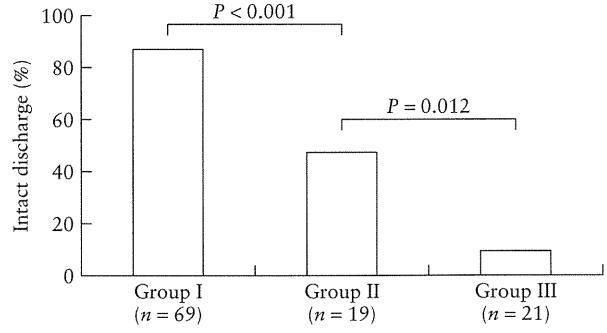

Figure 3 Our new grouping system of fetal patients with isolated left congenital diaphragmatic hernia using liver and stomach positions. Intact discharge rate was significantly different among the three groups (Group I, liver-down; Group II, liver-up and stomach position Grade 0–2; Group III, liver-up and stomach position Grade 3).

Stomach position has been used previously as a prenatal prognostic factor for CDH[5–7]. However, its prognostic role was replaced by LHR, and little attention has been paid to stomach herniation into the right chest cavity. Previously, we observed in a small series that stomach herniation into the right chest is an ominous sign in fetal left CDH[8]. To test the hypothesis that herniation into the right chest is related to poor outcome, we developed a new grading system, categorizing the degree of stomach

herniation into four grades. We found that stomach herniation into the right chest is not uncommon, especially in liver-up cases (Figure 2). Multivariate analysis revealed the strongest prognostic variable for intact discharge to be liver position, followed by stomach position. The OR of LHR was not statistically significant, possibly because it was not adjusted for gestational age at measurement or because of the retrospective study design involving multiple centers.

Finally, we propose a simple classification for fetal left CDH involving liver and stomach position (Figure 3). This classification may be useful in estimating the patient's prognosis and in planning perinatal management, including maternal transport to high volume centers or those offering fetal intervention, especially in cases in which o/e LHR is difficult to obtain. The fact that Group III patients had only a 9.5% chance of intact discharge shows the limitations of a gentle ventilation strategy, and could be the starting point for future trials of fetal intervention in Japan.

A major limitation of this study was late diagnosis of CDH. The initial measurement of LHR was conducted at a median of 31 weeks of gestation. This fact should be considered when using the present data to identify candidates for fetal intervention now being performed at 26–28 weeks of gestation[13]. However, neither liver nor stomach position changes dramatically over time; none of the 23 liver-up patients before 30 weeks was judged as being liver-down after 30 weeks. Similarly, only two of the 10 patients with Grade 3 stomach herniation before 30 weeks were judged as being Grade 2 later in gestation. Another limitation was the retrospective study design using case report forms. Interobserver variation cannot be discounted since it was not possible to have all prenatal imaging reviewed by a single person. Because it was not possible in every center to collect o/e LHR[22], MRI lung volume[27] and MRI lung intensity[28], these measurements were not analyzed. Therefore, our findings need to be confirmed in future prospective studies.

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