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Non-immune Hydrops Fetalis: A Prospective Study of 53 Cases

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Non-immune hydrops fetalis (NIHF) is a symptom caused by a heterogeneous group of conditions. Diagnostic investigations may constitute a real challenge. This study aimed to evaluate prospectively and systematically a series of NIHF cases using a research protocol expanded for studying inborn errors of metabolism (IEM) during 2 years—2010 and 2011. We also reviewed the frequency of IEM among the NIHF reported in literature. A clinical or etiopathogenic diagnosis was reached in 46 (86.8%) of the 53 studied cases. The main diagnostic groups were chromosomal anomalies (28.3%), syndromic (18.9%), isolated cardiovascular anomaly (7.5%) and congenital infection (7.5%). Metabolic causes were found in 5.7%, all lysosomal storage disorders (LSD). In seven (13.2%), no diagnosis was found in part because of incomplete evaluation. The hydrops was identified prenatally in 90.5% of cases. In 5.7% a spontaneous and complete resolution of the hydrops occurred during pregnancy. Overall mortality was 75.5%. The IEM frequency in the present study (5.7%) was higher than that usually reported. We suggest performing studies directed to IEMs if the more common causes are excluded. © 2013 Wiley Periodicals, Inc.

Key words: non-immune hydrops fetalis; etiology; protocol investigation; spontaneous resolution; inborn errors of metabolism, lysossomal storage disorder

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INTRODUCTION

Non-immune hydrops fetalis (NIHF) is a symptom caused by a heterogeneous group of conditions. The definition of HF in the literature varies from: (a) generalized subcutaneous edema with or without cavitary effusion [Bellini et al., 2010]; (b) generalized subcutaneous edema and at least one cavitary effusion [Liao et al., 2007]; to (c) two cavitary effusions or one cavitary effusion and generalized edema [Ratanasiri et al., 2009].

The wide diversity in causes makes NIHF a diagnostic challenge. A 2007 review of NIHF causes included >5,000 cases and reported absence of diagnosis in 17.8% [Bellini et al., 2009]. The paper reviewed retrospective case series except for two prospective studies [Haverkamp et al., 2000; Burin et al., 2004].

Some diagnostic groups are universally recognized as cause of NIHF such as cardiovascular defects, chromosomal anomalies, hematological disturbances, and congenital infections [Bellini

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et al., 2009]. Other conditions have been suggested to be underdiagnosed such as inborn errors of metabolism (IEM) [Piraud et al., 1996; Burin et al., 2004; Kooper et al., 2006], and lymphatic anomalies [Bellini et al., 2010].

The present study evaluated prospectively a series of NIHF cases using a research protocol that paid specific attention to metabolic disorders after exclusion of the most common causes. In addition, we reviewed the frequency of IEM among the NIHF series reported in literature.

MATERIAL AND METHODS

The present study included all patients with NIHF referred to the Perinatal Genetics Program at Woman's Hospital of the State University of Campinas (Unicamp) in Brazil during the period 2010–2011. NIHF cases were identified during antenatal care regardless of the gestational age or after birth (abortion, stillbirth or live birth).

As purpose of the present study, HF was defined as the presence of generalized subcutaneous edema with or without cavitary effusion or more than one cavitary effusion (pleural, pericardial, or peritoneal) in the absence of subcutaneous edema.

The investigation was based on the flowchart proposed by Bellini et al. [2009b]. The protocol was modified according to the local service and is described in Figure 1. The evaluation initially included the investigation of the most common causes of NIHF—heart defects, chromosomal anomalies, hematological disturbances, congenital infections, structural defects, twin-to-twin transfusion, and syndromes commonly associated with HF. When the initial examinations were normal, the metabolic investigation was performed using samples obtained during prenatal and/or at the postnatal period. From amniotic fluid (AF) supernatant (prenatal) or plasma (postnatal) activities of total hexosaminidase, β-D-glucuronidase, α-mannosidase, chitotriosidase were performed. From AF cultured cells (prenatal) and leukocytes (postnatal) the following examinations were performed: activities of *N*-acetylgalactosamine-6S-sulfatase, β-D-glucuronidase, β-D-galactosidase, β-D-glucosidase, α-iduronidase, sphingomyelinase, and α-D-neuraminidase. This latter enzyme was evaluated in fibroblasts (postnatal) in the following situations: increased levels of sialic acid in urine sample, β-D-galactosidase deficiency, and chromatography of oligosaccharides and sialoligosaccharides (urine) suggestive of sialidosis. From urine thin-layer chromatography of oligosaccharides and sialoligosaccharides, electrophoresis of glycosaminoglycans, and measurements of glycosaminoglycans, and sialic acid were performed. Finally the screening for CDG by serum transferrin isoelectric focusing was performed from serum. Thus, the following diseases were investigated: congenital defects of N-glycosylation and lysosomal storage disorders (LSD). These latter included gangliosidosis GM1, galactosialidosis, sialidosis, Niemann–Pick A disease, Gaucher disease, mucopolysaccharidosis I, IVA and VII, mucolipidosis II, infantile sialic acid storage disease (ISSD) and multiple sulfatase deficiency.

Regarding the etiopathogenesis, the cases were categorized according the 14 groups suggested by Bellini et al. [2009], with a little modification. The idiopathic cases were classified into two subgroups. The first one—true idiopathic, included the cases that despite of the investigation the etiology remained unknown. By the other hand, cases incompletely evaluated due to early intrauterine demise and with no basic complementary investigations were placed in the second subgroup—doubtful cases.

To assess the reported frequency of IEM among the different series of NIHF, the literature was revised from PubMed until July 31, 2012. The adopted criteria of inclusion were two: (1) paper published in English, Spanish or Portuguese, (2) series with 10 or more cases and at least one with diagnosis of metabolic disease. For this review cases with a single cavitary effusion were not considered because similar cases were not included in our own series. Additionally, the review articles were also excluded to avoid duplication of cases. Finally, the selected articles were classified into two groups: studies in which the main goal was the IEM investigation, and general surveys performed to evaluate causes of NIHF in general.

Frequencies were compared with the Fisher's exact test or χ^2 test according to the size of the sample. For the expected frequency the EpiInfo 7 software was used. The results were considered statistically significant when two-sided *P*-value was <0.05.

This study was approved by the Committee of Ethics in Research of the Faculty of Medicine of the State University of Campinas and the informed consent was obtained from all participants families.

RESULTS

During the period 2010–2011, 53 cases of NIHF were referred to Perinatal Genetic Program. An etiopathogenic diagnosis was reached in 46 (86.8%) cases. The cases distribution into the 14 diagnostic groups is showed in Table I. The main diagnostic groups were the chromosomal anomalies (15 cases: 28.3%), followed by syndromic cases (10 cases: 18.9%), isolated cardiovascular defect (four cases: 7.5%), and congenital infection (four cases: 7.5%). Metabolic etiology was identified in three cases (5.7%), all LSD. The lymphatic dysplasia and thoracic groups also had three cases (5.7%) each one. No cases of hematological or gastrointestinal abnormality, neither extra thoracic tumor were observed. One (1.9%) case presenting hepatic hemosiderosis was placed in the miscellaneous group. Finally, seven cases (13.2%) were classified as idiopathic, being three true idiopathic since these patients were extensively investigated, and four were identified as doubtful cases because they were not properly investigated due to early intrauterine demise and incomplete basic complementary investigation.

Among the syndromic cases the diagnosis was based on clinical findings. Molecular investigation (methylation analysis at IC1 and IC2 by MLPA) in the case referred as Beckwith–Wiedemann syndrome (BWS) was negative. Although another possibility for this child is Costello syndrome, her findings (phenotypic appearance including macrosomia with macroglossia, placentomegaly with chorangioama, nephromegaly, adrenocortical cytomegaly, and advanced carpal bone age) fit better with BWS than Costello syndrome. Unfortunately, none other molecular investigation was possible in this case. Two cases presenting Turner phenotype (female fetus with large cystic hygroma, generalized subcutaneous edema, lymphedema of hands and feet, and hypoplastic left heart) were placed in this group because of the absence of karyotype.

Cases with no specific clinical diagnosis and normal or inconclusive basic complementary investigation (karyotype,

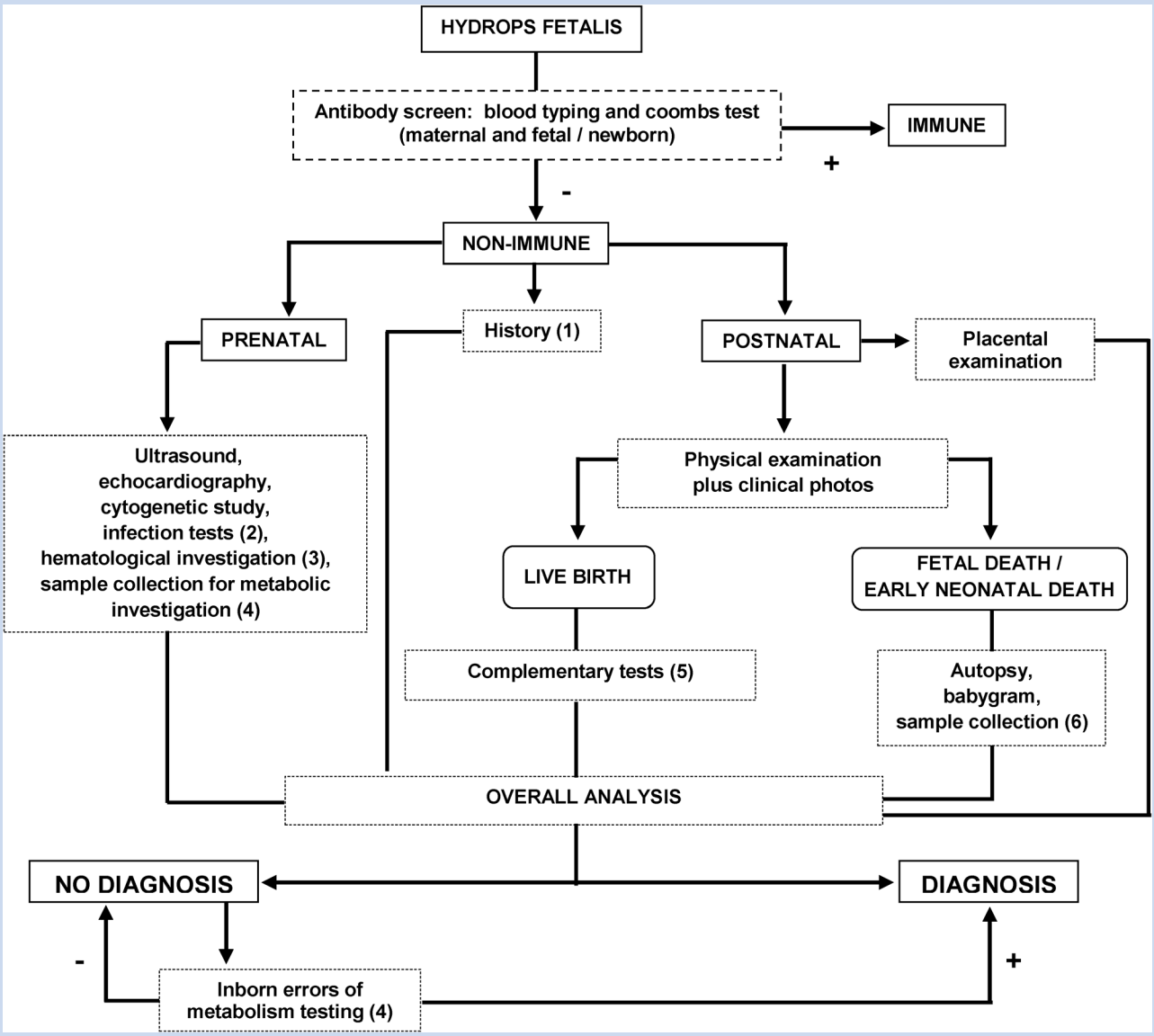

FIG. 1. Diagnostic Flowchart for Hydrops Fetalis. (1) History: maternal and familial data—previous and acute diseases, teratogenic agents, previous pregnancies, parental origin, consanguinity, recurrence of hydrops fetalis, previously identified genetic disorder or congenital malformation. (2) Infection tests: syphilis, *Toxoplasma gondii*, rubella virus, cytomegalovirus, hepatitis B and C, HIV and parvovirus B19 (if fetal anemia was presented or suspected). (3) Hematological investigation: during prenatal, fetal anemia was screened by measure of middle cerebral artery peak systolic velocity. Laboratorial tests: complete blood count, blood grouping and Rh. (4) Inborn errors of metabolism testing: prenatal (amniotic fluid) or postnatal (blood, urine and fibroblast). Investigated conditions: congenital defects of N-glycosylation and lysosomal storage disorder (gangliosidosis GM1, galactosialidosis, sialidosis, Niemann–Pick A disease, Gaucher disease, mucopolysaccharidosis I, IVA and VII, mucolipidosis II, infantile sialic acid storage disease and multiple sulfatase deficiency). (5) Complementary tests: were performed according to the prenatal examinations and included tests for conditions that were not investigated during pregnancy and/or tests for diagnostic confirmation. Further tests were carried out according to each specific case, for instance: X-ray, biopsy, CT, MRI, lymphoscintigraphy, other biochemical examinations. Molecular studies were performed according to the diagnosis in specific cases. (6) Sample collection: skin biopsy for fibroblast culturing, blood by intra cardiac puncture and urine. If there was intrauterine demise, placental specimen was an alternative for cell culturing.

echocardiogram, X-ray, autopsy, and serological tests) were labeled as multiple malformed. One case presented bilateral hydronephrosis, cerebral ventriculomegaly, camptodactyly of the 3rd–5th fingers, syndactyly of 2nd–3rd toes and clubfoot. The second one had cataracts, facial dysmorphisms, cervical hygroma, VSD, ASD, and clubfeet. The third case was a fetus of 19 weeks with brachydactyly, platyspondyly, narrow thorax and single umbilical artery. Finally, the last one had VSD, bilateral renal hypoplasia, and

TABLE I. Distribution of NIHF Cases and Comparison between Cases Reviewed by Bellini et al. [2009] and the Present Casuistic

	Present study			Bellini et al. [2009]		
Groups	**Cases (N)**	**%**	**Conditions (N)**	**Cases (N)**	**%**	***P*-value**
Cardiovascular	4	7.5	Ebstein anomaly + arrhythmia [1] Hypoplastic left heart syndrome [1] Truncus arteriosus communis [1] Vascular tumor [1]	1,181	21.7	0.010
Hematologic	0	0	—	564	10.4	0.005
Chromosomal	15	28.3	Monosomy X [9] Trisomy 21 [3] Trisomy 18 [1] Trisomy 13 [1] Isochromosome 18q [1]	727	13.4	0.003
Syndromic	10	18.9	Turner phenotype [2] Beckwith–Wiedemann [1] Ivemark [1] Lethal multiple pterygium syndrome [1] Meckel–Gruber [1] Multiple malformed [4]	237	4.4	<0.001
Lymphatic dysplasia	3	5.7	Cystic hygroma [2] Congenital lymphedema [1]	310	5.7	1.000
IEM	3	5.7	Gangliosidosis GM1 [2] Infantile sialic acid storage disease [1]	60	1.1	0.020
Infection	4	7.5	Cytomegalovirus [1] Parvovirus B19 [1] Syphilis [1] Toxoplasmosis [1]	366	6.7	0.780
Thoracic	3	5.7	Achondrogenesis type II [1] Chondrodysplasia punctate [1] Teratoma [1]	327	6.0	1.000
Urinary tract malformation	1	1.9	Polycystic kidney disease	127	2.3	1.000
Extra thoracic tumor	0	0	—	39	0.7	1.000
TTTS-placental	2	3.8	Acardic [1] Receptor [1]	304	5.6	0.760
Gastrointestinal	0	0	—	29	0.5	1.000
Miscellaneous	1	1.9	Hepatic hemosiderosis [1]	200	3.7	1.000
Idiopathic	7	13.2		966	17.8	0.030
Total	53	100.0		5,437	100.0	

N, number of cases.

skeletal changes characterized by long bones metaphyseal irregularity especially in humerus and femur.

The three cases classified into the lymphatic group had normal karyotype as well as absence of heart defect. The HF was spontaneously resolved during pregnancy in two of these cases. At birth, there were redundant neck skin suggesting residual cystic hygroma (two cases), and typical lymphedema of the hands and feet (one case).

In the metabolic group we found two cases with gangliosidosis GM1 and one with ISSD. In this latter case as well as in one case of GM1 the diagnosis of LSD was firstly suspected because of the skeletal findings (diffuse osteopenia, irregularity of metaphyses, coarse trabecular aspect of the long bones and execessive periosteal cloaking) and by the placental histological aspect (vacuolization of trophoblast and stromal cells) respectively. Unfortunately the second case of GM1 was born in another hospital and the placental could not be examined. The diagnosis of the three cases was confirmed on both, biochemical level and by molecular test. Two cases with gangliosidosis GM1 had β-galactosidase deficiency and mutations on *GLB1* (c.172insG in homozygous in one case and c.1515G>T and c.1643A>G in the second case). The third case with ISSD had high free sialic acid in AF, no mutations after sequencing of the *GNPTAB* and homozygous mutation (c.533delC) in the *SLC17A5*.

In the thoracic group besides a case with a teratoma localized at the anterior mediastinum near the great vessels of the heart, two cases of skeletal dysplasia were diagnosed. The first one was a typical case of achondrogenesis type II while the other had a severe form of chondrodysplasia puncatata. The clinical and radiological changes were compatible with a severe form of Conradi–Hünermann

syndrome. Unfortunately this unusual form could not be confirmed by molecular or enzymatic analyses so far.

Comparing the distribution of the present cases into the 14 diagnostic groups with that published by Bellini et al. [2009], differences in the frequency of the following groups were observed: cardiovascular, hematologic, chromosomal, syndromic, IEM, and idiopathic cases (Table I). The cardiovascular, hematologic and idiopathic groups were less frequent in the present study, while the chromosomal, syndromic and IEM groups have presented a higher proportion.

Regarding the adopted criteria for HF, the most cases (42–79.3%) presented edema and cavitary effusions. Five (9.4%) cases had only cavitary effusions (two or more)—Beckwith–Wiedemann syndrome, cystic hygroma, monosomy X, thoracic teratoma and an idiopathic case. Six (12.3%) cases presented only subcutaneous edema—achondrogenesis type II, chondrodysplasia punctata, cystic hygroma, lethal multiple pterygium syndrome, trisomy 21 and a twin-to-twin transfusion syndrome.

The main clinical features of NIHF cases are described in Table II. There were slightly more females (58.5%), and most were born preterm (88.7%). Overall mortality was 75.5% (40/53), prenatal demise occurred in 30/53 (56.6%). Median of demise of live born cases was 1.5 day.

Parental consanguinity was observed in 12% (6/50 informative cases), but none case with recessive inheritance was associated with consanguinity. Recurrence of NIHF occurred once (gangliosidosis GM1).

Hydrops was identified prenatally in 48 cases (90.5%), at a median age of 19.5 weeks (range 11–38 weeks). Chromosomal anomalies were detected in 14/25 (56%) if hydrops was detected $<$20 weeks, and in 3/23 (13%) if detected $>$20 weeks, ($P < 0.05$).

TABLE II. Summary of Clinical Features of NIHF Cases

Clinical features	N (%)
Gender	
Male	22 (41.5%)
Female	31 (58.5%)
Gestational age (weeks)	
<20	11 (20.7%)
21–36	36 (68.0%)
>36	6 (11.3%)
Outcome	
TOP[a]	4 (7.5%)
Abortion	10 (18.9%)
Stillbirth	16 (30.2%)
Live birth	23 (43.4%)
Mortality	
Prenatal	30/53 (56.6%)
Neonatal	10/23 (43.4%)
Overall	40/53 (75.5%)

N, number of cases; TOP, termination of pregnancy.
[a]Isochromosome 18q, Meckel–Gruber syndrome, lethal multiple pterygium syndrome and achondrogenesis type II.

Among the 48 cases in which the HF was identified during prenatal, eight cases had only one cavitary effusion observed at the first ultrasound examination, subcutaneous edema and/or other cavitary effusion were subsequently detected.

In three cases (congenital lymphedema, cystic hygroma and trisomy 21), all detected before 20 weeks, the prenatal follow up showed spontaneous and complete resolution of the HF during pregnancy. The interval between the hydrops detection and its resolution was variable—10 weeks for the congenital lymphedema case, 7 weeks for the cystic hygroma one, and 2 weeks in one case of trisomy 21.

Fetal anemia was suspected in three cases during prenatal US, but it was confirmed in only two ones (parvovirus B19 and syphilis).

In the literature review about NIHF 21 series of cases were found (Table III). There is a great diversity in the way as each series was studied and also a great difference among the IEM frequencies, being these usually higher in the studies whose goal was the specific IEM investigation than in those whose purpose was a general evaluation of the NIHF.

DISCUSSION

Since NIHF is a manifestation of a large number of conditions, it is impossible to have an ideal classification. Because the etiology is related to prognosis, follow up, and genetic counseling a classification based on etiology would be preferable. However, due to the great heterogeneity the two more relevant review about hydrops took into account both etiology and pathogenesis [Machin, 1989; Bellini et al., 2009]. This latter have provided the most extensive and detailed overview of causes of NIHF. After excluding 33,294 papers they systematically reviewed 51 articles, which described a total of 5,437 NIHF cases, and proposed a classification with 14 diagnostic categories.

In the series presented here we have used a flowchart based on that suggested by Bellini et al. [2009b] and systematically studied 53 cases during 2010–2011 period. In this study, a diagnosis was reached in 86.8% of the cases, which were distributed in 11 of the 14 etiopathogenic categories proposed by Bellini et al. [2009].

The main diagnostic group in the present series was that of chromosomal abnormality (28.3%). Probably the inclusion of abortions have contributed for this rate since aneuploidies are especially observed in the NIHF early detected during pregnancy [Heinonen et al., 2000; Has, 2001]. The second group more frequent was the syndromic cases (10–18.9%), and this could be associated with the high frequency of birth defects in our hospital that is around 6–7% (unpublished data). In addition, the role of the prenatal ultrasonography specially involved in the high frequency of structural defects easily diagnosed at prenatal have been showed since the early 2000's [Cavalcanti and Salomão, 2003]. High frequency of the syndromic group was also reported [Heinonen et al., 2000; Has, 2001].

The lower frequency of cardiovascular defects may be just coincidence due to our sample size. However, take into account the diversity of the series revised by Bellini et al. [2009] and also the fact that most of the cases were not followed up, it also is possible that in some series the frequency of cardiovascular defects can be

TABLE III. Diagnosis and Frequency of IEM in NIHF Studies

Refs.	Type of casuistic (gestational age)	[N] IEM/[N] NIHF	%	Diagnosis
	IEM in NIHF—specific investigation (LSD)			
Piraud et al. [1996]	Fetus (mainly 3rd trimester)	8/54	14.8	ISSD, galactosialidosis (2), Gaucher disease, MPS VII (2), sialidosis (2)
Groener et al. [1999]	Fetus (20–36 weeks)	1/17	5.8	MPS VII
Burin et al. [2004]	Fetus (16–32 weeks) and newborn	5/33	15.0	Galactosialidosis, MPS IVA, mucolipidosis II, NP-A, sialidosis
Kooper et al. [2006]	Fetus (14–36 weeks)	6/75	8.0	Gangliosidosis GM1, galactosialidosis, MPS VII (2), MPS—probable (2)
Total		20/179	11.2	
	IEM in NIHF—general investigation			
Etches and Lemons [1979]	SB, LB	1/22	4.5	LSD—not specified
Van Aerde et al. [1982]	SB, LB	1/10	10.0	Gangliosidosis GM1
Mahony et al. [1984]	Fetus	1/27	3.7	MPS—not specified
Im et al. [1984]	A, SB, LB	3/20	15.0	Gaucher disease
Mostoufi-Zadeh et al. [1985]	SB, LB—autopsy	1/40	2.5	Gaucher disease
McFadden and Taylor [1989]	Autopsy	4/90	4.4	Unespecified metabolic disease
Larroche et al. [1992]	SB, LB	3/38	7.9	LSD—not specified
Laneri et al. [1994]	SB, LB	1/45	2.2	Tyrosinemia
McCoy et al. [1995]	Fetus (>20 weeks GA)	2/82	2.4	Gaucher, NP-C
Rejjal et al. [1996]	LB	2/17	11.7	Gangliosidosis GM1 e MPS—not specified
Lallemand et al. [1999]	SB—autopsy (2nd–3rd trimester)	1/94	1.0	Cardiomyopathy secondary to mitochondrial pathology
Mascaretti et al. [2003]	LB	1/21	4.7	Gangliosidosis GM1
Favre et al. [2004]	Fetus	4/21	19.0	MPS VII, NP-C, sialidosis
Rodríguez et al. [2005]	Neonatal death (autopsy)	2/32	6.2	Glycogenosis type IX (cardiac phosphorylase kinase deficiency) and carnitine deficiency
Abrams et al. [2007]	LB	5/573	0.9	Not specified
Santo et al. [2011]	Fetus (>20 weeks GA)	4/71	5.6	MPS—not specified (2), NP-C, LSD—not specified
Fritsch et al. [2012]	Fetus	2/116	1.7	Mucolipidosis II, sialidosis
Total		34/1,229	2.7	

A, abortion; GA, gestational age; ISSD, infantile sialic acid storage disease; LB, live birth; LSD, lysosomal storage disorder; MPS, mucopolysaccharidosis; NP-A, Niemann–Pick type A; NP-C, Niemann–Pick type C; SB, stillbirth.

overestimated by the inclusion of cases presenting other anomalies beyond the heart defects.

The absence of hematologic disease in this sample could just be related to the low frequency of Bart's hemoglobin in the local population, or a real failure of diagnosis, since the investigation of anemia is usually made after 18 weeks in our hospital.

The proportion of IEM (3/53–5.7%) was higher than that reported in the large overview by Bellini et al. [2009] (60/5,437–1.1%). The systematic investigation following the flowchart (Fig. 1), probably have contributed to our higher proportion. However, this difference may also be explained by mere coincidence due to present small sample size.

Bellini et al. [2009] reported a rate of 17.8% for the idiopathic group, however an incomplete investigation of several cases in some articles as well as the great heterogeneity of the reviewed series can explain that high frequency. We believe that classifying cases incompletely investigated as idiopathic gives a false idea about diagnostic capacity. It is not the same, a hydropic fetus without diagnosis after to be exhaustively investigated compared with another one also without diagnosis but with none investigation. For instance, Santolaya et al. [1992] have reported 25% of idiopathic cases among a series of 66 NIHF ones, however over a half of the idiopathic cases had not the karyotype performed. For comparison purposes, we have classified seven cases (13.2%) as idiopathic, but we believe to be important to stress that four cases among them have died very early, even before to have a chance to be minimally investigated. Of course the evaluation of a hydrops fetus is not easy and many should remain incompletely investigated in the daily practice. Therefore, unlike of the retrospective series, in the prospective ones it is expected that the systematic study helps to reduce the number of idiopathic.

For the other diagnostic groups (lymphatic dysplasia, infection, thoracic, urinary tract malformation, extra thoracic tumor, TTTF-placental, gastrointestinal, miscellaneous) proportions were not different.

Most cases of this sample presented generalized subcutaneous edema and at least one cavity effusion. Considering that patients with a same condition, for instance trisomy 21, can present hydrops under different presentations and that hydrops is a dynamic process during pregnancy, the use of broader criteria for HF should be considered.

The occurrence of prenatal spontaneous resolution of HF is rarely described in NIHF series [Swain et al., 1999; Suwanrath-Kengpol et al., 2005]. The most cases were anecdotal reports associated with different conditions, like aneuploidy [Mostello et al., 1989] and lymphatic dysplasia [Ghalamkarpour et al., 2009]. However, more notably hydrops resolution has been related to parvovirus B19 infection [Petrikovsky et al., 1996]. The possibility of spontaneous resolution of HF during prenatal reinforces the importance of the follow up to identify this type of evolution and should not be neglected in prenatal genetic counseling.

The female predominance (58.5%) in the present series could be due to the high frequency of monosomy X (20.7%), but it is also reasonable to think this is just coincidence. Regarding the gestational age, the high frequency of prematurity (74% among live births and 88.7% overall) here reported is almost a constant finding in the NIHF series [Liu et al., 2002; Mascaretti et al., 2003; Teixeira et al., 2008]. Mortality also is usually high in NIHF, even when termination of pregnancy (TOP) is not considered [Ratanasiri et al., 2009]. In the present study, the overall mortality was 75.5% and neonatal mortality was 43.4%. Similar neonatal mortality rate was found by Mascaretti et al. [2003]—55% in a series of Brazilian live births. The high neonatal death rate observed in the present series might be explained by the fact that TOP in Brazil is legally authorized and performed only in exceptional circumstances.

Parental consanguinity among the families of the present series was 12%, what means four times the consanguinity rate of 3% observed in Campinas region (non published data). However, none of these families had a baby or fetus with a recessive autosomal disease. Thus, we believe that the high frequency may be just biased by the small sample.

Recurrence of NIHF was identified in our series in one family (gangliosidosis GM1). Familial recurrence has been reported and has been found more frequent in Asian populations where the alpha-thalassemia is a major cause of NIHF [Suwanrath-Kengpol et al., 2005; Liao et al., 2007].

Hydrops was identified by prenatal ultrasound examination in 90.5% of the cases in the present study. Similar results were described in other NIHF series [Mascaretti et al., 2003; Silva et al., 2005]. In our series the registry of HF have occurred predominantly in the 2nd trimester (median: 21 weeks), more early than that reported in old series (25–32 weeks) [Mahony et al., 1984; McCoy et al., 1995; Rejjal et al., 1996; Swain et al., 1999].

Regarding the literature review of the IEM among NIHF, there is such diversity in the way as each series was studied that probably this is one of the most important causes related to the great differences of IEM frequencies. For instance, the casuistic were not uniform with respect to the included individuals (live births, stillbirths or neonatal deaths). Furthermore, the series of cases were differently studied—some studies included specific tests through of biochemical assays [Piraud et al., 1996; Groener et al., 1999; Burin et al., 2004; Kooper et al., 2006] versus others that used indirect signs like evidence of autopsy [Larroche et al., 1992]. As it was expected the studies whose goal was the specific IEM investigation presented a higher IEM frequency than in those whose purpose was a general evaluation of the NIHF (Table III). In some studies, the high frequency of IEM can be related to bias, like preferential investigation of cases in a center of reference for metabolic diseases [Burin et al., 2004], the study of patients with specific characteristic (ascites) [Favre et al., 2004], or a very small number of cases [Van Aerde et al., 1982; Im et al. 1984; Rejjal et al., 1996; Favre et al., 2004]. In addition to the sample size, other possible bias in these studies could be related to the influence of some population aspects, for example, inbreeding coefficient and founder effect. Regarding this and considering the Brazilian as a heterogenous and large population with some regions presenting a high consanguinity rate [Freire-Maia, 1990], three articles listed on Table III were studies carried out in Brazilian population [Mascaretti et al., 2003; Burin et al., 2004; Fritsch et al., 2012]. They recorded different conditions, all from LSD group, and reported a widely variable IEM frequency ranging from 1.7% to 15.1%, probably related to the design of each study. Consanguinity data was mentioned only by Burin et al. [2004] that reported the highest rate of IEM—15.1%, despite no evidence of consanguinity in any case [Burin et al., 2004]. So, a higher proportion of IEM cannot be necessarily related to the inbreeding.

Despite this great heterogeneity the most common group of metabolic disease associated with NIHF was LSD in all reported series of cases. Although biochemical tests are required for the LSD diagnosis confirmation, findings from the other complementary investigations like demonstration of vacuolated cells in placenta and fetus [Kooper et al., 2006] and evidence of skeletal findings, especially in case of Mucolipidosis II and ISSD [Froissart et al., 2005; Cathey et al., 2010], can strongly suggest the diagnosis. In two metabolic cases here presented placental changes and skeletal findings have previously suggested a probably LSD. Therefore, based on these cases and also in the reported experience [Bouvier, 1997; Froissart et al., 2005; Unger et al., 2005; Kooper et al., 2006; Cathey et al., 2010], we reinforce that placental examination and also skeletal evaluation through of the babygram should not be neglected because they can raise the suspicion of a metabolic disease, specifically a LSD.

CONCLUSIONS

In spite of the complexity, the approach of NIHF with a systematic and broaden protocol of investigation allows to reach a high diagnostic rate. The main diagnostic groups found in this series were chromosomal anomalies, syndromic, isolated heart defects, and congenital infections. The frequency of IEM in NIHF (5.7%) suggests that this group of diseases may be more frequent than previously supposed. The investigation of IEM should begin after exclusion of the more common causes of NIHF.

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