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ORIGINAL ARTICLE

Role of Autopsy in Diagnosis and Genetic Counselling of Congenital Malformations: a Prospective Analytical Study

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Abstract The study aimed to determine the role of autopsy in refining the antenatal diagnosis of fetal anomalies and its effect on genetic counseling. The objectives of the study were to correlate the findings of antenatal ultrasound with that of perinatal autopsy, to determine the contribution of autopsy in refining the antenatal diagnosis and whether it altered the genetic counseling. This was a prospective, non-interventional analytical study conducted in the Department of Obstetrics and Gynaecology, JIPMER with the approval of Institutional Ethics Committee. Fourty-six fetuses which were stillborn/aborted or expired in newborn period due to congenital malformations were included. The findings of antenatal ultrasound were correlated with postnatal autopsy findings. The genetic counselling based on antenatal findings were compared with that based on postnatal findings and any change in the counselling given were noted and analysed. More than 70% of anomalies among the 46 fetuses included in the study were detected by the mid trimester anomaly scan. Male predominance was seen in this study (24 male fetuses). A definitive diagnosis or documentation of anomalies by autopsy was possible in all 46 fetuses (100%). Single system anomalies were noted in 24 (52.17%) fetuses and multisystem involvement was observed in 19 (41.3%) fetuses. Among fetuses with multiple malformation syndrome, 15.8% of fetuses had probable single gene etiology, 26.3% were probably sporadic and 57.9% were unclassified. Autopsy was valuable in prenatal genetic counseling by confirming the USG findings with (5) or without additional information (30) in 35/46 (76.1%) cases and by changing the diagnosis in 11/46 (23.9%) cases. Autopsy led to refinement of the recurrence risk in 23.9% of cases (increased in 19.6% and decreased in 4.3%). From the present study, we conclude that the results demonstrate a good correlation between prenatal diagnosis by USG and post mortem diagnosis by autopsy. Prenatal ultrasound and postnatal autopsy are complementary and supplement each other. The value of autopsy in reconfirming the ultrasound finding and providing additional information is irrefutable. Though autopsy may not provide exact diagnosis or establish definitive etiology, it is an invaluable tool for genetic counseling in fetal anomalies.

Keywords Fetal autopsy · Perinatal autopsy · Congenital malformations · Genetic counselling · Postnatal autopsy · Prenatal diagnosis

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Introduction

Congenital malformations remain a common cause of perinatal deaths accounting for 10–15% of perinatal mortality in India [1]. It is estimated that 3% of all the neonates have a major congenital malformation and 0.7% have multiple congenital malformations [2]. Even a single pregnancy that ends in perinatal death due to congenital malformation in the fetus causes distress to the entire family. Apprehensive women are often reluctant to conceive again and seek counseling regarding the cause of anomaly and their recurrence risk in future. This risk can vary from very low to a risk as high as 100% depending on the underlying etiology. Hence, every effort should be made in establishing the correct diagnosis to provide optimum genetic counseling.

There is a declining trend in the acceptability of perinatal autopsy globally [3]. This is due to various factors such as administrative constraints, consent issues, lack of knowledge about the implications of current diagnosis on future pregnancies. Although prenatal ultrasound can identify most of the major malformations, it has been observed that the final diagnosis based on additional inputs from fetal autopsy is often different from prenatal diagnosis. Many studies have evaluated the correlation of autopsy findings with antenatal ultrasound [4, 5]. There are only few studies which have addressed the influence of fetal autopsy on genetic counseling [6]. Most of these have been retrospective and data on the value of autopsy in redefining the ultrasound based diagnosis with an aim to improve the genetic counseling is limited. We undertook this study to derive an expected outcome of definitive diagnosis for perinatal deaths due to fetal anomalies and to provide more specific and targeted genetic counseling.

Aim and Objectives

The study aimed to determine the role of autopsy in refining the antenatal diagnosis of fetal anomalies and its effect on genetic counseling. The objectives were to correlate the findings of antenatal ultrasound with that of perinatal autopsy, to determine the contribution of autopsy in refining the antenatal diagnosis and if contributory, to determine whether it altered the genetic counseling.

Materials and Methods

Prospective, non-interventional analytical study conducted in the Department of Obstetrics and Gynaecology, JIPMER (Jawaharlal institute of postgraduate medical education and research), a tertiary level hospital in Pondicherry between March 2016 & December 2017. The study has the approval of Institutional Ethics Committee.

Inclusion Criteria

- Aborted fetuses with congenital malformations.
- Stillborn fetuses with malformations.
- Newborns that expired due to congenital malformations.

Exclusion Criteria

- Autolysed fetuses for whom autopsy could not be completed.
- Antenatal information from ultrasound was not available.

Sample Size

All cases meeting the inclusion criteria during the study period of 18 months were included. There were 46 cases of postnatal autopsy done in fetuses with prenatally diagnosed structural malformations.

Study Procedure

Study participants were either referred for anomalies detected on routine scans done at our institute or for detailed evaluation and counseling after detection of anomaly at other institute. Only fetuses for which both prenatal USG & autopsy were done in our institute were included in the study. Fetuses with major malformations diagnosed by antenatal ultrasound were evaluated with targeted genetic sonogram. Detailed findings were noted with system wise categorization of anomalies. Non-directive genetic counseling about the nature of malformations noted and their effect on prognosis was provided antenatally. Pregnancy was followed till termination or delivery and all parents of fetuses who expired were again counseled post-natally. Parents were informed about the option of autopsy and all the questions and concerns were addressed. Fetuses of consenting parents were included into the study. Autopsy was conducted in a systematic manner and findings were noted in pre-designed format. Patient demographic details, maternal history, medical history, drug history, past history, family history with three generation pedigree analysis and marital history were noted.

The final diagnosis & the risk of recurrence before and after autopsy were grouped and compared according to the following categories.

Category [7]	Contribution of autopsy to final diagnosis and genetic counseling
1.	No addition to USG diagnosis and the counseling remained same
2.	Added to the diagnosis but did not affect counseling
3a.	Added to diagnosis and decreased recurrence risk estimation
3b.	Added to diagnosis and increased recurrence risk estimation

Results

A total of 46 autopsies were performed and analysed critically in the department of Obstetrics and Gynecology, JIPMER, a tertiary level hospital in Pondicherry between March 2016 & December 2017.

Demography and Baseline Characteristics (Table 1)

The age of the mothers ranged from 16 to 32 years with an average of 22.4 years and 41.3% of fetal anomalies were found in women aged between 21 and 25 years. Of the 46 fetal autopsies, 24 babies (52.2%) were born to multigravida and 22 babies (47.8%) were born to primigravida. Previous history of anomalous fetuses was present in 8 out of 46 mothers (17.4%) and in 3 cases it was the recurrence of same type of anomaly. History of consanguinity was noted in 18/46 of mothers (39.1%). In the population studied, 9 out of 46 antenatal mothers were taking drugs for one or the other medical disorder. Thyroxine of varying doses was given to 7 mothers from first trimester. Levetiracetam and oxcarbamazepine were being given at the time pregnancy was diagnosed; and 1 one mother took Metformin at 28 weeks of gestation. Out of 46 antenatal mothers included in the study, 15 mothers found to have medical disorders complicating their pregnancy, majority being diabetic and hypothyroid, 6 cases each, 2 cases of seizure disorder on anti-epileptics and 1 case of Rh incompatible- not iso-immunized. There were no cases in whom infectious diseases were identified in prenatal period. The gestational age of 46 fetuses ranged from 13 to 38 weeks with an average of 24.6 weeks. Maximum number no of anomalies diagnosed between the gestational age group of 20–26 weeks.

Table 1 Demography of cases

Parameter	Frequency	Percent
Maternal age groups (Y)		
16–20	11	23.9
21–25	19	41.3
26–30	11	23.9
31–35	5	10.9
Parity		
Multiparity	24	52.2
Primiparity	22	47.8
Marital history		
Consanguinity	18	39.1
Gestational age at diagnosis		
< 15 weeks	2	4.3
15–20 weeks	12	26.1
21–26 weeks	16	34.8
27–32 weeks		
33–38 weeks	9	
7	19.6	
15.2		
Mode of termination		
Induced	35	76.09
Spontaneous	11	23.91
Sex of the fetus		
Male	24	52.17%
Female	18	39.13%
Indeterminate	4	8.70

After the prenatal diagnosis of congenital anomalies, 35 fetuses (76.09%) were induced and 11 fetuses (23.91%) delivered spontaneously. There were 14 macerated stillbirths due to intrauterine demise, 11 abortuses and 10 fresh still births. There were 11 fetuses who were alive at birth but expired at varying periods within 7 days. Out of 46 fetuses subjected to autopsy, 24 fetuses (52.17%) were male, 18 fetuses (39.13%) were female with a male to female ratio of 4:3 and 4 had ambiguous genitalia (8.70%).

System Wise Classification of Anomalies (Table 2)

System wise classification of anomalies and the correlation between ultrasound and autopsy are given in Table 2.

Single System Involvement

On autopsy, single system anomalies were noted in 24 out of 46 fetuses (52.17%). Respiratory system with 5 cases (21%) was the most frequently involved followed by gastro intestinal tract with 4 cases (16.6%), central nervous

Table 2 System wise classification of anomalies and correlation between ultrasound and autopsy findings

System involved[a]	Number of cases on ultrasound	Number of cases on autopsy	Correlation (%)
CNS	10	10	100
CVS	15	16	93.75
RS & Thorax	8	11	72.7
GIT	8	11	72.7
GUS	19	19	100
MS	10	12	83.3
CF	9	14	64.2
Others	11	12	91.6

[a]*CNS* Central nervous system, *CVS* cardiovascular system, *RS & Thorax* respiratory and thoracic system, *GIT* gastro-intestinal system, *GUS* gastro-urinary system, *MS* musculoskeletal, *CF* cranio-facial

system with 3 cases (12.5%) and genitourinary system with 3 cases (12.5%). Cardiovascular and musculoskeletal involvement was 8.3% with 2 cases each and there was 1 case of lymphangioma over the left jaw (4.2%) in cranio-facial system and other cases which could not be classified under single system (16.6%) were 4 cases of hydrops, 2 unexplained and 2 with cystic hygroma.

Multi-System Involvement

Involvement of more than one system was observed after autopsy in 19 fetuses 19/46 (41.3%). These fetuses were classified further into subgroups namely multiple malformation syndrome (MMS) with likely single gene etiology (3), MMS that are probably sporadic (5) and MMS unclassified for cases with no recognizable cause (11).

Category-Wise Classification After Correlation Between USG and Autopsy Findings

Contribution of autopsy to redefine the final diagnosis after categorization as described previously (Fig. 1).

Additional Observations

Placental and umbilical cord pathology were present in 6 cases (13%). There are 3 cases of mono chorionic placenta, 2 cases of single umbilical artery and 1 case of hyper coiled cord with replacement of cord by thin atretic band at the umbilical attachment site. Amniotic fluid abnormalities were seen in 50% of cases, oligohydraminos in 13%, anhydraminos in 6% and 4% of cases had polyhydramnios.

Additional Tests

Karyotyping was done in 17 cases. It was reported to be normal in 15 cases. Two cases showed abnormal karyotype, monosomy 18 and trisomy 18 each.

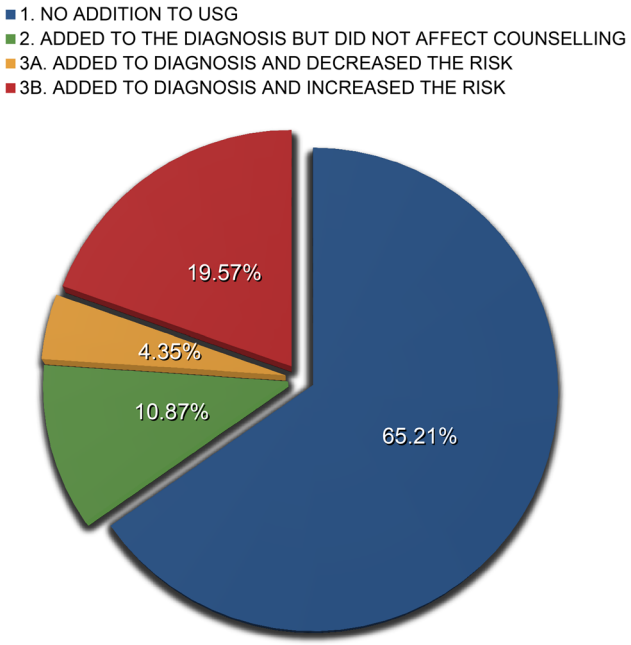

Fig. 1 Degree of agreement between ultrasound and autopsy

Viral Polymerase chain reaction **(PCR)** was done to screen the infectious etiology in one case of unilateral hydrothorax and found to be normal.

Clinical Exome sequencing (CES) was done in 3 case and 2 cases showed mutation and changed the final diagnosis in 1 case.

Case 1: No significant variants found in this fetus with bilateral anophthalmia and hydrocephalus and mother had history of previous anencephaly and spontaneous abortion.

Case 2: This mother had history of spontaneous abortion of three male fetuses and the current 4th male fetus showing bilateral renal dysplasia, post-axial polydactyly and anhydramnios resulting in fetal demise at 18 weeks of gestation. A heterozygous missense mutation (c.4970G > A) was found in exon 25 of C2CD3 gene indicating carrier status for Oro-facio-digital syndrome type XIV but not helpful in establishing the exact diagnosis. Extended

investigation with whole exome or genome sequencing was not done due to cost constraints.

Case 3: In this case with history of NIH in previous 2 male fetuses and the current fetus also showing NIH and cardiomegaly, we found hemizygous missense variant c.215G > A (p.C72Y) in the exon 2 of GPC3 gene. Glypican 3 is a heparan sulfate proteoglycan involved in regulation of signaling of WNTs, Hedgehogs, fibroblast growth factors, and bone morphogenetic proteins. GPC3 gene is a known cause for Simpson-Golabi-Syndrome Type 1. The mutation was validated by Sanger sequencing in the fetus and on testing the mother the same was found in heterozygous state indicating her carrier status. Non-immune hydrops though described as a typical presentation of SGS-2 has rarely been reported in SGS Type 1.

Histopathological examination was reported to be abnormal in 8 cases of total 46 (17.4%). One case each of polycystic kidney disease, Ebstein anomaly with atrialization of right ventricle, and 5 cases of single umbilical artery.

Discussion

Post mortem examination of the fetus significantly contributes to the diagnosis of intrauterine fetal death and congenital anomalies. The study of congenital malformations greatly influences the genetic counseling in successive pregnancies. The future reproductive decision of the couple depends on the cause of fetal death, which will in turn predict the recurrence risk and may provide options for the prevention of the same. Since the inception of the study, we found nearly 20 studies published in the literature with similar objectives [1, 4, 6–22], one such study involving 230 cases published by Nayak et al. in 2015 [14].Most of these have been retrospective and data on the value of autopsy in redefining the ultrasound based diagnosis with an aim to improve the genetic counseling is limited.

Baseline Characteristics

Age: Most of the women included in the study were between 21 and 25 years (41.3%) and only 10.9% of anomalous fetuses were born to mothers above 30 years of age. This higher proportion of women belonging to younger age group might be because the study was conducted in a tertiary health care center in South India where the prevalence of early marriages and child bearing is quite high. Many authors both from India and abroad have shown the higher incidence of congenital malformations in the fetus born to maternal age between 20 and 35 years [22].

Gestational age: In the present study, maximum number of anomalies were diagnosed between the gestational age of 21–26 weeks (35%), followed by 15–20 weeks (26%), 27–32 weeks (20%), 33–38 weeks (15%) and < 15 weeks (5%). The mean gestation at diagnosis was 24.6 weeks and more than 70% of anomalies were detected by the mid trimester anomaly scan.

Fetal sex: Male predominance was seen in this study with a male to female ratio of 4:3 with 24 male and 18 female fetuses. Many studies have documented male predominance amongst congenital malformed babies [23]. The reason for male predominance in the study is not clear, although there were 3 cases in which an X-linked pattern of inheritance was implied and few cases with cloacal anomalies mostly occurred in males. Cloacal anomalies have mainly been described in females most of them examined postnatally or as children and the male predominance of prenatally diagnosed cloacal anomalies is indicative of more severe end of the spectrum.

Diagnosis on Autopsy

Diagnosis or documentation of anomalies was possible in all 46 fetuses (100%) included in this study on autopsy. The anomalies observed in this study were grouped under two categories: those with single system involvement and those with multisystem involvement. Single system involvement was noted in 24 fetuses (52.2%) and involvement of more than one system was observed in 19 (41.3%). Involvement of respiratory and gastrointestinal system was the predominant observation in the present study compared to previous studies as detailed under Table 5. We observed lower frequency of central nervous system involvement (12.5%) compared with other studies (21–74.2%). This could be due to selection bias as fetuses with clear cut diagnosis are less likely to be referred to tertiary care center for fetal autopsy. Among the cases with multi-system involvement, majority of syndromic diagnosis fell under the subgroup unclassified an observation similar to previous studies. In cases with multiple malformations without any specific diagnosis, same malformations can be looked for in future pregnancies by USG evaluation even at the early gestational age.

Impact on Definitive Diagnosis and Genetic Counseling

The autopsy findings were compared with prenatal USG findings to analyze the role of autopsy in redefining the final diagnosis. To analyze the utility of autopsy on genetic counseling, the risk of recurrence in future pregnancies were compared before and after autopsy. Autopsy led to

refinement of the recurrence risk in 23.9% of cases. However, the recurrence risk remained the same pre and post autopsy in 76.1% of cases (Fig. 1).

There were two cases in category 3a that is the risk of recurrence estimated based on ultrasound was higher than that estimated following autopsy. In one case of non-immune hydrops, the risk of recurrence was estimated to be at least 25% assuming the underlying etiology to be autosomal recessive. However, on autopsy a diagnosis of urorectal malformation sequence was made which is usually a sporadic condition with very low risk of recurrence. A second case with prenatal risk estimation that of autosomal recessive disorder was reassigned after autopsy as VACTERL malformation hence bringing down the recurrence risk to that associated with sporadic/multifactorial disorders of 5–8%. The recurrence risk estimated after autopsy was higher compared to prenatal risk in 9 cases hence placing them under category 3b. Few prominent among them were Popliteal pterygium syndrome, Cornelia-de-Lange and Holoprosencephaly with bilateral cleft lip and palate. In these cases, findings such as presence of pterygium, polydactyly, increased hair distribution, dysmorphism (Fig. 2) were not reported following prenatal ultrasound. However, many of these features very easily evident following autopsy.

Comparison with Previous Studies

Various studies have compared prenatal USG findings with autopsy findings with an objective to evaluate if autopsy findings just confirmed the diagnosis or added to the diagnosis or changed the diagnosis [9–11, 19, 24]. Very few studies have looked into the impact of fetal autopsy on genetic counseling for affected families (Table 3). Although the results of different studies vary, they all establish the clinical utility of fetal autopsy. Variation in the results may be due to ultra-sonographer efficacy, quality of the equipment, type of fetal anomaly, differences in the gestational age at the time of examination or differences in the definition of outcome measures (Tables 4 and 5).

Table 3 Comparison of impact of autopsy on genetic counselling

Author	Recurrence risk redefined (%)
Boyd et al. [3]	27
Sankar et al. [7]	11.6
Phadke and Gupta [12]	33
Nayak et al. [15]	36
Present study	23.9

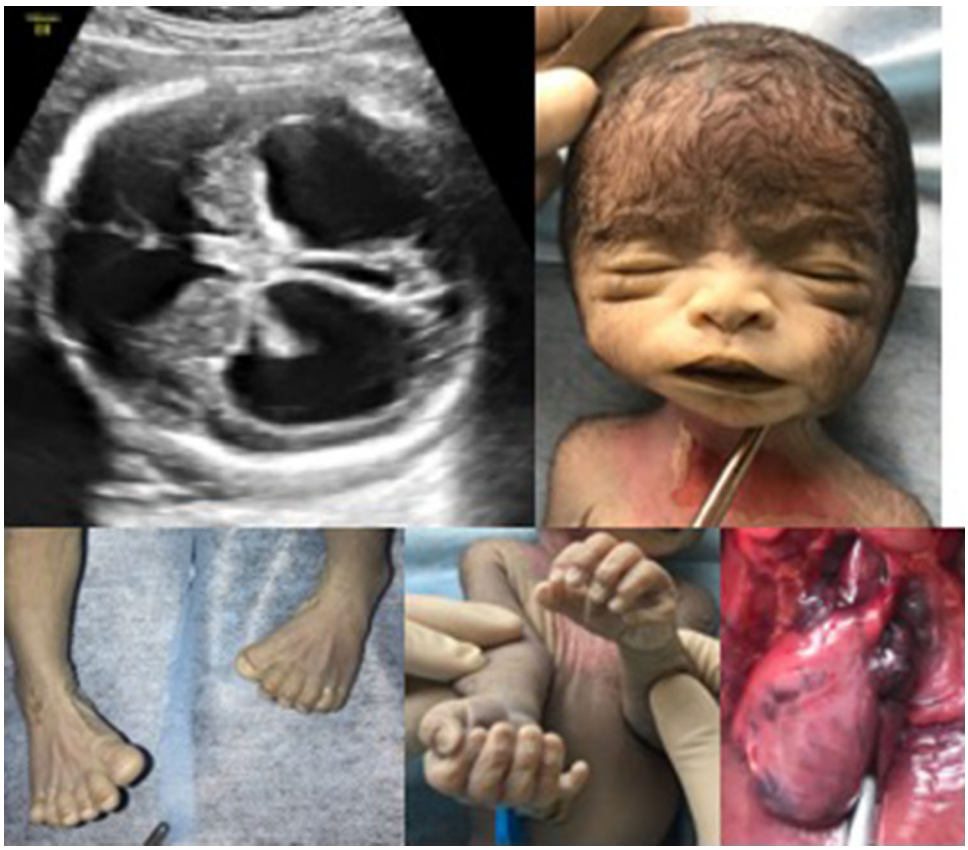

Fig. 2 Clock wise pictures showing Hydrocephalus on ultrsound, Hypetrichosis and hexadactyly of all limbs and pulmonary stenosis on autopsy

Table 4 Percentages of single organ system and multisystem involvement in various studies

Single system involvement									
Authors	N	CNS (%)	CVS (%)	RS & thorax (%)	GIT (%)	GUT (%)	MS (%)	CF (%)	Others (%)
Boyd et al. [3]	132	21	3	2	–	3	5	1	6
Sankar et al. [7]	206	74.2	4.9	2	–	17.2	–	0.8	–
Tomatir [24]	183	31.1				2.3	14.2	18.6	
Nayak et al. [15]	230	25	10.3	0.9	2	12	10	3.7	17
Present study (2017)	46	12.5	8.3	21	16.6	12.5	8.3	4.2	16.6

Multisystem involvement			
Authors	Single gene MMS N (%)	Sporadic MMS N (%)	MMS unclassified N (%)
Sankar et al. [7]	8/54 (14.8%)	10/54 (18.5%)	16/54 (29.6)
Nayak et al. [15]	36/92 (39.1%)		45/92 (48.95)
Present study (2017)	3/19 (15.8%)	5/19 (26.3%)	11/19 (57.9%)

Table 5 Cases with additional findings on autopsy thus increasing the estimated risk (Category 3b)

	Autopsy	Ultrasound
1	Popliteal pterygium syndrome	B/L renal agenesis
2	Holoprosencephaly, lissencephaly, bilateral cleft lip and palate	Holoprosencephaly
3	Bladder outlet obstruction with left renal agenesis	Megacisterna
4	Meningocele, horseshoe kidney with urethral atresia	Meningocele
5	Cloacal anomaly, left renal agenesis with right hydro uretro nephrosis, abnormal pulmonary artery and limb anomalies-	TOF, left renal agenesis with right hydrouretronephrosis
6	Simpson Golabi syndrome-1	Non-immune hydrops?Cardiomyopathy
7	Post axial polydactyly, hydrocephalus, hypertricosis and Pulmonary Stenosis- *Cornelia-de-Lange*	Hydrocephalus and Pulmonary Stenosis.
8	Fetal akinesia deformation sequence	Non-immune hydrops
9	Left CDH, monoventricle with common arterial trunk, persistent urogenital sinus & meso/meta nephric ducts & common cloaca	Left CDH, monoventricle & B/L dysplastic kidneys.

Additional Tests

The exact benefits of postmortem autopsy to affected parents are not limited to refining the risk of recurrence. A definitive diagnosis cannot be made sometimes, even after thorough post mortem examination and hence information given to parents may cover a wide range of possible diagnosis. In such situations, radiographs, histopathological examinations, evaluation for infections, karyotyping, molecular genetic tests such as microarray, sequencing tests can provide additional information needed to establish the cause. Storage of fetal samples for future genetic analysis is invaluable in unresolved cases and provides the hope of an accurate diagnosis at a much later date.

Evaluation of Additional Tests

Establishing etiology depends on deep phenotyping along with robustness and thoroughness of investigations. A systematic approach starting from easily available, low cost tests and moving on to advanced investigations in unresolved cases is reasonable. Gross autopsy, histopathology and fetogram are the baseline investigations but may not always provide answers. Fetal karyotyping is desirable in all cases of fetal anomalies, if cost is not a concern. Evaluation for infections may be needed for certain indications such as non-immune hydrops, hydrothorax, placentomegaly, intra-uterine growth restriction, oligohydramnios etc. If the indication to investigate further is very strong, especially with recurrent affected

pregnancies, advanced investigations like Microarray and sequencing tests are highly valuable in providing the diagnosis.

Radiographs

Fetograms are important in evaluation of bony structures and can provide important clues to the possible diagnosis. In this study, postmortem radiographs were performed in all cases. It aided in the diagnosis of cases like skeletal dysplasia (Atelosteogenesis), pentalogy of Cantrell and VACTER anomalies.

Histo-Pathological Examination (HPE)

HPE of fetal organs is of limited usefulness in fetuses with congenital malformations. An exception to this is the evaluation of fetal renal disease. It is difficult to distinguish between renal cystic diseases on an ultrasound scan as oligohydramnios is usually associated. Moreover, the differentiation between infantile polycystic renal disease (recurrence risk—25%) and cystic renal dysplasia (recurrence risk—3%) is possible based on tissue diagnosis.

We had 3 cases of multi cystic kidney disease (MCKD), 1 case of dysplastic and cystic renal dysplasia each. Out of 3 MCKD, one case turned out to be polycystic kidney disease (PCKD) thereby increasing the recurrence risk (25–50%) in future pregnancies as PCKD will be inherited in an AR/AD pattern. In this study, abnormality on HPE was found in 8 cases.

Karyotyping

KT was performed in 17 out of 46 cases either using amniotic fluid or fetal cord blood samples. Due to limited resources, KT was restricted to cases where there was strong and clear indication. The result was abnormal in 2 cases- Monosomy 18 and Trisomy 18 and in 2 cases it was culture failure.

Monosomy 18

Karyotype of amniotic fluid in a fetus with NIH at 24 weeks of gestation revealed Monosomy 18. Though USG and autopsy could rule out fetal anemia and structural anomalies, they were inconclusive in establishing the cause.

Trisomy 18

Trisomy 18 was detected on amniotic fluid karyotype in another fetus with multiple anomalies. There was enlarged cisterna magna, cerebellar hypoplasia, large CDH and micro-retrognathia in the fetus on ultrasound. Autopsy findings correlated with USG with additional findings of B/L Rocker-bottom feet and Eventration of diaphragm instead of CDH. Trisomy 18 or *Edward syndrome* is estimated to have a recurrence risk of 1% [25, 26].

Molecular Studies

Clinical Exome sequencing (CES) was done in 3 cases with previous history of anomalous fetuses. **CES** played an important role in clinching the diagnosis in one case ***(Case no.39)*** in which ultrasound and autopsy were both unrewarding. Mother had history of recurrent non-immune hydrops fetalis in previous two pregnancies. Both the prior fetuses were still born and male. Dilated cardiomyopathy was reported during prenatal scan in these two previous male fetuses. In the current pregnancy, the fetus had similar presentation of NIH and dilated cardiomyopathy. Fetal karyotyping was normal. CES done on the fetal DNA showed a missense mutation in exon 2 of the **GPC3 gene** (chrX:133087199; C > T;c.215G > A). On screening the parents, mother was found to be a carrier of the same mutation. GPC3 gene is a known cause for Simpson-Golabi-Syndrome Type 1 which is characterized by pre- and postnatal macrosomia, craniofacial features like macrocephaly and multiple congenital anomalies including congenital heart defects, diaphragmatic hernia and skeletal anomalies. Affected children often have mild to severe intellectual disability. Non-immune hydrops is not typically described in SGS-1 but this feature in our case could be the result of dilated cardiomyopathy as suspected on prenatal ultrasound. This fetus was also male and still born. Hence, in this case the risk of recurrence could be stated as 50% in subsequent male off springs. Following the establishment of etiology, mother could avail prenatal diagnosis in two of her future pregnancies, both of which were unaffected.

In two other cases, etiology could not be established even after autopsy and CES. In one of these (Case no. 9) there was history of early fetal demise by 18–20 weeks in previous 3 males and the fourth pregnancy was evaluated under our study. This fetus was also a male and showed bilateral renal dysplasia and anhydramnios at 16 weeks of gestation resulting in early fetal demise by 18 weeks. Fetal karyotype was normal. Since mother had post-axial polydactyly of lower limbs and her brother had facialfeatures suggestive of Oro-facio-digital syndrome, this disorder which is most commonly X-linked was suspected to be the cause for repeated mishaps. However, clinical Exome sequencing of fetal DNA revealed a heterozygous missense variation in exon 25 of the C2CD3 gene (chr11:73768571; C > T;c.4970G > A)). This gene is associated with OFD XIV which is a type of OFD with autosomal recessive inheritance. As the mutation found in this fetus was

heterozygous indicating a carrier state not accountable for the phenotype the diagnosis remained unestablished. In the other case with history of one previous anencephaly, one spontaneous abortion and present fetus showing bilateral anophthalmia and hydrocephalus (Case no. 1), even extensive tests proved futile. All the investigations including USG, autopsy, karyotyping and CES could not provide a definitive diagnosis though a genetic cause was strongly suspected. In cases such as this, a more detailed genetic work up including whole exome sequencing may provide clues to possible etiology.

Others

DNA banking for future study has been done in 1 case of recurrent non immune hydrops where only limited investigations could be done due to financial concerns.

Non Immune Hydrops

Investigative work up for infectious and metabolic disorders plays an important role in cases of NIH were no obvious cause has been identified. The present study had 9 cases of hydrops, of which 5 cases were associated with other anomalies. These were lethal skeletal dysplasia, laryngeal atresia, bronchial atresia, arthrogyposis and Dilated cardiomyopathy. Among the four where no other associated anomaly could be identified, two cases had cystic hygroma and two were cases of isolated hydrops. Fetal autopsy combined with the results of microbiological, cytogenetic and metabolic investigations provides an etiological diagnosis in 65–85% of NIH as per literature from previous studies. In this study, the low diagnostic yield in hydrops is possibly due to incomplete genetic workup in addition to insufficient investigation for infective and metabolic disorders.

Benefits and Limitations of Autopsy

Autopsy is considered to be the gold standard investigation for evaluating the causes of perinatal mortality. Quantifying the value of autopsy is not easy. In majority of cases, in which prenatal USG findings are confirmed, parents can gain comfort that their fetus had the prenatally suspected condition. The finding of additional malformations, as well as in some cases changing the diagnosis itself, may be helpful in targeting tests in a future pregnancy. Autopsy was very valuable in confirming the USG based diagnosis in many of our cases. Confident documentation of anomalies suspected on ultrasound was possible by autopsy. It was easier to establish the phenotype by dysmorphology, examination of external orifices, establishing patency of bowel, ureter, esophagus and examination of extremities including digits. For example, in the present study some findings like band between finger and scalp, lobulations of lung, bifid thymus, colonic atresia, absence of ureters and hypertrichosis which could almost never be picked up on ultrasound were easily documentable by autopsy. In some cases, though autopsy did not add greatly to the diagnosis, as in Laryngeal atresia and Bladder outlet obstruction, visual confirmation of the exact level and type of obstruction was possible by autopsy. Some anomalies like popliteal pterygium, hexadactyly of all four limbs, sirenomelia and cloacal anomalies, though suspected on ultrasound, were easily evident on autopsy. Contribution of autopsy in evaluating various organ systems varies. We found USG/ECHO to perform better at evaluation of heart as it enables functional study, while autopsy was best at evaluation of dysmorphology, ear, limbs, genital and digital defects. USG can perform equally well and complementary to autopsy in evaluation of internal organs such as heart and evaluation of hydrops.

Though autopsy is very useful in confirmation of ultrasound findings or providing additional information and establishing the phenotype, it has limited utility in confirmation of exact etiology. As many fetuses with major anomalies die in utero or expire during the termination process, there is limited availability of adequate non-autolysed samples to conduct informative, histopathological examination and do genetic tests. Karyotyping by cell culture and molecular genetic tests by DNA extraction are likely to fail if samples are taken long after the foetus is dead. Hence, it is preferable to do collect the prenatal amniotic/foetal blood/chorionic villus samples whenever possible. Autopsy by itself is an incomplete tool in evaluation of foetuses with anomalies, though when supplemented by clinical history, pedigree analysis and ultrasound it can narrow down the probable diagnosis. Newer investigations such as chromosomal microarray, Next-generation sequencing for clinical exome/whole exome are likely to assume more importance in future. However, these tests are currently highly expensive, limited in availability and expertise in interpretation is still evolving at present.

Autopsy is one of the least expensive, easily reproducible and documentable investigation. Despite many limitations, description and documentation of anomalies by autopsy are valuable in providing prenatal diagnosis in future pregnancies by monitoring the foetus by ultrasound for similar defects. Hence, autopsy still remains an indispensable component of perinatal investigation and should always be the first step in evaluation of perinatal deaths. Our study highlights the benefits, limitations and complementary value of both USG and fetal autopsy and also

stresses the role of fetal autopsy in providing accurate genetic counseling.

Summary

The study was conducted in the department of Obstetrics & Gynaecology, JIPMER, a tertiary level hospital in Pondicherry over a period of one and half years extending from March 2016 & December 2017. A total of 46 women who fulfilled the inclusion criteria were recruited into our study. Maternal age of majority of recruited cases was between 21 and 25 years (41.3%) and 24/46 fetuses (52.2%) were born to multigravida mothers. Maximum number of anomalies were diagnosed between 20 and 26 weeks with a mean of 24.6 weeks. Diagnosis or documentation of anomalies was possible in all 46 fetuses (100%) included in the present study on autopsy. Anomalies involving a single system were noted in 24/46 fetuses (52.17%) and multisystem involvement were observed in 19/46 fetuses (41.3%). Autopsy was valuable in prenatal genetic counseling by confirming the USG findings with (5) or without additional information (30) in 35/46 (76.1%) cases and by changing the diagnosis in 11/46 (23.9%) cases. Autopsy led to refinement of the recurrence risk in 23.9% of cases (increased in 19.6% and decreased in 4.3%).

Conclusion

Results from the present study demonstrate a good correlation between USG prenatal diagnosis and autopsy post mortem diagnosis, reflecting the potential and limitations of both examinations. The value of autopsy in reconfirming the ultrasound finding and providing additional information is irrefutable. Prenatal ultrasound and postnatal autopsy are complementary and supplement each other. DNA storage of the fetal sample is of utmost importance in establishing an etiology and counseling the couple for recurrence risk. Though autopsy may not provide exact diagnosis or establish definitive etiology, it is an invaluable tool in the field of genetic counseling.

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Compliance with Ethical Standards

Conflict of interest The author(s) declare that they have no competing interests.

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