"FETAL AUTOPSY STUDY TO ASSESS RELATIVE FREQUENCY OF PULMONARY HYPOPLASIA AND EVALUATION OF VARIOUS ANOMALIES ASSOCIATED WITH PULMONARY HYPOPLASIA"

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ABSTRACT

Background/ Introduction- Fetal autopsy study helps in determination of aetiology of pregnancy loss and may help in determining the cause of Fetal death. Pulmonary hypoplasia (PH) is one of the commonest causes of neonatal morbidity and mortality. The suggested diagnostic criteria for PH are Lung weight: Body weight (LW: BW) ratio ≤ 0.012 and/or Radial Alveolar Count (RAC) ≤ 4.1. Hence present study was done to determine the relative frequency of pulmonary hypoplasia (PH) in Fetal autopsy cases by evaluation of LW: BW ratio and RAC. Evaluation of associated conditions/anomalies with PH was also done.

Objectives-

1. To determine the relative frequency of pulmonary hypoplasia in fetal autopsy study by Lung weight: Body weight ratio and Radial alveolar count.
2. To evaluate frequency of various anomalies associated with pulmonary hypoplasia.

Material & Methods- A prospective observational study was done on Fetal autopsy specimens received in the Department of Pathology from December 2019 to July 2021. External examination, anthropometric data of foetus and grossing was done as per the standard format of Fetal autopsy study. Evaluation of PH was done in all cases by LW: BW ratio and RAC.

Diagnostic criteria for PH were taken as LW:BW ratio less than 0.012 and/ or RAC less than 4.1.

Results- Out of 62 cases studied, diagnosis of PH was rendered in 45 cases. Concordance between LW: BW ratio and RAC was observed in 33 cases amounting to 53.23%. In these cases, RAC was less than 4.1 and LW: BW ratio was also less than 0.012. In 12 cases discordance was noted between LW: BW ratio and RAC. In these cases, RAC was less than 4.1 and LW: BW ratio was more than 0.012. Out of these 12 cases 4 cases were of NTD, 2 cases were of CCAM, 1 case of gastroschisis and 5 cases were of IUD.

Conclusion- Evaluation by LW: BW ratio and RAC provides a reliable index of lung growth & should be an essential part of Fetal autopsy study. In the present study PH was incidentally discovered in some cases which may be the cause of mortality.

Key words- Foetal autopsy, Lung weight to Body weight ratio (LW: BW), Pulmonary hypoplasia (PH), Radial alveolar count (RAC).

LIST OF ABBREVIATIONS USED

ACD	Alveolar capillary dysplasia
AMC	Arthrogryposis multiplex congenita
BPD	Bronchopulmonary dysplasia
CCAM	Congenital cystic adenomatoid malformation
CDH	Congenital diaphragmatic hernia
CTEV	Congenital Talipes Equinovarus
CT	Computed tomography
DNA	Deoxyribonucleic acid
E	Embryonic days
FBM	Fetal Breathing Movements
GA	Gestational age
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IUD	Intrauterine death
LW:BW	Lung weight: Body weight
MRI	Magnetic resonance imaging
NTD	Neural tube defect
PC	Postconceptional days
PH	Pulmonary hypoplasia
PROM	Premature rupture of membranes
RAC	Radial Alveolar Count
SD	Standard deviation
USG	Ultrasonography
WHO	World Health Organization

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INTRODUCTION

Pulmonary hypoplasia (PH) is one of the commonest causes of neonatal morbidity and mortality.[1] It is defined as unilateral or bilateral defective or incomplete development of lung parenchyma, airway, and vessels or incomplete development of lung which is not appropriate for gestational age.[1-3]

The incidence of pulmonary hypoplasia is 9 to 11 per 10000 in general population and its reported prevalence in perinatal autopsy is between 7.8% to 26%.[2,3]

Pathologically lung hypoplasia is defined as a decrease in the Lung Weight to Body Weight (LW: BW) ratio and reduced Radial Alveolar Count (RAC) based on findings of autopsy examination.[3] Simple method to determine pulmonary hypoplasia is a direct measure of lung weight and its comparison with body weight. LW: BW ratio is the consistent and better method of diagnosing pulmonary hypoplasia.[2] Lower limit for LW:BW ratio for an infant older than or equal to 28 weeks is 0.012. LW:BW ratio in fetus less than 28 weeks of gestation is 0.015.[3]

Pulmonary Hypoplasia is also diagnosed by RAC which is measured by the number of alveoli which are traversed by a perpendicular line drawn from the center of a respiratory bronchiole to the nearest connective tissue septum.[4]

PH may be primary or secondary. Primary PH is rare as compared to secondary PH. Exact etiology is not known in primary PH. However, deficiency of Vitamin A and viral infection during pregnancy are considered as possible etiological conditions.

Genetic or iatrogenic factors are also mentioned as possible causes for primary PH.[5] Secondary PH can occur due to abnormalities in the thoracic cavity, heart, kidneys, abnormal fetal breathing movement and decrease in the volume of amniotic fluid.[1,2] In majority of the cases PH is due to the reduction in thoracic volume, followed by reduced production of amniotic fluid due to renal anomalies.[1] Reduced production of amniotic fluid usually occurs due to renal anomalies.

Fetal autopsy study helps to determine the PH by evaluating LW:BW ratio and RAC and thus may help to evaluate the relative frequency of pulmonary hypoplasia in fetal autopsy study and this may provide the explanation for loss of pregnancy. Hence this study was undertaken to determine the frequency of PH by LW:BW ratio and RAC and also to determine the various conditions/ anomalies associated with PH.

OBJECTIVES OF THE STUDY

1. To determine the relative frequency of pulmonary hypoplasia in fetal autopsy study by Lung weight: Body weight ratio and Radial alveolar count.

2. To evaluate the frequency of various conditions/ anomalies associated with pulmonary hypoplasia.

REVIEW OF LITERATURE

Autopsy Overview

The word "Autopsy" is derived from a Greek word. Autopsy means seeing for oneself.[6,7] Autopsy examination is also called post mortem examination. Herophilus, from Greece, was the first person to dissect the human body in 335-280BC to look into causes of death. Galen, the scientist from Rome, gave anatomical descriptions of the human body in 138-201AD. Vesalius based on his observations from 1514 to 1564 corrected the errors of Galenic anatomical findings. Morgagni in his study done from 1682 to 1771 confirmed that all human diseases cause morphological changes in various organs. Rokitansky correlated Morgagni principles with clinical presentation and the role of pathologists in human diseases. Virchow, Father of Modern Pathology gave the concept that any disease in the body starts in the cell.[6]

Autopsies can be divided into two major types, hospital autopsies and medicolegal autopsies. Hospital autopsies are done at the request of clinicians with the consent of diseased kin. Medicolegal autopsies are forensic autopsies.[7] Autopsy study aims to identify the cause of death in medicolegal cases and thus helps the forensic pathologist. Autopsy study also helps to teach undergraduate and postgraduate medical students about the etiopathogenic mechanism and morphological changes in various disorders. In addition to these, Fetal autopsies will also help in identifying congenital and hereditary disorders to counsel parents, which will help them to undergo genetic tests to plan for further management.[8,9]

Four principal techniques of autopsy are technique of Virchow, technique of

Rokitansky, technique of Ghon and technique of Letulle. In the technique of Virchow organs are removed one by one starting from the cranial cavity, spinal cord, thoracic cavity, followed by cervical and abdominal cavity. The technique of Rokitansky is characterized by in situ dissections. In Ghon's technique organs are removed as organ blocks- en bloc removal. In the Letulle technique organs of cervical, thoracic, abdominal and pelvis are removed as one organ block- en masse removal.[10] Letulle technique is recommended for the perinatal autopsies to demonstrate organ anomalies instead of organ removal.[10]

Pediatric and Perinatal autopsy forms one of the important to ol in the determination of the cause of death.[10,11] Parents who have lost a baby would want to know the cause of death. Autopsy will explain whether the loss of fetus was preventable or whether recurrence is possible in future pregnancy. Thus, fetal autopsy study will guide parents and clinicians to plan future pregnancies.[11,8] Information obtained by autopsy study is important for formulating the guidelines for better outcome of subsequent pregnancies. Thus, perinatal morbidity and mortality rate can be reduced.[11,9] Based on autopsy study proper hematological, biochemical, immunological, microbiological and cytogenetic tests can be done which may help in determining the cause of death.[8]

Pediatric or perinatal autopsies differ from adult autopsies and are usually done by pathologists who are experienced in perinatal pathology.[10] External examination is very important in fetal autopsy and should be looked for dysmorphic features of the face, cleft lip, cleft palate, amniotic bands, neural tube defects, skeletal deformities, external genitalia and imperforate anus.[10] In perinatal autopsies significant findings are observed in 40%-70%.[12,13]

The future of autopsy is changing with many advanced techniques. The current post mortem research includes less invasive autopsy, restricted autopsy, no incision autopsy which includes radiological post mortem magnetic resonance imaging, computed tomography and tissue sampling. Restricted autopsy gives consent for examination of parts that are only asked for. Tissue sampling in restricted autopsy include needle biopsy and aspiration of body fluids. Future development in this field are Biobanks which store tissue samples which can be studied for genomics, transcriptomics, proteomics, metabolomics. These techniques help in improved understanding of the etiology of diseases, discovery of targeted treatment. This may lead to changes in clinical practice and may help in formulating new guidelines and rules to prevent diseases.[13,14]

Embryogenesis and Development of Respiratory System

Respiratory system consists of upper and lower respiratory tract. Upper respiratory tract consists of nose, nasopharynx and oropharynx. Lower respiratory tract consists of trachea, bronchi and respiratory units.

Embryogenesis and Development Upper Respiratory Tract

Nose and mouth development begins at the 5th week of gestation. It is derived from five main facial processes, frontonasal prominence, paired maxillary processes and paired mandibular processes. Upper lip is formed by the fusion of frontonasal prominence and maxillary processes. Lower lip is formed by the fusion of mandibular processes. The intervening space between fused maxillary processes and mandibular processes becomes primitive mouth. Thickening of the epithelium on either side of the midline of the frontonasal process forms circular nasal discs or placodes which ultimately burrows and forms the anterior nares.[8,15]

At the 7th week of gestational period, the nasobuccal membrane breaks posteriorly to form the communicating posterior choanal space. Anteriorly, the nasobuccal membrane remains as the primary palate, the remainder being replaced by the secondary or definitive palate derived from horizontal palatine processes. Fusion commences at the primary palate and extends posteriorly.[8,15]

The epithelium of the larynx develops from the endodermal lining of the laryngotracheal tube and cartilages. Muscles of the larynx are derived from the mesenchyme of 4th to 6th branchial arches.[8,16]

Embryogenesis and Development of Lower Respiratory Tract

Primitive trachea forms by the 4th week of gestation from the posterior surface of foregut by the tracheoesophageal septum.[8,15]

Lung Development

Lung development is subdivided into three main periods:[17, 18]

1. Embryonic period: This period ranges from 4 to 7 weeks of gestation. Features of this stage are anlage of two lungs, organogenesis, formation of major airways and formation of pleura. On 26th day of post-conception the right and left lung anlage appears from ventral wall of primitive foregut as two independent outpouchings. The two lung buds are located right and left to anlage of trachea. Lung buds begin to elongate, branch and grow into surrounding mesenchyme called branching morphogenesis. The visceral pleura originates from the splanchnic mesoderm and fold into the lung separating the tissues surrounding the lobar bronchi which form lobar

fissure separating the lung lobes. Parietal pleura originates from somatic mesoderm. Closure of pleural cavities takes place by fusion of pleuropericardial folds and two pleuroperitoneal membranes.[17,19] Structural malformations during this period include pulmonary agenesis or aplasia, pulmonary valve stenosis, diaphragmatic hernia, pulmonary hypoplasia, tracheoesophageal fistula and esophageal atresia.[17]

2. Fetal period: Consists of pseudo glandular, canalicular and saccular stages.

   The pseudo glandular stage ranges from 5 to 17 weeks of gestation. Features of this stage are formation of bronchial tree up to terminal bronchioles and the birth of acinus. The name pseudo glandular means resemblance of growing bronchial tree to a tubular gland. The lining epithelium of bronchial tree is tall columnar. Outgrowth of the terminal bud into the surrounding mesenchyme is followed by branching and formation of the next generation airways, that are loosely embedded in the mesenchyma. During this stage, approximately first 20 generations of the future airways are formed along with it first few generations of alveolar ducts are also laid down.[17,19] Pseudo glandular stage depends on mechanical stimuli. Around 10 weeks of post conception, Fetal breathing movements start in humans and cause additional stretching of the lung tissue and move fluid in and out of the lungs. A congenital diaphragmatic hernia leads to pulmonary hypoplasia and pulmonary hypertension.[17]

   Canalicular stage ranges from 16 to 26 weeks of gestation. Features of this stage are formation of most distal airways up to respiratory bronchioles, formation of the first air-blood barrier and acini. Differentiation of the

epithelia into type 1 and type 2 epithelium takes place and this helps in the morphological distinction between conducting and respiratory airways. The alveolar epithelium comes into close contact with the mesenchymal capillary network leading to formation of the first air-blood barrier.[17,19] Alveolar capillary dysplasia (ACD) is a malformation, where the alveolar epithelium is surrounded by mesenchyma containing small blood vessels instead of capillaries. This malformation results in a reduced capillary density, reduced air–blood interface, thickened alveolar septa and pulmonary hypertension.[17]

Saccular stage ranges from 24 to 38 weeks of gestation. Feature of this stage is expansion of gas exchanging area. Expansion of air spaces takes place which are called saccules. At this stage branching of morphogenesis ceases.[17,19] Prematurely born infants use their lungs for gas exchange at the end of the canalicular stage and during the saccular stage alveolarization does not progress well and this leads to a disease called bronchopulmonary dysplasia (BPD). Infants showing BPD presents with fever and are having larger alveoli with insufficient vascularization and airflow limitations.[17]

3. Postnatal period: Consists of stages of first phase of classical alveolarization, second phase of continued alveolarization and microvascular maturation.

   First phase of classical alveolarization ranges from 38weeks of gestation to 3 years. In this stage, the existing airspaces are divided by new septa that are lifted off from immature pre-existing septa. The new septum rises to its full height and the first alveoli is formed. Alveolar septa contain double

capillary network.[17]

Second phase of continued alveolarization ranges from 2 years to 21 years. Alveolar septa now contain an effective single layer capillary network.[17] Microvascular maturation continues till 21 years and is characterized by remodeling, maturation of interalveolar septa and capillary bed.[20] Alveolarization and microvascular maturation continue till young adulthood.[17,20]

According to Reid, normal lung growth can be summarized by three laws.[21] Law I states that the bronchial tree is developed by the 16th week of gestation. Law II states that by birth, majority of alveoli develop and increases in number and size of alveoli continue until the age of 8 years and until the growth of chest wall is finished. Law III states that pre-acinar arteries and veins parallel the development of the airways and intra-acinar vessels follow the development of the alveoli.[21]

Physical forces are vital for regulating Fetal lung growth and maturation. Lung fluid which is produced by epithelial cells helps in expanding the airways. This expansion causes growth factor release.[18] In utero, the fetus exhibits low amplitude rapid breathing movements which are diaphragmatic in origin and this may allow influx of amniotic fluid in lungs. Distended pressure by fluid within the airways is the primary physical force stimulating the lungs.[8] The fetus exhibiting episodic Fetal breathing movements (FBMs) are part of normal human lung development.[22]

Histology Of Lower Respiratory tract

Respiratory tract consists mainly of conducting and respiratory portions. Conducting

system conducts air into respiratory portion where gas exchange takes place. Respiratory portion consists of respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli. Conducting portion is lined by pseudostratified columnar ciliated epithelium with goblet cells and is surrounded by glands, cartilage and blood vessels. Respiratory bronchioles are lined by low columnar or cuboidal and may be ciliated in the proximal portion of tubules. Alveolar sacs are clusters of alveoli and alveoli are lined by type 1 and type 2 pneumocytes. The junction between conducting and respiratory portion is marked by terminal bronchiole which exhibits mucosal folds and is lined by columnar to cuboidal ciliated epithelium that lacks goblet cells.[23]

Fetal Deaths

Congenital malformations are major cause of perinatal deaths.[24] Congenital anomalies account for 8–15% of perinatal deaths and 13–16% of neonatal deaths in India.[25] Prevalence of congenital anomaly in prenatal diagnosis is 10.98 per 1000 births and the rate of pregnancy termination for congenital anomaly is 4.39 per 1000 births.[26]

Congenital anomalies are defects which are present at birth that are detected at that time or later. These defects can be structural, functional and/ or biochemical.[25,27] Lethal congenital anomalies are the important causes for spontaneous abortion and stillbirths. Less severe congenital anomalies are responsible for prolonged intrauterine survival and handicap births.[28] Causes of congenital anomalies are grouped into four categories- genetic causes, environmental causes, multifactorial causes and unknown causes.[28] Genetic causes include chromosomal aberrations (10–15%) and Mendelian inheritance (2–10%). Environmental causes include maternal or placental infections (2-3%), maternal diseases (6-8%), irradiation injury (1%) and drugs or chemicals

(1%). Multifactorial causes (20-25%) are due to interactions of genetic and environmental factors. In 40-60% of anomalies, exact etiology is not known. Mattingly P[29] also reported in his study on evaluation of fetal death that unknown causes of fetal death can occur in 25-60% of cases.

Fetus is a product of conception, regardless of the duration of pregnancy.[30] Fetal death is defined as death occurring prior to the complete expulsion of the products of conception, and is indicated by absent breathing, heartbeat, pulsating umbilical cord, or muscular movement.[31,32] It is divided further as early fetal deaths when gestational age is less than 22 weeks. Intermediate fetal deaths when gestational age is between 22 to 27weeks and late fetal deaths when gestational age equal to or more than 28weeks.[30] Early fetal deaths are considered abortions whereas intermediate fetal deaths and late fetal deaths are known as stillbirths.[30] Perinatal deaths include stillbirths and early neonatal deaths.[30-33]

The cause of fetal death can be divided into fetal, maternal and/or placental pathology.[29,34]

- Fetal causes include intrauterine growth restriction, congenital anomalies, genetic abnormality, infection (parvovirus B19, Cytomegalovirus, Listeria), hydrops, etc.

- Maternal causes include prolonged pregnancy (>42 weeks), poorly controlled diabetes mellitus, systemic lupus erythematosus, antiphospholipid syndrome, infections, hypertension, preeclampsia, eclampsia, hemoglobinopathy, advanced maternal age, Rh disease, uterine rupture, inherited thrombophilias,

maternal trauma or death, etc.

- Placental causes include cord accident, abruptio placenta, premature rupture of membranes, vasa Previa, fetomaternal hemorrhage, placental insufficiency, etc.

It is essential to know the different periods of infancy which are divided into the following:[32,33]

- Neonatal period ranges from 1st day of birth to 28 days of life.
- Early neonatal period is first 7 days of life.
- Late neonatal period ranges from 8th day to 28 days of life.
- Post neonatal period ranges from 28 days to 1 year of life.

More than a quarter of global deaths among neonates occur in India accounting for 0.76 million deaths each year.[31] Odisha has the highest stillbirth rate in India that is 10 per 1000 live births. Odisha also has the highest perinatal mortality rate in India that is 34 per 1000 live births.[33]

A methodical analysis of global, regional and national causes of child mortality in 2013 reported preterm birth complications (43.7%) and infections (20.8%) to be the two major causes of neonatal deaths in India followed by perinatal asphyxia (19.2%) and malformations (8.2%) to be the next other two important causes.[31,35]

Horn L C[36] reported rate of congenital anomalies as the cause of death with range between 23 and 34.5%. Bonetti L R[37] reported 21.73% cases of congenital anomalies

as fetal cause of deaths in their study. Zanconato G *et al*[38] reported 13.5% cases of congenital anomalies in their study. In study done by L Donna *et al*[34] to find out cause of death over a period of 2 years, 10.8% congenital anomalies were noted.

Pulmonary Hypoplasia (PH)

Respiratory disorders are major cause of neonatal morbidity and mortality.[31] Pulmonary hypoplasia is defined as unilateral or bilateral, defective or incomplete development of lung parenchyma, airway and vessels or incomplete development of lung which is not appropriate for gestational age.[1,2,3]

PH incidence in perinatal autopsies is found to be 14%- 22%.[1] PH incidence in the general population is of 9 to 11 cases per 10,000 live births and prevalence of PH in perinatal autopsy case series ranges between 7.8%- 26%.[3] Husain and Hessel[39] found 26% cases of pulmonary hypoplasia at autopsy. Wigglesworth and Desai[40] reported an incidence of 14.5% of pulmonary hypoplasia in a series of perinatal autopsies.

In most cases, pulmonary hypoplasia is secondary to an underlying abnormality. Fetal breathing movements, positive pressure of fetal lung fluid and normal amniotic fluid volume are required for normal lung growth in utero. Primary pulmonary hypoplasia is very rare, but occasionally structurally normal but hypoplastic lungs are found at autopsy and no underlying cause can be identified.[39]

Anomalies associated with pulmonary hypoplasia are:[1,2,3,39]

- Congenital diaphragmatic hernia (CDH)
- Congenital cystic adenomatoid malformation (CCAM)
- Bronchogenic cyst

    - Bilateral renal agenesis
    - Renal dysplasia
    - Bladder outlet obstruction
- Skeletal malformations:
    - Osteogenesis imperfecta
    - Thanatophoric dwarfism
    - Skeletal dysplasia
- Rhesus and non-rhesus hydrops
- Central nervous system and neuromuscular anomalies:
    - Anencephaly
    - Fetal akinesia
- Cardiac lesions:
    - Hypoplastic chambers
    - Pulmonary stenosis
- Abdominal wall defects:
    - Omphalocele
    - Gastroschisis
- Syndromes associated with pulmonary hypoplasia:
    - Trisomy 13
    - Trisomy 18
    - Trisomy 21
    - Robert syndrome
    - Pena-Shokier and Jarcho-Levin syndrome

Most cases of pulmonary hypoplasia are lethal. Less lethal cases of PH may present with

clinical manifestations which range from severe respiratory failure, pulmonary hypertension, persistent fetal circulation, pulmonary hemorrhage, bronchopulmonary dysplasia to death. Associated facial abnormalities in PH includes Potter facies with large, low set, floppy ears, small chin, flattened nose, hypertelorism, epicanthal folds. Limb defects include spade like hands, flexion contractures at elbow, knee, feet and talipes equinovarus.[22] Radiological criteria for PH include small lung fields with diaphragmatic domes elevated up to the seventh rib, downward sloping of ribs, barrel shape thorax and pneumomediastinum.[22]

A study done by Wigglesworth J S and Desai R[40] on use of DNA as estimation for growth assessment in normal fetal lungs and hypoplastic fetal lungs observed that total DNA of lung increased with gestational age and used this DNA measurement as index of cell population. They mentioned that at 17 weeks, lung DNA is 35mg per kg body weight and 480mg per kg body weight at term. In pulmonary hypoplasia they mentioned cut off of less than 100mg of lung DNA per kg body weight.

Pathologically pulmonary hypoplasia is defined as decrease in the Lung weight: Body weight (LW: BW) ratio and reduced Radial Alveolar Count (RAC) during post mortem examination.[3] Simple and most consistent method of diagnosing pulmonary hypoplasia is direct measurement of lung weight (LW) and its comparison with body weight (BW).[2] Lower limit for Lung weight: Body weight for an infant older than or equal to 28 weeks is 0.012 and for fetus less than 28 weeks of gestation is 0.015.[2]

In 1960, Emery and Mithal introduced a method to access to study structure of the terminal respiratory unit, called Radial Alveolar Count (RAC)[41]. The radial alveolar

count provided a reliable index of lung growth in intrauterine and postnatal development.[41,42,43] RAC is measured by the number of alveoli which are traversed by a perpendicular line drawn from the center of a respiratory bronchiole to the nearest connective tissue septum.[4]

Normal RAC at various gestational age stated by Husian A[44] were 2,3,4 and 5 at 24,30,35 and 40 weeks of gestational age respectively. Betz P *et al*[45] found RAC of less than 2, 2.2, 3.3, at less than18, 18, 20 weeks of gestation age respectively and stated that RAC increased with gestation age and found to be 2.5 to 4 between the gestation age of 25 to 30 weeks and 6.3 between gestation age of 37 to 39 weeks. Projected RAC values for various ages were given by Cooney in 1982 were 1.5, 1.7, 2.3, 3, 4, 5.4, 6.8 at gestation age of 18, 20, 25, 30, 35, 40, 44 respectively.[42]

Askenazi S and Perlman M[4] in their study mentioned that LW: BW ratio of 0.012 and/or RAC of less than 4.1 as a diagnostic criteria of PH.[4] They also compared LW: BW ratio with RAC and observed that RAC was more reliable criterion of PH. They also recommended strategies for the diagnosis of PH when LW: BW ratio was less than 0.009, PH was very likely, RAC was not mandatory. When LW: BW ratio was between 0.010-0.012, PH was probable and RAC was indicated for confirmation. When LW: BW ratio was between 0.013- 0.017, PH was possible and RAC was needed. When LW: BW ratio was more than 0.018, PH was unlikely and RAC not indicated.

Lung pathologies in modern days are diagnosed by radiological techniques such as X rays, ultrasonography (USG), Computed Tomography (CT) scan, Magnetic Resonance Imaging (MRI) scan, Bronchoscopy, Bronchography and Pulmonary angiography.[5]

The dynamic process of lung growth requires meticulous work with correlation of clinical details, investigations and pathological examination. Routine assessment of lung growth should be a part of Fetal autopsy as there are significant incidental reported cases of PH in clinically unsuspected cases.[4]

A study done by Ernst M[46] showed that even with emerging technologies, the conventional perinatal autopsy remains the gold standard for defining the cause of death and the final summary of all pathologic results. Perinatal autopsy will also provide information about pathological processes and checking the accurateness of clinical diagnosis.

MATERIALS AND METHODS

Source of data: A prospective observational study was done on fetal autopsy specimens sent to the histopathology section of the Department of Pathology, B.L.D.E. (Deemed to be University) Shri B M Patil Medical College Hospital & Research Centre, Vijayapura.

Study period: 01 December 2019 to 31 July 2021.

Type of study: Prospective study- Observational study.

Method of collection of data:

1. Fetal autopsy specimens of IUD, still births and neonatal deaths sent to histopathology section of the Department of Pathology, B.L.D.E. (Deemed to be University) Shri B M Patil Medical College Hospital & Research Centre, Vijayapura was processed according to the standard protocol with modifications. Detailed obstetric history and USG finding details were collected.

2. Anthropometric data of fetus and external examination was recorded as per the details mentioned in Table 1.

3. Internal examination was done by taking I shaped incision and en-mass dissection was done. Internal examination for gross organ anomaly was done and details of measurements, weight, gross and microscopic examination findings of various organs were recorded as mentioned in the Table 2.

4. Gross examination of the lung was done as per the study done by Husain A & Hessel R.[44]

   Lungs were separated from the heart, thymus, and mediastinal tissues. Trachea was cut above its bifurcation. Weight of both the lungs was recorded in fresh state. Lungs were fixed in 10% buffered formalin. After fixation lungs were serially sectioned in the sagittal plane and the middle slice was submitted for routine processing of the tissue.

5. Gross examination of other organs was done as per the standard protocol and tissue bits were given from each organ and processing was done as per the standard method.[47]

6. Sections of 3-6 μ thickness were taken for all organs and hematoxylin and eosin-staining was done and microscopic examination findings were recorded as mentioned in Table 2.

7. RAC of both right and left lung was recorded by evaluating the number of alveoli cut by a line which was dropped at right angles to the bronchial epithelium from the center of terminal respiratory bronchioles to the nearest connective tissue septum".[4,41] RAC was evaluated in 10 high power fields in two sections taken from lung. Average of it is taken as RAC.[4,41] Bronchioles partly lined by epithelium was also selected.[22]

8. Diagnostic criteria for PH were taken as LW:BW ratio less than 0.012 and/ or RAC less than 4.1.

Inclusion criteria: All the fetal autopsy specimens of IUD, stillborn and neonatal deaths which were sent to Department of Pathology were included.

Exclusion criteria: Macerated foetus with extensive autolytic changes in the lungs were excluded from the study.

Sample Size: With anticipated Prevalence of Pulmonary Hypoplasia 7.8-26% in study done by Pena Y *et al*[3] the minimum sample size calculated was 62. Hence 62 fetal autopsies were included in the study with 5% level of significance and 10% absolute error.

Sample size calculation: Formula used

$$n = \frac{z\ 2\ p*q}{d\ 2}$$

Where Z= Z statistic at α level of significance

d 2 = Absolute error

P= Proportion rate

q= 100-p

Statistical analysis:

1. Mean values of LW:BW ratio and RACs and Standard Deviation (SD) of the mean were calculated.

2. Numerical variables were presented as mean ±SD, and categorical variables were presented as frequency (%) and diagrams.

3. Association of categorical variables was found by using chi square or fisher's exact test.

Statistical methods used: The data collected was put into Microsoft Excel format and then analyzed using the 20th version of Statistical Package for Social Sciences (SPSS) for Windows. Categorical variables were presented as numbers and continuous data was presented as Mean±standard deviation and charts. The association between qualitative data was determined by applying Chi-square test. The continuous data was compared by Student T-test and Krsuskal walli's test wherever required. P value < 0.05 was taken as significant.

Table No.1: Anthropometric Data and External Examination of Fetus [47]			
Weight of Fetus			
Crown Heel Length (CHL)			
Crown Rump Length (CRL)			
Head Circumference (HC)			
Biparietal Diameter (BPD)			
Abdomen Circumference (AC)			
Chest Circumference (CC)			
Signs of maceration	Yes/no		
Umbilical cord (UC)	Length-	No. of vessels	Tied/ torn/ any other
Placenta-	Weight- Dimensions-	Umbilical cord- Attached/not attached	Any signs of disease
Foreign bodies in external orifices-	Yes/no		
Skin-	Normal/abnormal	If abnormal	
Head-	Intact/not intact	Shape-globular/flat/depressed	
Skull bones-	Normal/abnormal	Fontanelle-	Flat/raised/depressed
Eyes/ears/nose/lips/chin/ oral cavity-	Normal/abnormal	If abnormal- details	
Neck-defects-	Yes/no	Rest of head and neck-	Normal/abnormal
Limbs-symmetry-	Normal/abnormal	If abnormal- details	
Digits-upper limbs- **Lower limbs-**		Position of palms/soles-	
Upper limbs- **Hand/forearm/finger/nails/ crease**	Normal/abnormal	**Lower limbs-** **thigh/leg/foot/finger/nail/crease**	Normal/abnormal
Scars/signs of injury-	Yes/no	If yes-	
Thorax and abdomen symmetry-	Normal/abnormal	Nipples/sternum/abdomen-	Normal/abnormal
External genitalia-	Normal/abnormal	If abnormal-	
Gender- **male/female/ambiguous**			
Anal opening-	Normal/abnormal		
Signs of violence	Head-yes/no	Face-yes/no, mouth- yes/no	Neck-yes/no

Table No. 2: Gross and Microscopic Details of Various Organs [47]				
ORGAN	**WEIGHT**	**MEASUREMENTS**	**GROSS EXAMINATION FINDINGS**	**MICROSCOPIC EXAMINATION**
THYMUS				
RIGHT LUNG				RAC-
LEFT LUNG				RAC-
HEART				
LIVER				
SPLEEN				
RIGHT KIDNEY WITH ADRENAL				
LEFT KIDNEY WITH ADRENAL				
OTHER				

RESULTS AND ANALYSIS

Total numbers of fetal autopsies included in the study were 62. Detail history and ultrasonography (USG) findings were collected for all cases from the records available in case files. Evaluation of PH was done in all cases by LW: BW ratio and RAC. Also, evaluation of associated conditions and anomalies associated with PH was done.

Table No.3: Distribution of Fetal autopsy cases according to Gestational Age

Gestational Age(weeks)	Number of cases (N)	Percentage (%)
10-15	06	9.68
16-20	17	27.42
21-25	15	24.20
26-30	08	12.90
31-35	04	6.45
36-40	12	19.35
Total	62	100.0

Majority of the fetal autopsy cases received in the present study were having gestational age of 16 to 20 weeks followed by 21 to 25 weeks of gestational age.

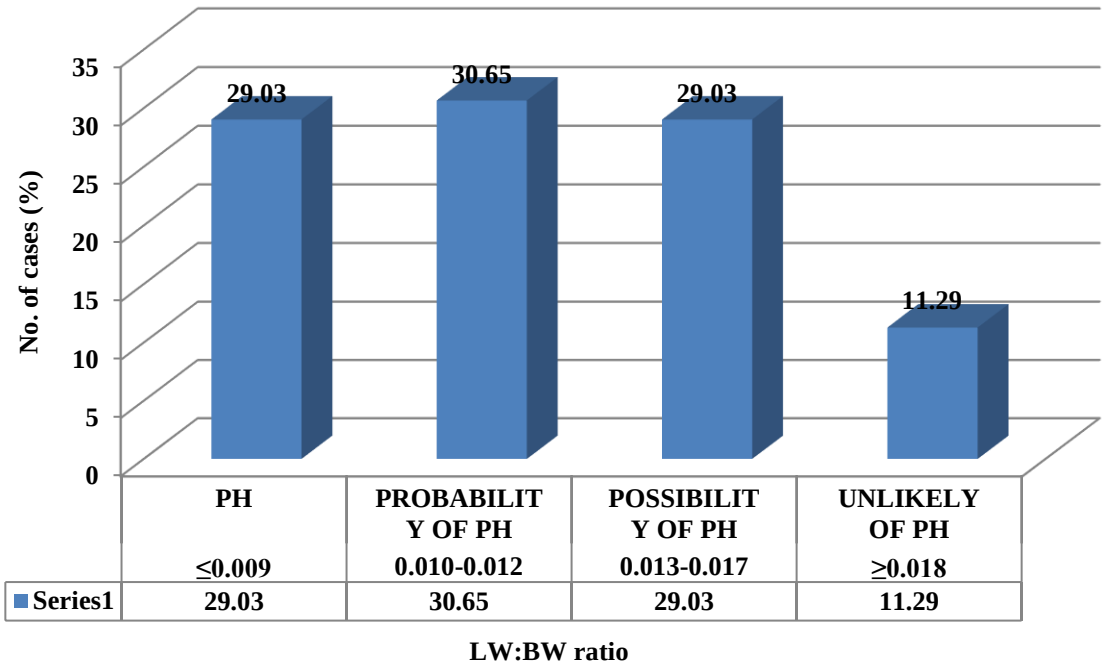
Table No.4: Demographic profile of Fetal autopsy: Gestational age (GA) & Gender

GA (weeks)	Male		Female		Ambiguous	
	N	%	N	%	N	%
10-15 (n=6)	2	33.33	2	33.33	2	33.33
16-20 (n=17)	9	52.94	8	47.06	0	0.0
21-25 (n=15)	9	60.00	6	40.00	0	0.0
26-30 (n=8)	3	37.50	5	62.50	0	0.0
31-35 (n=4)	1	25.00	3	75.00	0	0.0
36-40 (n=12)	6	50.00	6	50.00	0	0.0

Male to female ratio was 1:1 having 30 cases each of male and female. In two cases gender ambiguity was noted.

Table No.5: Categorization of Fetal autopsy cases based on LW:BW ratio			
LW:BW	**Category**	**Number of cases (N)**	**Percentage (%)**
≤0.009	Pulmonary hypoplasia	18	29.03
0.010-0.012	Probability of pulmonary hypoplasia	19	30.65
0.013-0.017	Possibility of pulmonary hypoplasia	18	29.03
≥0.018	Unlikely of pulmonary hypoplasia	07	11.29

Figure 1: Categorization of Fetal autopsy cases based on LW:BW ratio



Categorization of PH in fetal autopsy was done based on LW: BW ratio into four groups. 18 cases (29.03%) had LW: BW ratio less than and/or equal to 0.009. These cases were diagnosed as PH. 19 cases (30.65%) had LW: BW ratio between the ranges of 0.010 to 0.012. These cases were categorized as cases having probability of PH. 18 cases (29.03%) had LW: BW ratio between the ranges of 0.013 to 0.017, where possibility of PH was considered. 7 cases (11.29%) had LW: BW ratio more than and/or equal to 0.018, in these cases PH was unlikely as per LW: BW ratio.

Table No.6: Distribution of Fetal autopsy cases based on LW:BW ratio with associated conditions/anomalies		
	Number of cases (N)	**Percentage (%)**
≤0.009 (n=18)		
IUD	7	38.88
Oligohydramnios	6	33.33
Hydrops fetalis	2	11.11
CDH	1	5.56
PROM	1	5.56
Ascites	1	5.56
0.010-0.012 (n=19)		
IUD	9	47.37
Renal pathology	4	21.06
Neural tube defect	2	10.53
Ascites	1	5.26
AMC	1	5.26
PROM	1	5.26
Uteroplacental insufficiency	1	5.26
0.013-0.017 (n=18)		
IUD	11	61.11
Neural tube defect	3	16.66
Gastroschisis	1	5.56
Oligohydramnios	2	11.11
Ascites	1	5.56
≥0.018 (n=7)		
IUD	2	28.57
Neural tube defect	3	42.86
CCAM	2	28.57

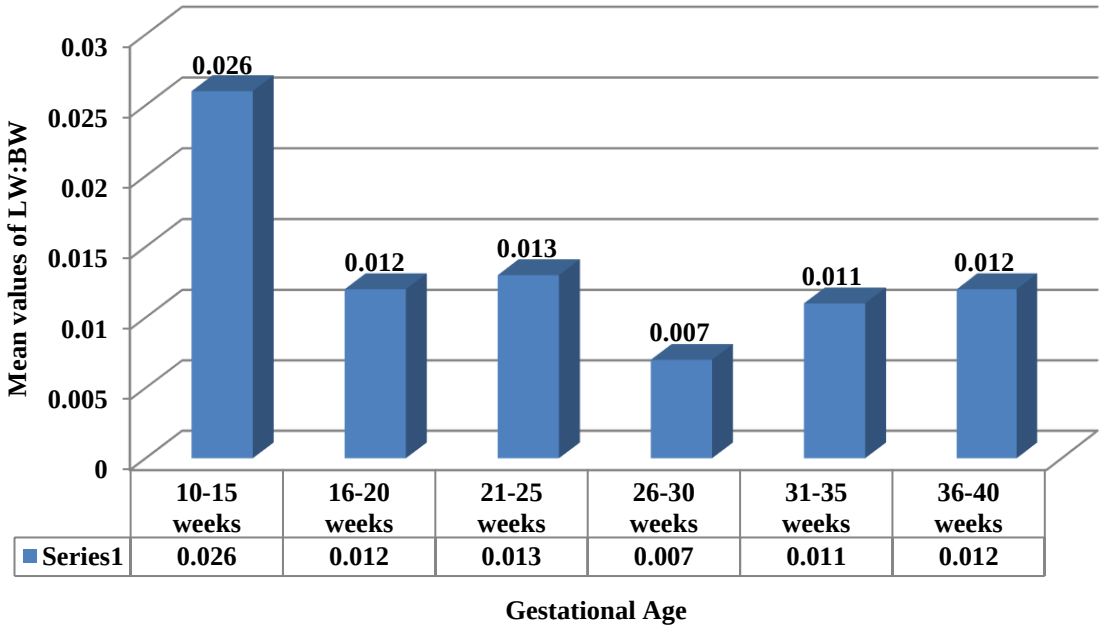
IUD was the commonest associated condition followed by oligohydramnios, neural tube defect and renal pathology.

Table No.7: Distribution of Fetal autopsy cases according to LW:BW ratio range and GA									
GA (weeks)	**Normal range of LW:BW ratio**	**Below normal**		**Within normal**		**Above normal**		**Chi square test**	**p value**
		N	**%**	**N**	**%**	**N**	**%**		
10-15 (n=6)	-	-	-	-	-	-	-	13.708	0.0083*
16-20 (n=17)	0.014-0.042	14	31.82	3	25	0	0.0		
21-25 (n=15)	0.022-0.037	13	29.54	2	16.66	0	0.0		
26-30 (n=8)	0.026-0.036	8	18.18	0	0.0	0	0.0		
31-35 (n=4)	0.022-0.028	4	9.10	0	0.0	0	0.0		
36-40 (n=12)	0.013-0.022	5	11.36	7	58.34	0	0.0		
Total (56)		44	78.57	12	21.43	0	0.0		
*: Statistically significant (p value <0.05)									

Association of normal reference range of LW: BW ratio and GA was done in 56 cases as normal reference range for LW: BW ratio for GA range of 10 to 15 weeks could not find after extensive literature search. In these 56 cases it was observed that in 44 cases amounting to 78.57% LW: BW ratio was below normal range. Maximum numbers of below normal range cases were noted in GA range of 16 to 20 weeks followed by 21 to 25 weeks of gestational age.

Table No.8: Distribution of Fetal autopsy cases according to mean LW:BW ratio and GA				
GA (weeks)	No. of cases	LW:BW		Kruskal Walli's test p value
		Mean	SD	
10-15	6	0.026	0.011	KW=17.615 P=0.0035*
16-20	17	0.012	0.006	
21-25	15	0.013	0.008	
26-30	8	0.007	0.004	
31-35	4	0.011	0.002	
36-40	12	0.012	0.002	
Total	62			
*: Statistically significant (p value <0.05)				

Figure 2: Distribution of Fetal autopsy cases according to mean LW:BW ratio and GA



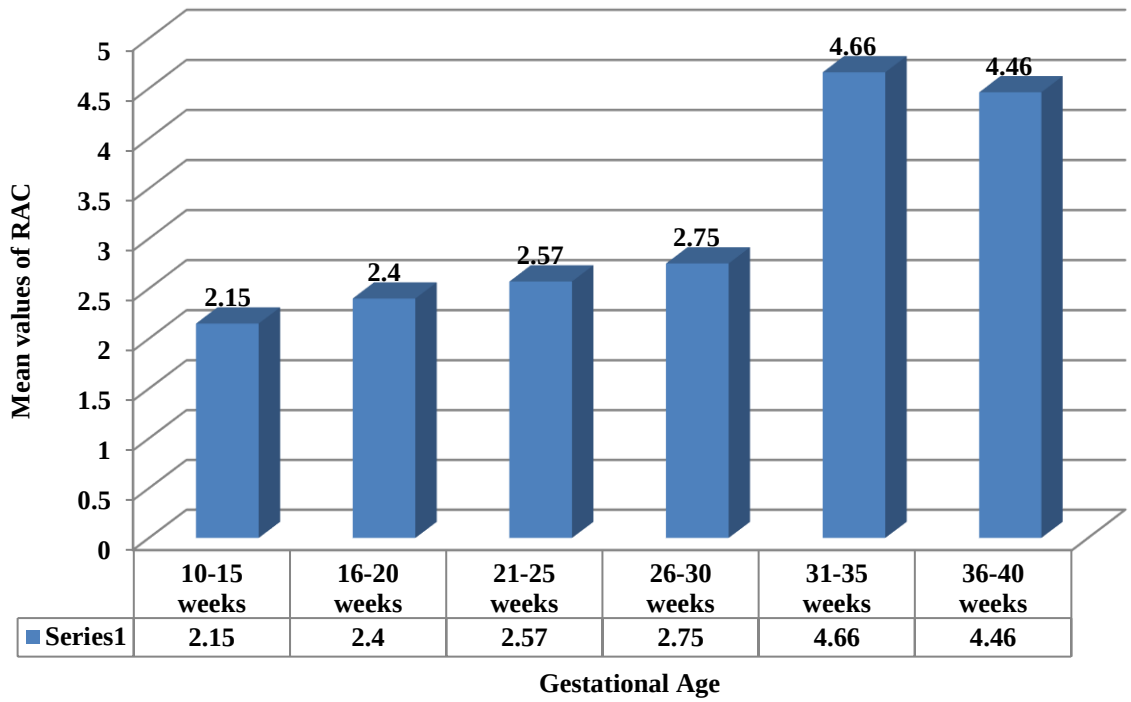
Mean LW: BW ratio was highest at the GA range of 10 to 15 weeks and was lowest at GA range of 26 to 30 weeks and the difference is statistically significant having p value less than 0.05.

Table No.9: Distribution of Fetal autopsy cases according to RAC and GA									
GA (weeks)	**Normal range of RAC**	**Below normal**		**Within normal**		**Above normal**		**Chi square test**	**p value**
		N	**%**	**N**	**%**	**N**	**%**		
10-15 (n=6)	-	-	-	-	-	-	-	31.425	0.0001*
16-20 (n=17)	1.2-1.7	0	0.0	1	5.88	16	94.12		
21-25 (n=15)	1.8-2.3	2	13.33	6	40.0	7	46.67		
26-30 (n=8)	2.4-3	2	25.00	4	50.00	2	25.00		
31-35 (n=4)	3.2-4	0	0.0	1	25.00	3	75.00		
36-40 (n=12)	4.3-5.4	4	33.33	8	66.67	0	0.0		
Total (56)		8	14.29	20	35.71	28	50.00		
*: Statistically significant (p value <0.05)									

Association of normal reference range of RAC and GA was done in 56 cases as normal reference range for RAC for GA range of 10 to 15 weeks could not find after extensive literature search. In these 56 cases it was observed that in 08 cases amounting to 14.29 % RAC was below normal range. Maximum numbers of below normal range cases were noted in GA range of 36 to 40 weeks followed by 21 to 25 weeks and 26 to 30 weeks of gestational age. In 50% cases RAC was above normal range with maximum number of cases noted in the 16 to 20 weeks GA.

Table No.10: Distribution of Fetal autopsy cases according to mean RAC and GA				
GA (weeks)	No. of cases	RAC		Kruskal Walli's test p value
		Mean	SD	
10-15	6	2.15	0.39	KW=23.103 P=0.003*
16-20	17	2.40	0.42	
21-25	15	2.57	0.86	
26-30	8	2.75	0.61	
31-35	4	4.66	0.97	
36-40	12	4.46	1.387	
Total	62			
*: Statistically significant (p value <0.05)				

Figure 3: Distribution of Fetal autopsy cases according to mean RAC and GA



Mean RAC was highest at the GA range of 31 to 35 weeks followed by 36 to 40 weeks and was lowest at GA range of 10 to 15 weeks and the difference is statistically significant having p value less than 0.05.

Table No.11: Distribution of Fetal autopsy cases showing concordance/discordance between LW:BW ratio and RAC			
LW:BW	**RAC**	**Number**	**%**
≤0.009 (n=18)	<4.1	18	100
	>4.1	0	0
0.010-0.012 (n=19)	<4.1	15	78.94
	>4.1	4	21.06
0.013-0.017 (n=18)	<4.1	11	61.12
	>4.1	7	38.88
≥0.018 (n=7)	<4.1	7	100
	>4.1	0	0

In 18 cases LW: BW ratio was less than 0.009 and RAC less than 4.1. These cases were diagnosed as PH based on diagnostic criteria. In 19 cases where LW: BW ratio was between the range of 0.010 to 0.012, as per LW: BW ratio probability of PH was considered in these cases. Out of 19 cases in 15 cases RAC was less than 4.1 thus 15 cases were diagnosed as PH. However, in 4 cases RAC was higher than 4.1, hence these cases were not categorized as PH as per RAC. In 18 cases where LW: BW ratio was between the range of 0.013 to 0.017, as per LW: BW ratio possibility of PH was considered in these cases. Out of these 18 cases in 11 cases RAC was below 4.1, however 2 cases of IUD had GA of 14weeks. According to review of literature lungs are in pseudoglandular stage at less than 15 weeks of GA where developing airways resemble glands and there is no definitive lining of bronchioles. Hence RAC in this age group is not conclusive. Hence out of 11, 9 cases were considered as PH as per LW:BW ratio and RAC. In 7 cases where LW: BW ratio was more than 0.018, as per LW: BW ratio where PH was unlikely in these 7 cases but RAC was less than 4.1. Out of 7 cases in 4 cases GA was less than 15 weeks with lungs in pseudoglandular stage. Hence these 4 cases were not considered as PH. Thus, out of 7 cases, 3 cases as per

RAC were considered as PH.

37 cases amounting to 59.67% had LW:BW ratio less than 0.012. 51 cases amounting to 82.25% had RAC less than 4.1. 33 cases amounting to 53.23% showed concordance between LW:BW ratio and RAC. Discordance between LW:BW ratio and RAC was noted in 22 cases amounting to 35.48%. 7 cases amounting to 11.29% were not considered as PH both by LW:BW ratio and RAC.

Table No. 12: Association of mean gestational age, LW:BW ratio and RAC with associated conditions/anomalies									
Cases/ Diagnosis	Cases (N=62)	Gestational age (mean)	LW:BW			RAC			P value
			Mean	SD	Range	Mean	SD	Range	
IUD	29	25.31	0.012	0.007	0.003-0.042	3.179	1.379	0.8-5.4	<0.0001*
Oligohydramnios	8	25.87	0.008	0.004	0.002-0.014	3.455	1.325	1.65-5.4	0.0002*
Neural tube defect	8	24.25	0.020	0.010	0.010-0.037	2.262	0.435	1.7-3.2	0.0001*
Renal pathology	4	21.75	0.010	0.0005	0.010-0.011	2.437	0.675	1.65-3.0	0.0286*
Hydrops fetalis	2	30	0.023	0.020	0.006-0.042	2.56	0.847	1.7-3.9	0.3333
CCAM	2	23	0.032	0.0007	0.032-0.033	2.075	0.53	1.7-2.45	0.3333
Ascites	3	28.33	0.010	0.003	0.007-0.013	3.7	1.609	2.2-5.4	0.1000
PROM	2	29.5	0.009	0.001	0.008-0.010	3.5	2.26	1.9-5.1	0.3333
CDH	1	-	-	-	-	-	-	-	-
Gastroschisis	1	-	-	-	-	-	-	-	-
AMC	1	-	-	-	-	-	-	-	-
Uteroplacental insufficiency	1	-	-	-	-	-	-	-	-
Total	62								
*: Statistically significant (p value <0.05)									

Mean LW: BW ratio was lowest in oligohydramnios as compared to other associated conditions/ anomalies and the difference is statistically significant having p value of 0.0002. Mean LW: BW ratio and RAC in IUD and renal pathology was also lower as compared to other anomalies and the difference was statistically significant having p value of less than 0.0001 and 0.028 respectively. RAC was lowest in CCAM as compared to other associated conditions/ anomalies however only 2 cases of CCAM were noted in the present study. In neural tube defect RAC was slightly higher than CCAM but lower than other associated conditions/ anomalies, and the difference is statistically significant with p value of 0.0001.

Table No.13: Association between gestational age and meconium aspiration cases					
Gestational Age (weeks)	**Meconium aspiration features**			**Chi square test**	**P value**
	NO	**YES**	**Total**		
10 – 15 (n=6)	6	0	6	27.719	0.0001*
%	100%	0.0%	100%		
16 – 20 (n=17)	16	1	17		
%	94.1%	5.9%	100%		
21 – 25 (n=15)	11	4	15		
%	73.3%	26.7%	100%		
26 – 30 (n=8)	3	5	8		
%	37.5%	62.5%	100%		
31 – 35 (n=4)	0	4	4		
%	0.0%	100%	100%		
36 – 40 (n=12)	3	9	12		
%	25%	75%	100%		
Total	39	23	62		
	62.9%	37.1%	100%		
*: Statistically significant (p value <0.05)					

Out of 62 cases meconium aspiration was noted in 23 cases amounting to 37.1%. Maximum number of cases of meconium aspiration was noted in the cases having GA range of 31 to 35 and 36 to 40 weeks amounting to 100% and 75 % respectively. Meconium aspiration was not observed in lung specimen of fetal autopsy having gestational age less than 15 weeks. Frequency of meconium aspiration increased as the GA advanced.

GROSS PHOTOGRAPHS

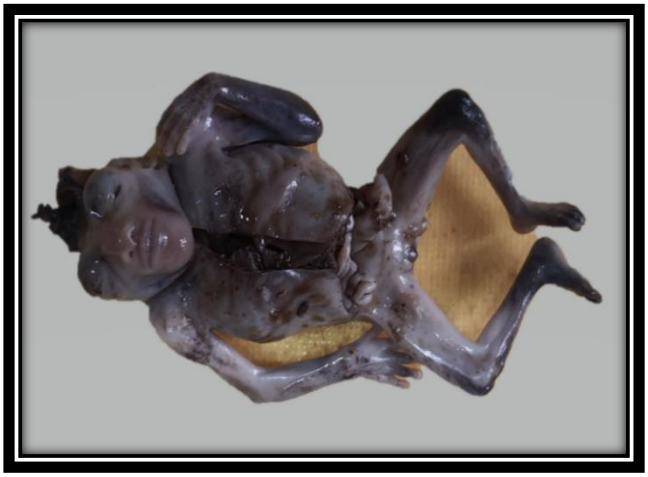


Fig. No.4 - Gross photograph of fetal autopsy specimen showing neural tube defect of anencephaly with protrusion of eyes.

Fig. No.5- Gross photograph of fetal autopsy specimen showing neural tube defect- anencephaly with bilateral cleft lip and protrusion of eyes.

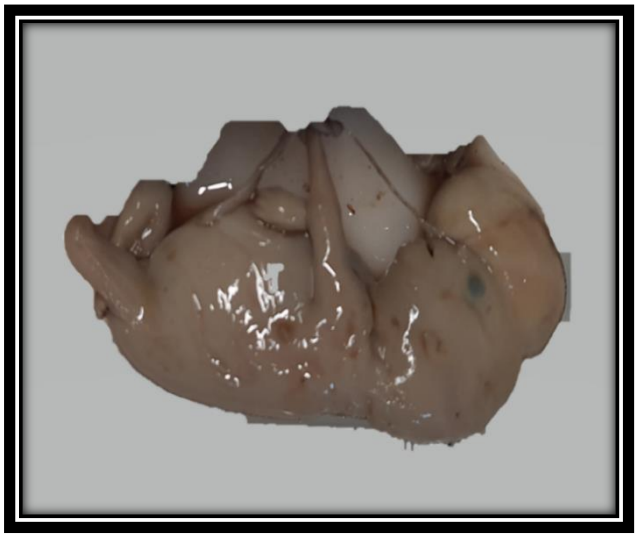


Fig. No.6- Gross photograph of fetal autopsy specimen with neural tube defect- exencephaly with presence of amniotic band and skeletal deformity.

Fig. No.7- Gross photograph of fetal autopsy specimen showing neural tube defect with open spina bifida.

Fig. No.8- Gross photograph of fetal autopsy specimen showing hydrops fetalis in Rh negative mother.

Fig. No.9- Gross photograph of fetal autopsy specimen showing gross ascites.

Fig. No.10– Gross photograph of fetal autopsy specimen with CTEV.

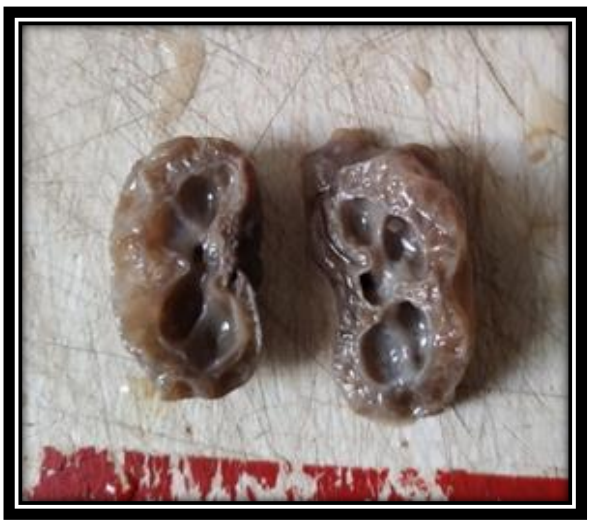


Fig. No.11- Gross photograph of bilateral multicystic renal dysplasia.

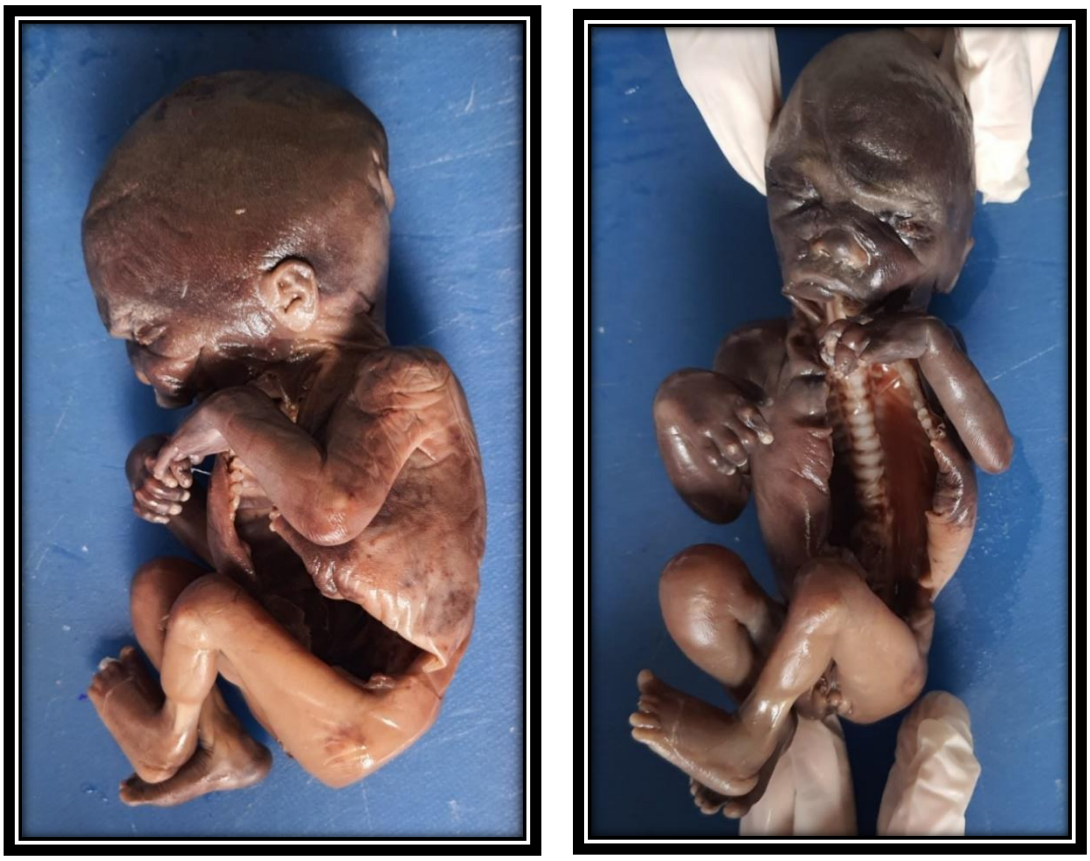


Fig. No.12– Lateral and front view of gross photographs of fetal autopsy specimen with skeletal deformity showing multiple contractures of wrist and interphalangeal joints of bilateral upper limbs. Diagnosed as AMC on USG.

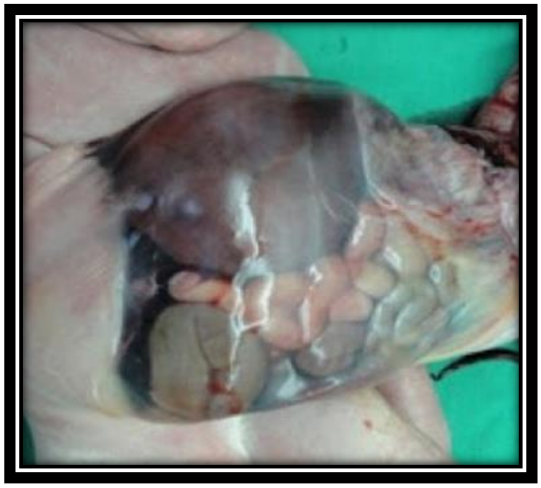


Fig. No.13- Gross photograph of fetal autopsy specimen with gastroschisis.

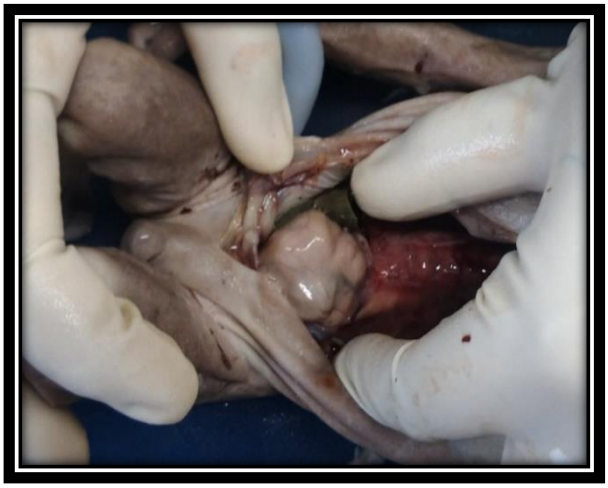


Fig. No.14- Gross photograph of fetal autopsy specimen showing unilateral renal agenesis with single lobulated kidney in pelvic region.

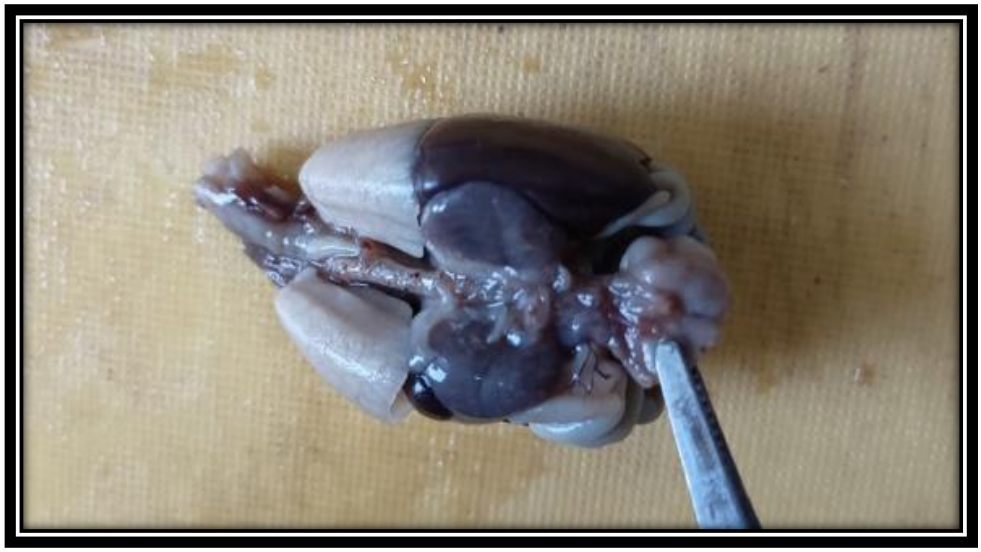


Fig. No.15- Gross photograph of en mass showing fused midline kidneys.

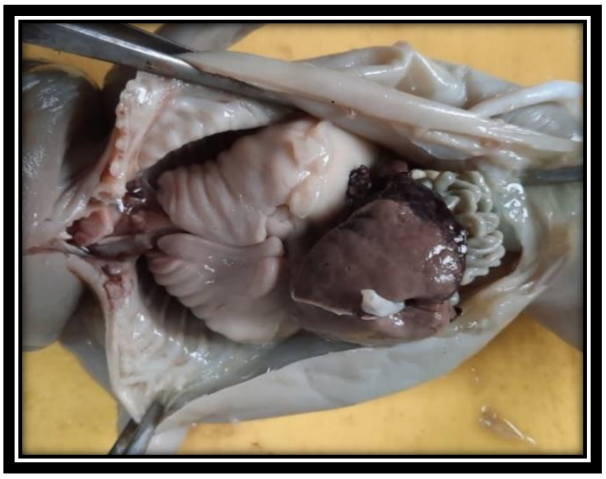


Fig. No.16- Gross photograph of autopsy specimen showing CCAM of lungs. Imprints of rib cage seen on lungs.

Fig. No.17- Gross photograph of cut section of CCAM of lungs.

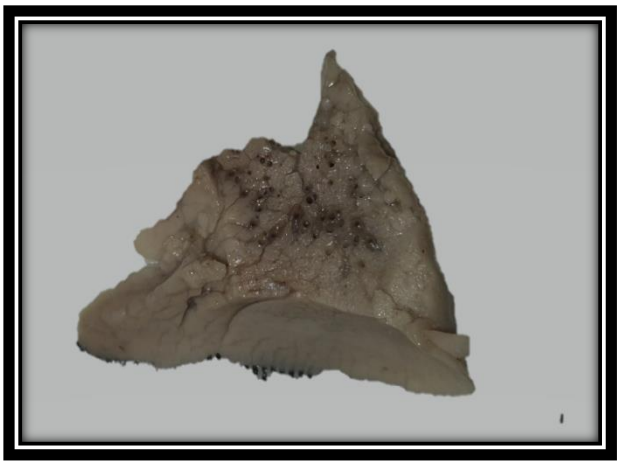


Fig. No.18- Gross photograph of cut section of CCAM of lung with multiple small cystic spaces in lung parenchyma.

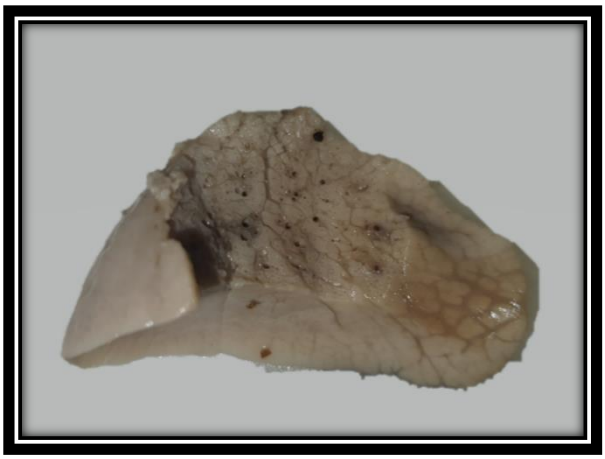


Fig. No.19- Gross photograph of cut section of CCAM of lung showing multiple cystic spaces in lung parenchyma.

Fig. No.20- Gross photograph of congenital diaphragmatic hernia with intestinal loops in chest cavity.

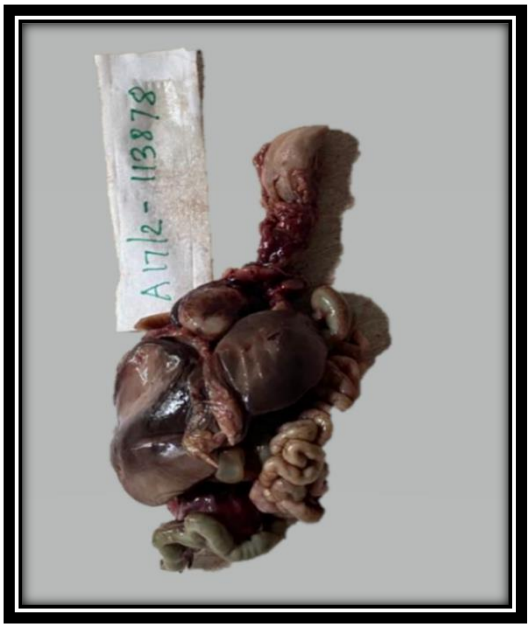


Fig. No.21- Gross photograph of en mass of congenital diaphragmatic hernia.

MICROPHOTOGRAPHS

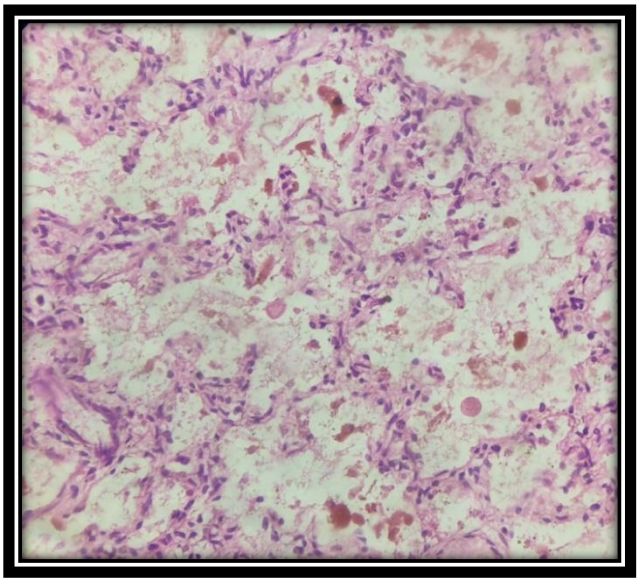


Fig. No.22- Microscopy of lungs showing aspirated squames in alveolar spaces and rugby ball appearance of meconium. (H&E stain, 400X)

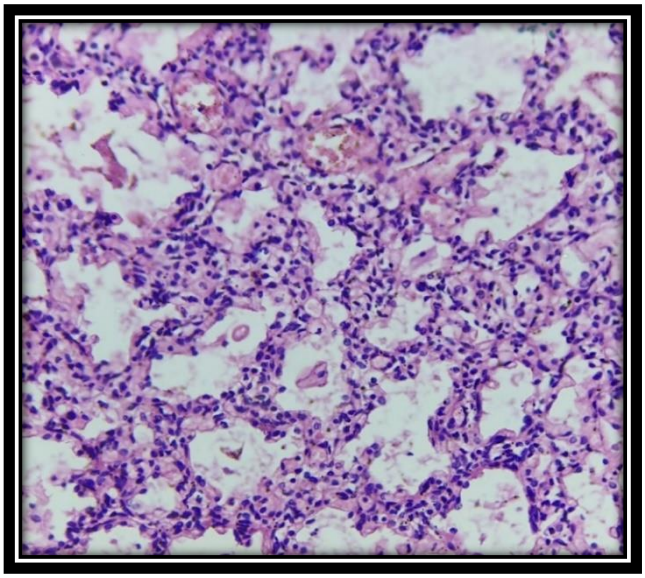


Fig. No.23- Microscopy of lungs showing aspirated squames in alveolar spaces. (H&E stain, 400X)

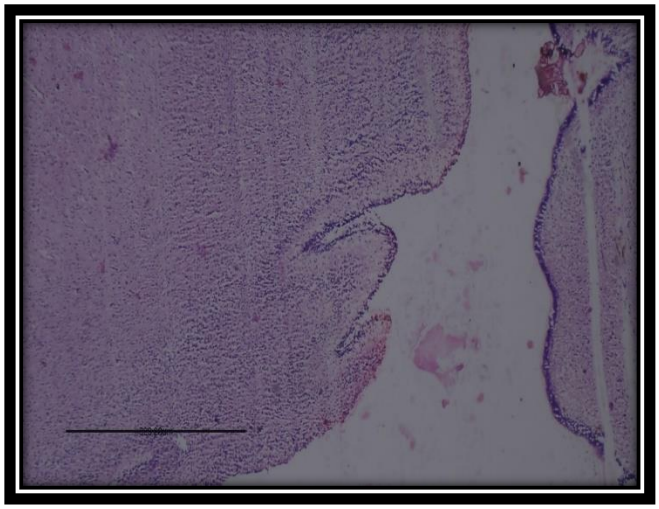


Fig. No.24: Microscopy of brain section of Encephalocele. (H&E stain, 100X)

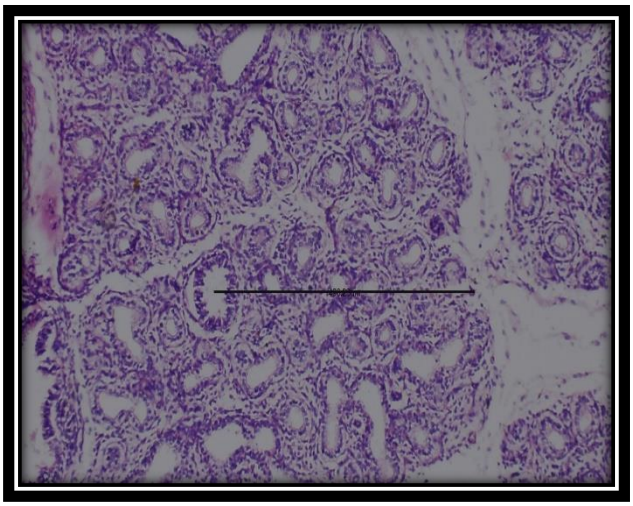


Fig. No.25- Microscopy of lung section with mean RAC of 2.6 in a case of Encephalocele. (H&E stain, 100X)

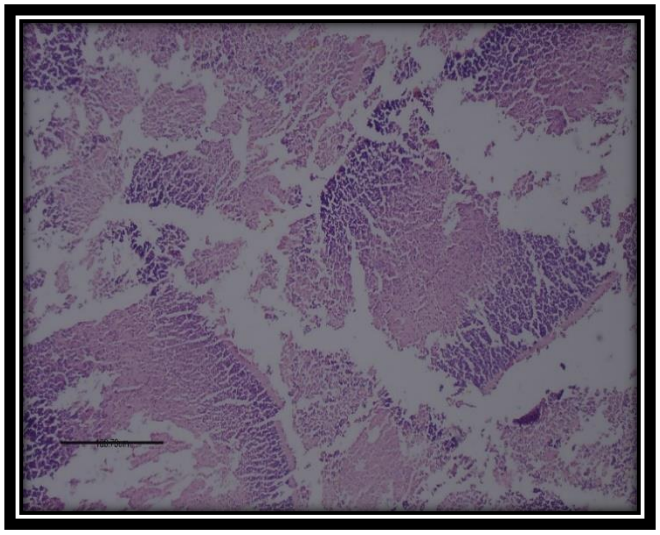


Fig. No.26- Microscopy of brain section showing disorganised brain parenchyma in a case of exencephaly. (H&E stain, 100X)

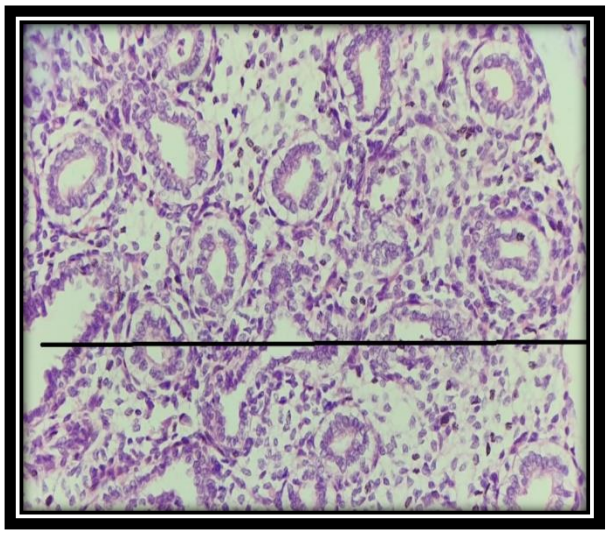


Fig. No.27- Microscopy of lung section with mean RAC of 2 in case of exencephaly. (H&E stain, 400X)

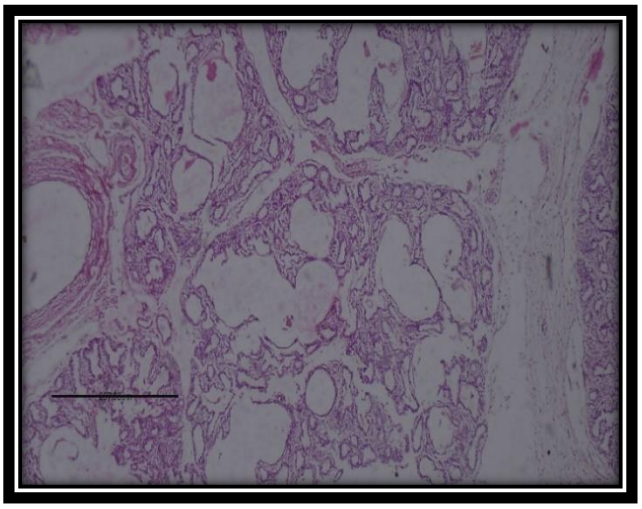


Fig. No.28- Microscopy of lung section showing cystic spaces lined by flattened to cuboidal epithelium in case of CCAM. (H&E stain, 100X)

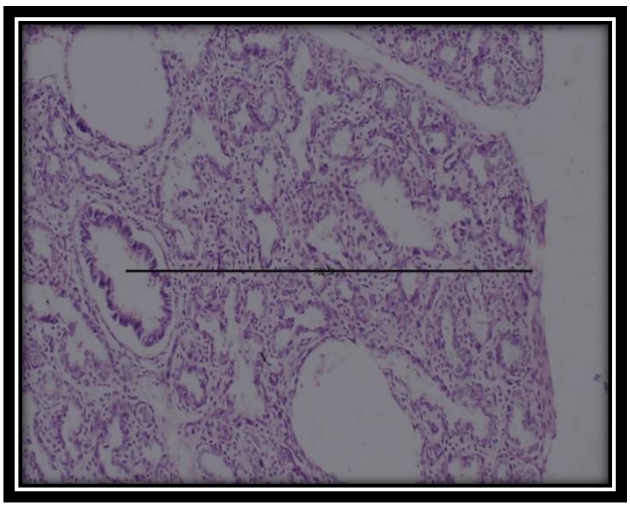


Fig. No.29- Microscopy of lung section with mean RAC of 2.45 in case of CCAM. (H&E stain, 100X)

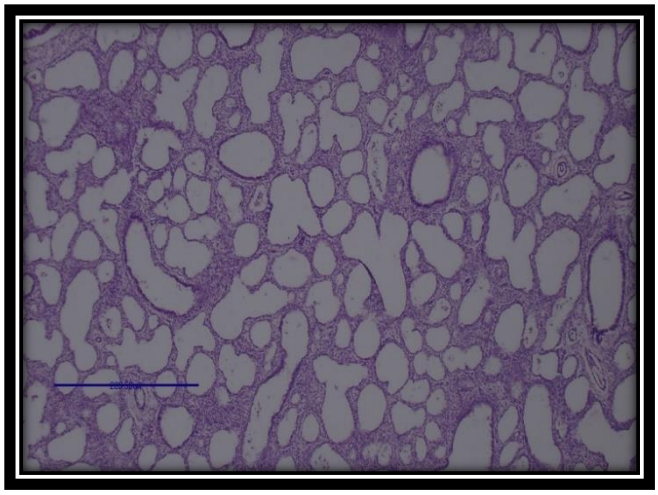


Fig. No.30- Microscopy of lung section showing cystic spaces lined by flattened to cuboidal epithelium in another case of CCAM. (H&E stain, 100X)

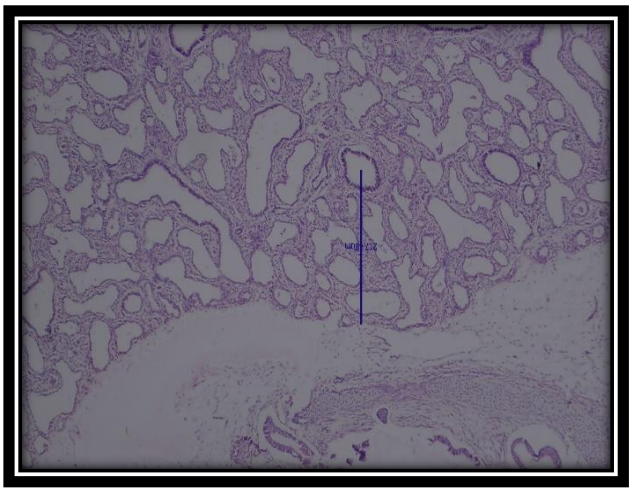


Fig. No.31- Microscopy of lung section with mean RAC of 1.7 in another case of CCAM. (H&E stain, 100X)

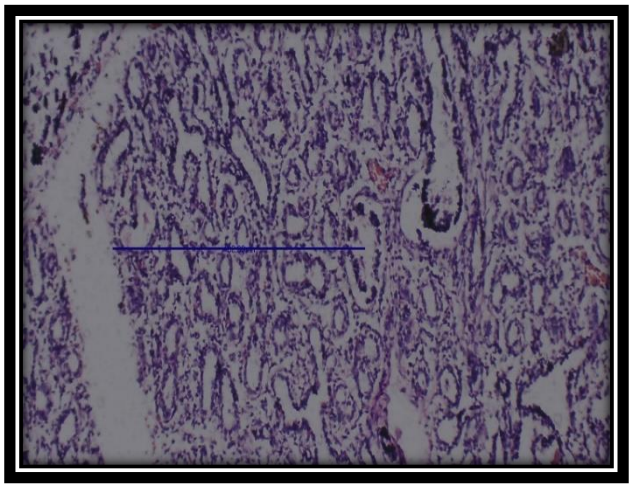


Fig. No.32- Microscopy of lung section with mean RAC of 2.55 in a case of oligohydramnios. (H&E stain, 100X)

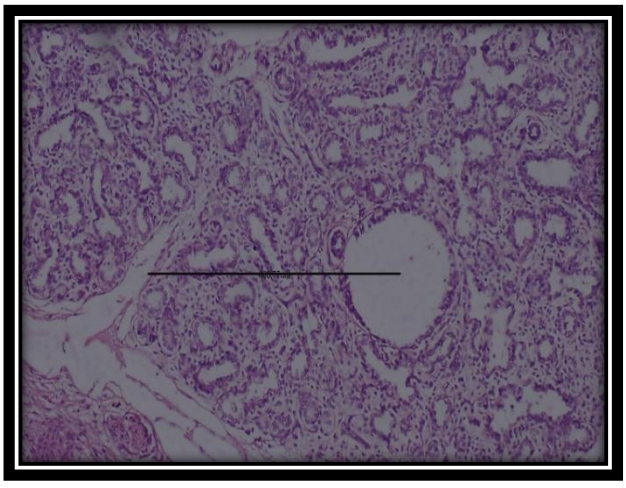


Fig. No.33- Microscopy of lung section with mean RAC of 2.1 in a case of IUD. (H&E stain, 100X)

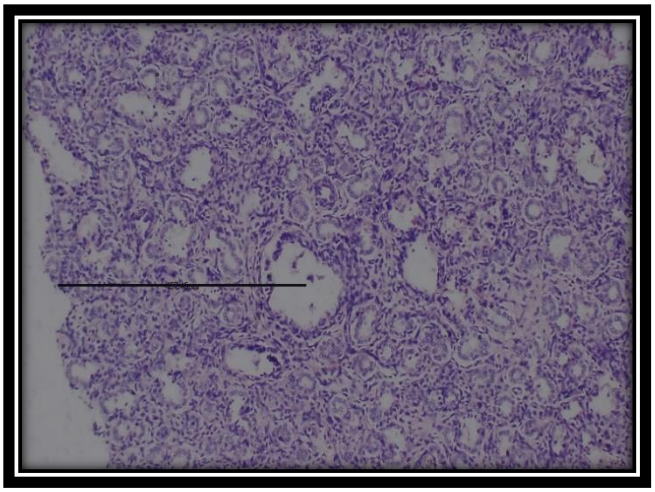


Fig. No.34- Microscopy of lung section with mean RAC of 2.3 in a case of CDH. (H&E stain, 100X)

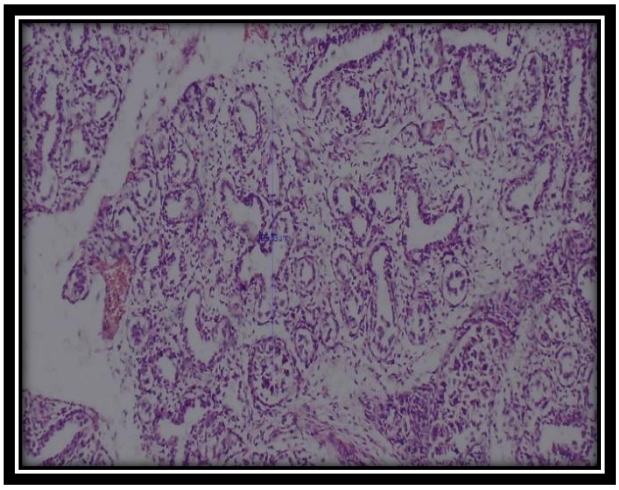


Fig. No.35- Microscopy of lung section with mean RAC of 2.2 in a case of gastroschisis. (H&E stain, 100X)

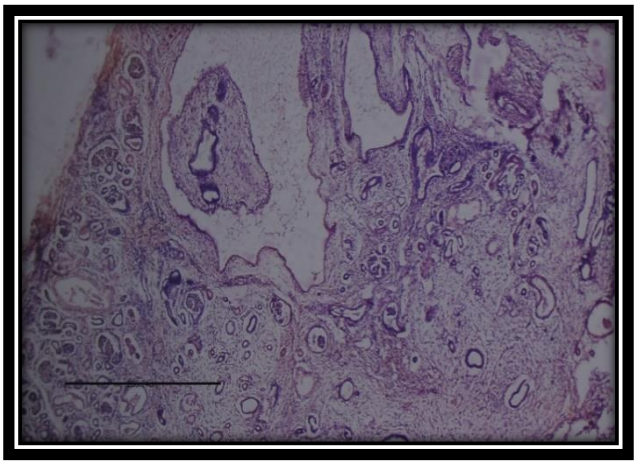


Fig. No.36- Microscopy of kidney section showing renal parenchyma arranges in disorganised pattern and primitive tubules and cysts lined by flattened to cuboidal epithelium in case of renal dysplasia with CTEV. (H&E stain, 100X)

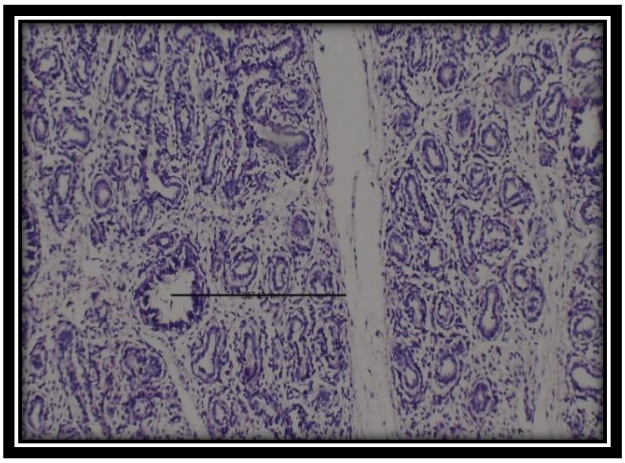


Fig. No.37- Microscopy of lung section with mean RAC of 1.65 in case of renal dysplasia with CTEV. (H&E stain, 100X)

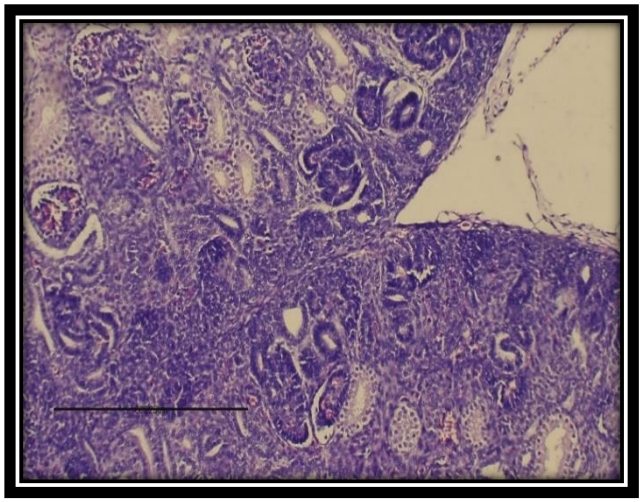


Fig. No.38- Microscopy of kidney section showing fused renal cortex in a case of fused midline kidneys. (H&E stain, 100X)

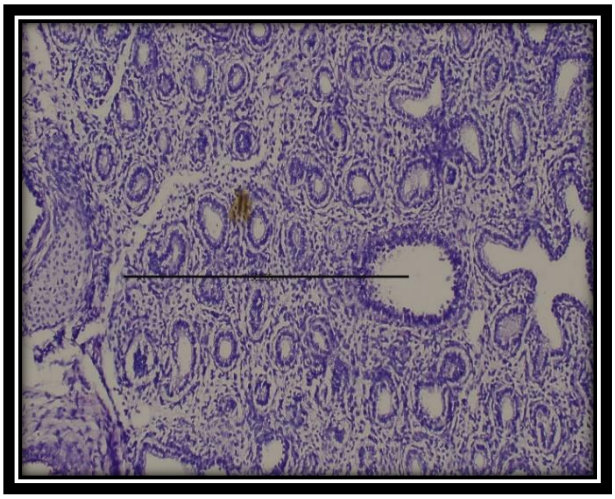


Fig. No.39- Microscopy of lung section with mean RAC of 2.1 in a case of fused midline kidneys. (H&E stain,100X)

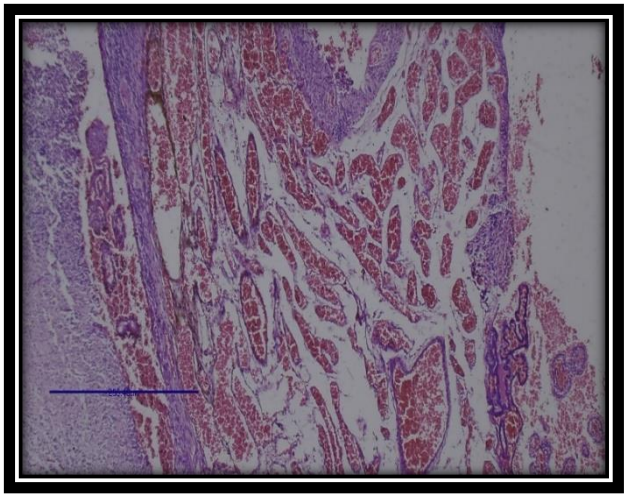


Fig. No. 40– Microscopy of case of anencephaly case showing angiomatous mass. (H&E stain, 100X)

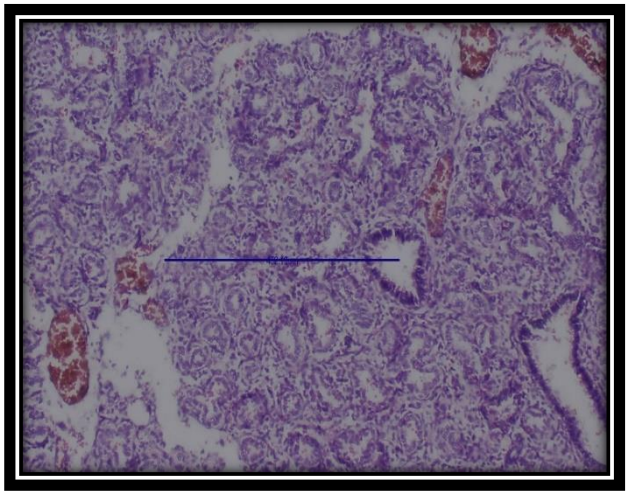


Fig. No.41–Microscopy of lung section in a case of anencephaly with mean RAC of 1.7. (H&E stain, 100X)

DISCUSSION

PH is congenital anomaly. PH is caused due to arrest in normal development of the lungs during intrauterine growth. Arrest in normal development is due to insults in thoracic cavities and extra thoracic cavities.[1-3,5,39]

Askenazi S and Perlman M[4] in their study mentioned that LW: BW ratio of 0.012 and/or RAC of less than 4.1 is a diagnostic criterion for PH. They also recommended that when LW: BW ratio is less than 0.009, PH is very likely and RAC is not mandatory. When LW: BW ratio is between 0.010-0.012, PH is probable and RAC should be indicated for confirmation. When LW: BW ratio is between 0.013- 0.017, PH is possible and RAC is must. When LW: BW ratio is more than 0.018, PH is unlikely and RAC is not indicated. In present study PH evaluation was done by LW:BW ratio and RAC as mentioned in the study done by Askenazi S and Perlman M[4].

In study done by Cherian *et al*[48] on "Evaluation of Pulmonary Hypoplasia in Various Congenital Anomalies with a Comparison of Two Conventional Methods of Assessment: Radial Alveolar Count (RAC) and Lung Weight: Body Weight Ratio (LBW)" mentioned that, PH is noted in more than 10% of neonatal autopsies and more than 85% of cases occur in association with another malformation/ malformations. In present study out of 62 cases of fetal autopsy study, in 45 cases amounting to 72.58% of cases PH was noted. Associated anomalies and conditions were noted in 27 cases amounting to 53.34%.

In study done by Aghabiklooei A *et al*[1], Wigglesworth and Desai[40], Husain A and Hessel R[44] and Cherian *et al*[48] observed that PH was noted in 11.3%, 14.5%, 26%, and 43% respectively. In present study PH was noted in 72.58%.

Concordance between LW:BW ratio and RAC was noted in 33 cases amounting to 53.23%. In these cases, RAC was less than 4.1 and LW: BW ratio was also less than 0.012. Discordance between LW:BW ratio and RAC was noted in 22 cases amounting to 35.48%. Further evaluation of these cases was done to conclude the diagnosis of PH. Out of 22 cases, 4 cases had LW:BW ratio between 0.010 to 0.012 however RAC was more than 4.1. These cases were not considered as PH. Out of 22 cases, 11 cases had LW:BW ratio between 0.013 to 0.017 and RAC was less than 4.1. Out of these 11 cases, 3 cases were of neural tube defects, 1 case of gastroschisis and 7 cases of IUD. Out of 11 cases, 2 cases were having GA less than 15 weeks. After extensive review of literature as normal reference range for RAC and LW:BW ratio was not available for GA less than 15 weeks. Lung examination of these cases showed pseudoglandular stage of development. RAC was not possible to evaluate for GA less than 15 weeks as true bronchial epithelium is still underdeveloped at this GA. Hence out of 11 only 9 cases were considered as PH. In 7 cases LW:BW ratio was more than 0.018 and RAC was less than 4.1. Out of 7 cases, 2 cases were of CCAM, 1 case of neural tube defect and 4 cases were with GA less than 15 weeks. Hence only 3 cases were considered as PH. Thus, out of 22 cases, 12 cases were considered as cases of PH and 10 cases were not considered as PH. Concordance between LW:BW ratio and

RAC was noted in 33 cases of PH amounting to 53.23%. In these cases, RAC was less than 4.1 and LW: BW ratio was also less than 0.012. In 12 cases discordance was noted between LW: BW ratio and RAC. In these cases, RAC was less than 4.1 and LW: BW ratio was more than 0.012. Out of these 12 cases 4 cases were of NTD, 2 cases were of CCAM, 1 case of gastroschisis and 5 cases were of IUD. Total 45 cases amounting to 72.58% were considered as cases of PH by diagnostic criteria.

Askenazi S and Perlman M[4] observed discrepancy between LW:BW ratio and RAC in cases of PH with neural tube defects(anencephalies). They mentioned that this discrepancy could be due to low body weight due to the absence of brain leading to more LW:BW ratio and delay in alveolar development due to absence of pituitary gland leading to low RAC. They observed neural tube defects had mean GA of 38 weeks, mean LW:BW ratio of 0.016 and mean RAC of 3.1. In present study 6 cases of neural tube defects amounting to 9.67% had mean GA of 28.1 weeks, mean LW:BW ratio of 0.016 and mean RAC of 2.3, which was corelating with their study.

Nimrod C *et al*[49] on their study on effect of PROM on oligohydramnios and fetal development found that there is greater impact on fetal growth when PROM occurred before 26 weeks of GA. PROM leading to oligohydramnios syndrome consists of tetrad of PH, skeletal deformities, Potter's facies and intrauterine growth retardation. Rotschild A *et al*[50] observed PH in 16% of fetuses that experienced oligohydramnios following rupture of membranes before 29 weeks of GA. Husain A and Hessel R[44] in their study observed that

4 cases with oligohydramnios had PH with mean LW:BW ratio of 0.011 and mean RAC of 2.7. In present study 6 cases of oligohydramnios and one case of PROM amounting to 11.29% were considered as PH with all cases having GA less than 29 weeks. These cases had mean GA of 22.85 weeks, mean LW:BW ratio of 0.006 and mean RAC of 2.65 which was corelating with these studies.

Chikkannaiah P *et al*[51] in their study on 2 case reports of congenital cystic adenomatoid malformation (CCAM) on lungs mentioned enlargement of affected lungs by CCAM and revealed multiple cysts of varying sizes. Dos Reis AR *et al*[52] in their study on CCAM with GA of 25 weeks mentioned their autopsy findings that affected lungs weighed 75.9gm and showed varying sized cysts with RAC less than 3. In present study, 2 cases of CCAM of lungs was seen with mean gestational age 23 weeks. Both cases showed enlarged lungs with one case showing imprint of rib cage on lung surface. Mean lung weight was 19.12gm and mean RAC was 2.075.

In present study 6.45% cases of renal pathology had mean GA of 21.2 weeks, mean LW:BW ratio of 0.010 and mean RAC of 2.43. Our study findings are corelating with study by Askenazi S & Perlman M[4] and Husain A & Hessel R[44], in their study they observed that renal pathology cases at mean GA of 36 weeks and 31 weeks had mean LW:BW of 0.010 and 0.012 and mean RAC of 3.2 and 2.6 respectively.

In present study 3.22% cases of hydrops fetalis had mean GA of 30 weeks, mean LW:BW ratio of 0.007 and mean RAC of 3.1. Our study findings are corelating with study by Askenazi S & Perlman M[4] and Husain A & Hessel R[44], in their study they observed that hydrops fetalis cases at mean GA of 34 weeks and 24 weeks had mean LW:BW of 0.013 and 0.011 and mean RAC of 4.4 and 2.2 respectively.

In present study 72.58% (n=45) cases fulfilled for PH according to diagnostic criteria. Out of these 40% cases were of IUD, 13.34% cases were of NTD, 13.34% were cases of oligohydramnios, 8.90% cases were of renal pathology, 4.44% cases each were of CCAM, hydrops fetalis and ascites, 2.22% cases each were of PROM, gastroschisis, CDH, AMC and uteroplacental insufficiency. Thus, 53.34% cases showed associated anomalies/conditions with PH in present study. In 40%(n=18) of IUD cases, PH finding was incidental finding. Out of which 50% (9 cases) showed meconium aspiration features in lungs. No significant anomalies or conditions were detected in another 50% of cases.

Table No.14- Comparison of PH associated with other conditions and anomalies with other author studies.				
Conditions/ anomalies associated with PH.	**Aghabiklooei A *et al* [1]**	**Pena Y *et al* [3]**	**Cherian DM *et al* [48]**	**Present study**
NTD	18.7%	-	15.2%	13.34%
Oligohydramnios	-	3.3%	23.7%	13.34%
Renal pathology	11.4%	10%	16.9%	8.90%
Lung pathology	7.3%	6.6.%	-	4.44%
Hydrops fetalis	8.3%	5%	-	4.44%
Diaphragmatic hernia	12.5%	40%	-	2.22%
Musculoskeletal abnormality	15.6%	-	-	2.22%
PROM	-	3.3%	-	2.22%
Gastroschisis	-	-	-	2.22%
Secondary causes of PH	92.7%	-	-	53.34%

Aghabiklooei A *et al*[1] in their study found 11.3% of primary causes of PH. Husain A and Hessel R[44] found 22% cases of primary PH where one third cases where not associated with any congenital malformations. In present study, 18 number of cases of PH had no congenital malformation or other associated conditions.

In a study done by Ward C and Caughey AB[53] observed that as gestational age increases the risk of meconium aspiration syndrome increases. In their study they mentioned that Meconium aspiration syndrome was 1.3% at 38 weeks of GA and 4.8% at 42 weeks of GA. They also mentioned that the risk of meconium aspiration syndrome increased by 30% with each week of GA. Fischer *et al*[54] also stated that risk of meconium aspiration syndrome and meconium-stained amniotic fluid increases with advancing gestational age. Meconium aspiration syndrome in their study was 0.11% at 37-38 weeks of GA, 0.20% at 39–41 weeks of GA and 0.49% at 42-43 weeks of GA. Our study findings also corelate with the observations done by these authors. In present study, meconium aspiration was noted in 37.1% of cases. Maximum number of cases of meconium aspiration were noted in the cases having GA range of 31 to 35 and 36 to 40 weeks amounting to 100% and 75 % respectively.

SUMMARY

A prospective study was done on 62 Fetal autopsies in the Department of Pathology for evaluation of PH from December 2019 to July 2021. Detail history and ultrasonography (USG) findings were collected for all cases. In all cases LW:BW ratio and RAC was evaluated. Diagnostic criteria for PH were taken as LW:BW ratio less than 0.012 and/ or RAC less than 4.1.

Majority of the fetal autopsy cases amounting to 27.42% were having gestational age of 16 to 20 weeks followed by 24.20% of cases having 21 to 25 weeks of gestational age. Male to Female ratio of the fetuses was 1:1 with two cases having ambiguity in sex determination.

IUD was the commonest associated condition followed by oligohydramnios, neural tube defect and renal pathology. Out of 62 cases, 29 cases were of IUD, 8 cases each of oligohydramnios and NTD and 4 cases of renal pathology.

In 44 cases LW: BW ratio was below normal range. Maximum numbers of below normal range cases were noted in GA range of 16 to 20 weeks followed by 21 to 25 weeks of gestational age. Mean RAC was highest at the GA range of 31 to 35 weeks followed by 36 to 40 weeks and was lowest at GA range of 10 to 15 weeks and the difference is statistically significant having p value less than 0.05.

In 18 cases LW: BW ratio was less than 0.009 and RAC was less than 4.1. Hence these cases were diagnosed as PH.

In 19 cases LW: BW ratio was between the range of 0.010 to 0.012. As per LW: BW ratio probability of PH was considered in these cases. Out of 19 cases in 15 cases RAC was less than 4.1 hence these 15 cases were considered as cases of PH. 4 cases with RAC more than 4.1 were considered cases without PH.

In 18 cases LW: BW ratio was between the range of 0.013 to 0.017. As per LW: BW ratio possibility of PH was considered in these cases. Out of these 18 cases in 11 cases RAC was below 4.1. However, in 2 cases GA was less than 15 weeks. Hence these cases were not considered as PH. Thus, 9 cases were diagnosed as cases of PH. 7 cases with RAC more than 4.1 were considered cases without PH.

In 7 cases LW: BW ratio was more than 0.018. As per LW: BW ratio where PH was unlikely. In these 7 cases RAC was less than 4.1. Out of 7 cases in 4 cases GA was less than 15 weeks. Hence these 4 cases were not considered as PH. Hence 3 cases out of 7 were considered as PH. Thus in 45 cases diagnoses of PH was given based on LW:BW ratio and RAC.

Out of 62 cases, 45 cases amounting to 72.58% were considered as PH based on diagnostic criteria. Cconcordance between LW: BW ratio and RAC was observed in 33 cases amounting to 53.23%. In these cases, RAC was less than 4.1 and LW: BW ratio was also less than 0.012. In 12 cases discordance was noted between LW: BW ratio and RAC. In these cases, RAC was less than 4.1 and LW: BW ratio was more than 0.012. Out of these 12 cases, 4 cases were of NTD, 2 cases were of CCAM, 1 case of gastroschisis and 5 cases were of IUD.

Meconium aspiration was noted in 37.1% of cases. Maximum number of cases of meconium aspiration was noted in the cases having GA range of 31 to 35 and 36 to 40 weeks amounting to 100% and 75 %. Meconium aspiration was not observed in lung specimen of fetal autopsy having gestational age less than 15 weeks. Frequency of meconium aspiration increased as the GA advanced.

Also, in the present study evaluation of PH with other associated conditions and anomalies was done. Mean LW: BW ratio was lowest in oligohydramnios as compared to other associated conditions/ anomalies and the difference was statistically significant having p value of 0.0002. Mean LW: BW ratio and RAC in IUD and renal pathology was lower as compared to other anomalies and the difference was statistically significant having p value of less than 0.0001 and 0.028 respectively. RAC was lowest in CCAM as compared to other associated conditions/ anomalies however only 2 cases of CCAM were noted in the present study. In neural tube defect RAC was slightly higher than CCAM but lower than other associated conditions/ anomalies, and the difference is statistically significant with p value of 0.0001.

CONCLUSION

Pulmonary etiologies are one of the commonest causes of neonatal deaths. PH is defined as unilateral or bilateral defective or incomplete development of lung parenchyma, airway, and vessels or incomplete development of lung which is not appropriate for gestational age. Pathologically diagnosis of PH was done based on two criterions, LW:BW ratio and RAC. In present study diagnosis of PH was done based on diagnostic criteria of LW:BW less than 0.012 and/or RAC less than 4.1. As per these diagnostic criteria, 45 cases were concluded as PH. Concordance between LW:BW ratio and RAC was noted in 53.23%. Discordance between LW:BW ratio and RAC was noted in 19.35%. Diagnosis of PH was rendered in a greater number of cases when both LW:BW ratio and RAC were considered. Evaluation by LW: BW ratio and RAC provides a reliable index of lung growth and should be essential part of fetal autopsy study. In present study PH was incidentally discovered in some cases which may be cause of mortality. Hence both LW:BW ratio and RAC should be routinely evaluated in all cases of fetal autopsies to avoid underestimation of PH and to detect incidental cases of PH. Early diagnosis and evaluation will help in counseling of parents for future pregnancies.

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ANNEXURE-I

ETHICAL CLEARANCE

IEC/131/19
22-11-2019

B.L.D.E. (DEEMED TO BE UNIVERSITY)
(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)
The Constituent College
SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The ethical committee of this college met on 13-11-2019 at 3-15 pm to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Fetal autopsy study to assess relative frequency of pulmonary hypoplasia and evaluation of various anomalies associated with pulmonary hypoplasia

Name of PG student: Dr Aparna Sajjan,, Department of Pathology

Name of Guide/Co-investigator : Dr Surekha U Arakeri, Professor & HOD Department of Pathology

DR RAGHVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

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ANNEXURE-II

BLDE (DEEMED TO BE UNIVERSITY)
SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL
AND RESEARCH CENTERVIJAYAPURAA-586103

INFORMED FETAL AUTOPSY CONSENT FORM

I am the…… (Enter relationship) of the deceased baby……(enter baby's name or mothers name) and I am his/ her parent/ substitute decision-maker.

I give consent to an autopsy (Post Mortem examination) to find out the reasons why my baby died and understand that there is a possibility that a cause of death may not be found. I understand that autopsy may also be considered for confirmation of ultrasound findings, investigation of chromosomal/ metabolic abnormalities, obtain tissue for storage for future investigations or identify a syndrome which may be related to but not represent the final event causing death. I have been given the opportunity of having a social support person (partner/ parent/ close friend) present during these discussions.

The Doctor has also informed me that information given by me, observations made by the investigator will be kept secret and not assessed by the person other than me or my legal hirer. My questions and concerns are discussed and are answered to my satisfaction.
I am aware that once consent is given it is not possible to withdraw back. On the basis of above statements, I give consent to the autopsy.

Consent to the pathologist performing:

- Full post mortem examination.
- Limited post mortem examination, which only involves the following organs or regions of the body: (specify)

Name of the parent/ substitute decision-maker:

Signature of the parent/ substitute decision-maker:

Witness:

1.

2.

Signature of the Doctor:

Date:

Place:

ANNEXURE-III

PROFORMA

- Autopsy No-
- OP/IP No -
- Intrauterine age/ Gestational age-
- Obstetric History of Mother -
- Biohazard Status of Mother during Antenatal period- HIV/HCV/HbsAg-
- USG Findings of Mother during Antenatal check-up-
- X-ray of Fetus -
- Other significant history/ finding -

Anthropometric Data and External Examination of Fetus			
Weight of Fetus			
Crown Heel Length (CHL)			
Crown Rump Length (CRL)			
Head Circumference (HC)			
Biparietal Diameter (BPD)			
Abdomen Circumference (AC)			
Chest Circumference (CC)			
Signs of maceration	Yes/no		
Umbilical cord (UC)	Length-	No. of vessels	Tied/ torn/ any other
Placenta-	Weight- Dimensions-	Umbilical cord- Attached/not attached	Any signs of disease
Foreign bodies in external orifices-	Yes/no		
Skin-	Normal/abnormal	If abnormal	
Head-	Intact/not intact	Shape-globular/flat/depressed	
Skull bones-	Normal/abnormal	Fontanelle-	Flat/raised/depressed
Eyes/ears/nose/lips/chin/ oral cavity-	Normal/abnormal	If abnormal- details	
Neck-defects-	Yes/no	Rest of head and neck-	Normal/abnormal
Limbs-symmetry-	Normal/abnormal	If abnormal- details	
Digits-upper limbs- **Lower limbs-**		Position of palms/soles-	
Upper limbs- **Hand/forearm/finger/nails/ crease**	Normal/abnormal	**Lower limbs-** **thigh/leg/foot/finger/nail/crease**	Normal/abnormal
Scars/signs of injury-	Yes/no	If yes-	
Thorax and abdomen symmetry-	Normal/abnormal	Nipples/sternum/abdomen-	Normal/abnormal
External genitalia-	Normal/abnormal	If abnormal-	
Gender- **male/female/ambiguous**			
Anal opening-	Normal/abnormal		
Signs of violence	Head-yes/no	Face-yes/no, mouth- yes/no	Neck-yes/no

Gross and Microscopic Details of Various Organs				
ORGAN	**WEIGHT**	**MEASUREMENTS**	**GROSS EXAMINATION FINDINGS**	**MICROSCOPIC EXAMINATION**
THYMUS				
RIGHT LUNG				RAC-
LEFT LUNG				RAC-
HEART				
LIVER				
SPLEEN				
RIGHT KIDNEY WITH ADRENAL				
LEFT KIDNEY WITH ADRENAL				
OTHER				

KEY TO MASTER CHART

- A. No.- Autopsy number
- AMC- Arthrogryposis multiplex congenita
- B/L - Bilateral
- CCAM - Congenital cystic adenomatoid malformation
- CDH - Congenital diaphragmatic hernia
- CTEV - Congenital talipes equinovarus
- G - Gravida of mother
- GA -Gestational age in weeks
- IUD - Intrauterine death
- LT - Left
- LW:BW - lung weight to body weight ratio
- M - Multigravida
- MA - Maternal age in years
- NAD - No abnormality detected
- P - Primigravida
- PROM - Premature rupture of membranes
- RAC - Radial alveolar count
- RT - Right
- Sl. No.- Serial number
- TGA - Transposition of great arteries
- WT – Weight in grams

MASTER CHART

SL NO	A. NO	MA	G	GA	CLINICAL DIAGNOSIS	GENDER OF FETUS	EXTERNAL ANOMALY	FETUS WT (gm)	RT LUNG WT (gm)	LT LUNG WT (gm)	LUNGS WT (mean) (gm)	LW:BW	RAC	UMBILICUS	THYMUS	HEART	B/L LUNGS	LIVER	SPLEEN	B/L KIDNEYS	B/L ADRENALS	NERVOUS SYSTEM
1	A/77/19	35	M	20	Hydrops fetalis	male	nil	300	2.1	1.9	2	0.006	2.3	2 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
2	A/78/19	28	P	28	Oligohydramnios	male	nil	950	9.8	8.2	9	0.009	3	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
3	A/79/19	29	M	40	Hydrops fetalis	male	nil	2500	22.3	19.6	20.95	0.008	3.9	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
4	A/80/19	21	P	23	IUD	male	nil	210	0.8	0.5	0.65	0.003	3.2	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
5	A/81/19	25	M	23	PROM	female	nil	356	3.3	2.6	2.95	0.008	1.9	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
6	A/82/19	22	P	27	Renal pathology-Right renal agenesis	male	nil	750	9.2	8.7	8.95	0.011	3	3 vessels	NAD	NAD	NAD	NAD	NAD	Rt kidney-agenesis	NAD	NAD
7	A/83/19	24	M	27	IUD	female	nil	900	4.6	3.6	4,1	0.004	3.25	2 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
8	A/84/19	20	P	39	Ascites	male	nil	2700	36.3	36.1	36.2	0.013	5.4	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
9	A/85/19	27	M	38	Neural tube defect-Anencephaly	male	anencephaly with frog like facies	100.5	1.6	1.4	1.5	0.014	1.7	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	anencephaly
10	A/90/19	23	M	28	IUD	female	nil	1070	7.2	5.5	6.35	0.005	3.2	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
11	A/91/19	25	M	37	Oligohydramnios	female	nil	1291	17.5	17.1	17.3	0.013	5.4	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
12	A/92/19	20	P	38	IUD	female	nil	2707	36	36	36	0.013	5.3	2 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
13	A/93/19	19	P	20	Oligohydramnios	male	nil	395	1.5	1.3	1.4	0.003	2.55	2 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

14	A/94/19	28	P	38	IUD	female	nil	2774	37	36.5	36.75	0.013	5.4	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
15	A/95/19	27	M	25	Uteroplacental insufficiency	male	nil	502	6.3	4.5	5.4	0.01	3.25	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
16	A/98/19	23	M	26	Oligohydramnios	female	nil	395	3.5	2.5	3	0.007	1.65	2 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
17	A/99/19	30	M	19	Gastroschisis	male	Gastroschisis	217	3.5	3.3	3.4	0.015	2.2	3 vessels	agenesis	NAD	NAD	NAD	agenesis	NAD	NAD	NAD
18	A/100/19	30	P	20	IUD	female	nil	350	4.4	3.8	4.1	0.011	3	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
19	A/1/20	28	M	20	IUD	female	nil	75	10	8	9	0.012	2.1	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
20	A/2/20	35	M	19	IUD	male	nil	50	2.1	1.9	2	0.013	2.2	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
21	A/3/20	28	M	22	IUD	male	nil	420	7	8	7.5	0.017	2.3	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
22	A/4/20	29	M	14	IUD	female	nil	110	2	1.5	1.9	0.017	2.1	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
23	A/5/20	22	P	37	Neural tube defect-Anencephaly	male	anencephaly with frog like facies	1700	18	17	17.5	0.01	3.2	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	anencephaly
24	A/6/20	23	M	29	IUD	female	nil	1300	20	18	19	0.014	1.9	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
25	A/9/20	23	P	20	IUD	male	nil	180	4	2	3	0.016	2.1	2 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
26	A/11/20	20	P	33	IUD	female	nil	1650	18	17	17.5	0.01	3.25	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
27	A/12/20	27	P	38	IUD	male	nil	890	15	15	15	0.016	5.3	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
28	A/13/20	19	M	16	Neural tube defect-Anencephaly	male	anencephaly with frog like facies	100	3	3	3	0.03	2.2	2 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	anencephaly
29	A/17/20	29	M	20	IUD	male	nil	500	6	6	6	0.012	2.15	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
30	A/18/20	22	P	22	Neural tube defect-Anencephaly	female	anencephaly with frog like facies and open spina bifida	350	6	6	6	0.017	2.2	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	Anencephaly with meningomyelocele

31	A/19/20	22	P	38	IUD	male	nil	2674	35	35	35	0.013	5.3	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
32	A/20/20	23	M	20	Oligohydramnios	female	nil	350	1	0.5	0.75	0.002	2.55	2 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
33	A/25/20	34	M	38	Neural tube defect-Anencephaly	female	anencephaly with frog like facies	100.5	1.6	1.4	1.5	0.011	2.1	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	anencephaly
34	A/27/20	24	M	23	CCAM	male	Ascites	650	21.3	21.5	21.4	0.032	2.45	3 vessels	NAD	NAD	CCAM	NAD	NAD	NAD	NAD	NAD
35	A/29/20	19	P	14	Neural tube defect-Anencephaly	male	anencephaly with frog like facies	35	1	1	1	0.028	2.1	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	anencephaly
36	A/30/20	23	M	14	IUD	male	nil	90	1.5	1.5	1.5	0.016	2.1	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
37	A/40/20	35	P	33	Oligohydramnios	male	nil	1850	26.5	26.1	26.3	0.014	5.3	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
38	A/41/20	28	M	34	IUD	female	nil	2300	24	23.1	23.5	0.01	4.8	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
39	A/42/20	35	M	36	IUD	female	nil	2800	28	28.2	28.1	0.01	5.4	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
40	A/43/20	23	M	19	Oligohydramnios	male	nil	320	3	3	3	0.009	3.4	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
41	A/44/20	26	M	14	IUD	female	nil	110	2	2	2	0.018	1.7	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
42	A/45/20	26	P	20	Arthrogryposis multiplex congenita	female	contractures of wrist and interphalangeal joints of upper limbs	300	4	3.5	3.75	0.012	2.75	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
43	A/46/20	25	M	20	IUD	female	nil	18	2	1.5	1.75	0.009	2.2	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
44	A/47/20	27	M	34	IUD	female	nil	1630	16.3	16.5	16.4	0.01	5.3	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
45	A/48/20	30	M	21	Ascites	male	nil	270	3	3	3	0.011	2.2	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

46	A/49/20	29	M	16	Renal pathology-Fused midline kidneys	female	nil	116	1.4	1.1	1.25	0.01	2.1	3 vessels	NAD	NAD	NAD	NAD	NAD	bilateral fused kidneys	NAD	NAD
47	A/50/20	23	P	25	Ascites	male	nil	1000	8.6	6.2	7.4	0.007	3.5	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
48	A/1/21	20	P	24	IUD	male	nil	700	10.8	7.9	9.35	0.013	3.95	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
49	A/3/21	20	M	18	Neural tube defect	female	occipital cystic swelling	192	3	3	3	0.015	2.6	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	encephalocele
50	A/4/21	28	M	36	PROM	female	nil	2500	25	25.3	25.1	0.01	5.1	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
51	A/5/21	30	P	20	IUD	female	nil	400	4	4	4	0.01	2.75	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
52	A/16/21	18	P	24	Oligohydramnios	female	nil	456	4	3.8	3.9	0.008	3.55	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
53	A/17/21	20	P	24	CDH	male	nil	555	3.5	3.5	3.5	0.006	2.3	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
54	A/18/21	23	M	22	IUD	female	nil	350	4	4	4	0.011	3.3	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
55	A/21/21	26	P	16	Renal pathology-Multicystic dysplastic kidneys	male	CTEV	130	1.5	1.5	1.5	0.011	1.65	3 vessels	NAD	NAD	NAD	NAD	NAD	multicystic renal dysplasia	NAD	NAD
56	A/23/21	24	M	24	IUD	male	nil	600	4	4	4	0.006	1.95	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
57	A/24/21	31	P	23	CCAM	female	nil	510	17.1	16.6	16.85	0.033	1.7	3 vessels	NAD	TGA	CCAM	NAD	NAD	NAD	NAD	NAD
58	A/25/21	21	P	25	IUD	female	nil	700	6	6	6	0.008	0.8	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
59	A/26/21	20	M	29	IUD	female	nil	1200	4	4	4	0.003	3	3 vessels	NAD	NAD	meconium aspiration	NAD	NAD	NAD	NAD	NAD
60	A/27/21	29	P	11	Neural tube defect-Exencephaly	ambigous	Exencephaly	24	1	0.8	0.9	0.037	2	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	exencephaly
61	A/28/21	28	P	11	IUD	ambigous	nil	35	1.5	1.5	1.5	0.042	2.9	3 vessels	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
62	A/29/21	24	M	28	Renal pathology-left kideny agensis, IUD	male	nil	440	4.8	4.5	4.65	0.01	3	not attached	agenesis	NAD	NAD	NAD	agenesis	rt kidney-NAD. Lt kidney-agenesis	rt adrenal-NAD. Lt adrenal-agenesis	NAD