Intrauterine Growth Retardation (IUGR) is an important entity and goes by a variety of names including small for date, dysmaturity, chronic malnourished fetus, undergrown fetus and placental insufficiency. Clifford (1954) described and classified the appearance of the so-called dysmature infant.

According to the recommendations of a WHO Committee (1961) newborn babies weighing less than 2500 g at birth are classified as low birth weight (LBW) infants irrespective of the period of gestation. It includes both preterm babies and those who are small for gestation (small-for-date), due to intrauterine growth retardation.

Lubchenco's (1963) definition which defines IUGR as birth weight below 10th percentile has problems in specificity. Because of the distribution of fetal weight in general population, at least 7% of normal babies will be classified as growth retarded when 10th percentile is used to differentiate normal from abnormal fetuses. Also it does not take into account growth retarded fetuses whose birth weight falls above 10th percentile line. Indeed, it is now known that the fetuses affected by IUGR do not constitute
a homogenous population and that a varying degree of
compromise in height, weight and soft tissue mass can be
observed.

According to Albermann and Butter (1969), birth
weight less than 2.5 kg does not imply IUGR as it does not
take into account the gestational age.

Usher, Maclean and Greunwald (1969) defined
IUGR as birth weight more than 2 standard deviation (2 SD)
below the mean birth weight.

By birth weight and gestational age, newborns
are divided into 3 categories (Bhargava et al, 1974):

(i) Preterm (gestational age less than 37 weeks)
   (a) Appropriate for gestation (AGA) and birth
       weight between ± 1 SD.
   (b) Small for date (SFD) and birth weight below 2 SD.
   (c) Large for date (LFD) and birth weight above 2 SD

(ii) Term (gestational age 37-41 weeks)
   (a) Appropriate for gestation (AGA) and birth weight
       between ± 1 SD.
   (b) Small for date (SFD) and birth weight below 2 SD
   (c) Large for date (LFD) and birth weight above 2 SD
(iii) Post term (gestational age 42 weeks or more)
   (a) Appropriate for gestation (AGA) and birth weight between $\pm 1$ SD.
   (b) Small for date (SFD) and birth weight below 2 SD
   (c) Large for date (LFD) and birth weight above 2 SD

Singh et al (1978) has subdivided small for date babies on the basis of severity of intrauterine growth retardation.

(i) **Mild IUGR**

Babies whose birth weight falls between 3rd and 10th percentile of the standard, appropriate for gestational age.

(ii) **Severe IUGR**

Babies whose birth weight falls below 2 SD or 3rd percentile of the standard weight for gestational age.

Intrauterine growth retardation is a major perinatal problem in developing countries (Miller et al, 1971, Urrastietal, 1972). These growth retarded babies do not constitute a homogenous group. For the diagnosis of IUGR, it is imperative that gestational age be determined accurately.
ESTIMATION OF GESTATIONAL AGE:

Because of the irregularity in menstrual cycle, a significant number of patients will not recall their last menstrual period (LMP). Wenner and Young (1974) observed that only 33% of mothers could be relied upon to give accurate date of LMP. However, Hertz et al. (1978) reported that only 18% of patients were able to give a reliable date of their LMP.

Gestational age can also be estimated by:

1. BIOCHEMICAL METHOD:

   (i) Lecithin/sphingomyelin ratio (L/S < 2 indicates gestational age less than 34 weeks. Ratio which is > 2 reflects a gestational age of more than 38 weeks (Deter et al., 1982; Singh, 1985).

   (ii) Shake test

   Undiluted amniotic fluid mixed with various strengths of saline when shaken for 15 secs with equal volume of 95% ethanol, a complete ring of bubbles persist at the meniscus after 15 minutes of standing the sample indicating a positive test. If positive in dilution of 1:2 or greater it indicates that the fetal lung is satisfactorily mature hence gestational age is more than 35 weeks (Singh, 1985) and correlates well with mature L/S ratios and absence of hyaline membrane disease.
(iii) Creatinine level in liquor amnii \( \geq 2 \text{ mg/100 ml} \) indicates gestational maturity of at least 37 weeks. It also indicates mature renal function (Singh, 1985).

2. CYTOLOGY:

Nile blue sulfate test of liquor amnii

Lipid containing epithelial cells from sebaceous glands of fetal skin are shed into the amniotic fluid. With 0.1% Nile blue sulfate these lipid cells stain orange. If there are more than 20% orangophilic cells it indicates gestational age of 36 weeks or more (Edward et al, 1968).

3. ANTERIOR VASCULAR CAPSULE OF LENS:

Examination of the disappearance of anterior vascular capsule of lens (pupillary membrane) was found useful to detect preterm infants with gestational age between 27 and 34 weeks (Hittner et al, 1977). This criterion was further confirmed by Narayan et al (1981).

4. ROENTGENOGRAM:

(i) Distal Femoral Epiphysis - Ossification centre appears between 32-38 weeks of gestation (Bhargava et al, 1977).

(ii) Proximal Tibial Epiphysis - Ossification centre appears by 40 weeks of gestation (Singh, 1985).
Besides roentgenography fetal maturity has been assessed by long bone measurement by Bhargava et al (1977).

5. **ULTRASOUND**:

Hellman (1969) has suggested that linear measurement of gestational sac, which appears at $4\frac{1}{2} - 5$ weeks after the last menstrual period, be utilized as parameter of gestational age.

Crownrump length which can be measured at $6\frac{1}{2} - 7$ weeks of gestation has been shown to increases parabolicly with gestational age (Robinson et al, 1973).

Hadlock et al (1982) has shown that head circumference is a better predictor of gestational age than biparietal diameter.

Femur length is a more reliable ultrasonographic parameter of fetal age during later gestation (Campbell et al, 1982).

6. A comprehensive assessment of gestational age, based on certain neurological and physical criteria has been devised by Dubowitz et al (1970).

**ETIOLOGY OF IUGR**:

In the third world countries, 25% to 40% babies have low birth weight (weight $\leq 2.5$ kg) and two third of
them are growth retarded. It is a well known fact that growth retarded babies differ from term-appropriately-grown infants in etiology, morbidity, mortality and later development (Bhargava et al, 1974 and Khatua et al, 1979).

Placental abnormality due to infarction is more likely to give rise to intrauterine infection, causing a high level of Ig M (Chandra et al, 1970) and intrauterine growth retardation. Placental dysfunction due to the toxaeemia of pregnancy was noted by Schutte et al, 1971 and played an important role in the etiology of intrauterine growth retardation as observed by the author.

Maternal toxaeemia, hypertension (blood pressure of more than 140/90 mmHg), edema and proteinuria have a direct effect on the placental dysfunction (Schutte et al, 1971). These factors are responsible for the birth of hypoplastic, apathetic, growth retarded children. Bhatia et al (1990) have shown that in toxaeemia of pregnancy, the insult for growth retardation operates late in gestation and the babies of such mothers demonstrate a catch-up growth for weight, crown heel length and head circumference after birth.

In developed countries, intrauterine undernutrition is more common than postnatal undernutrition. The etiology of poor intra-uterine nutrition is complex but
is related to the factors altering placental passage of nutrients, including poor placental blood supply to the fetus, maternal undernutrition, multiple pregnancies, maternal disease, genetic disturbances and intrauterine infections (Chase et al, 1971).

Maternal malnutrition contributes to intrauterine growth retardation. Maximum brain growth takes place during later half of foetal life with a peak at the time of birth. Approximately 2/3rd of human brain cells as represented by DNA are present prior to birth and 1/3rd are added after birth from 2-5 months of age. Malnutrition also adversely affects the nervous system (Schutte et al, 1971) and these babies have poor tone and reflex activity. The ponderal index defined as:

\[ \frac{\text{Weight in grams}}{(\text{Length in cm})^3} \times 100 \]

Miller and Hussanei (1971) observed more than 2.2 in those babies whose mothers experienced marked food deprivation in the last trimester. It reflected disproportionate growth in which weight was more affected than length.

Ghosh et al (1971) observed in Northern Indian women, that chronic malnutrition before and during pregnancy lead to birth of small infant with respect to weight, length and head circumference, at all gestational ages. The ponderal index was, however, normal in such cases.
Bhargava et al (1977) found the delay in the appearance of epiphyseal centres at the distal end of femur and proximal end of tibia of babies, born to malnourished mothers.

Bhatia et al (1984) found the lowest mean values for weight, crown heel length and head circumference in IUGR babies of malnourished mothers.

Immunological system in man is known to develop largely before birth. Intrauterine nutritional deprivation is bound to affect the immunocompetence of such a neonate. Bharadwaj et al (1987) undertook a study to evaluate the immunological effects of intrauterine growth retardation in forty five full term neonates with birth weight less than 2.5 kg. They observed elevated levels of immunoglobulin A and M in 4.4% of IUGR babies, indicating some kind of intrauterine infection leading to intrauterine growth retardation.

Tobacco smoking and living at high attitude may also contribute to fetal growth failure. At high attitude chances of hypoxia are much more which affects the growing fetus (Singh, 1986).
The malformed babies, born as a result of maternal irradiation and ingestion of teratogenic agents during first trimester of pregnancy, are also growth retarded (Singh, 1985).

Multiple pregnancy is an important factor which usually affects the growth of the fetus. Upto 35 weeks of gestation both the fetuses can be nourished satisfactorily. Beyond this period, single placenta is unable to sustain normal growth of two fetuses (Singh, 1985).

Diminished fetal growth may be due to genetic and chromosomal disorders in the form of trisomy syndrome, Turner syndrome and various types of short limb dwarfism. Also, the babies of small sized mothers suffer most in crown heel length followed by weight (Bhatia et al, 1984).

**INTRAUTERINE INFECTIONS**

Intrauterine growth retardation is one of the common manifestations of intrauterine infection. It is responsible for the significant mortality and long term morbidity. There are indications that micro-organisms like rubella, cytomegalovirus, toxoplasma, herpes and syphilis infect as many as 2% of fetuses and upto 10% infections occur during delivery or in first few months of life after birth (Seth et al, 1985).
Hence decreased crown heel length, small head circumference, loss of adipose tissue and muscle mass as well as decreased organ weight have been associated with IUGR. However, recent studies have shown that these findings are not always associated with low birth weight indicating that growth retardation has more than one form. Hence, more than one parameter may be required to identify all affected fetuses, in which infection is one of the etiological factors. Seth et al, 1985 described that these babies have usually hepatomegalgy, jaundice, adenopathy, skin lesions, petechiae, purpurpa, maculopapular exanthems meningoencephalitis, microcephaly, hydrocephaly, intrauterine calcification, hearing defects, congenital defects, glaucoma, cataract. Diagnosis of maternal infection was a prerequisite for suspecting potential fetal or neonatal involvement, observed the author.

**Rubella:**

At present, rubella is considered a threat to fetus during the first 20 weeks of gestation. Antibody may appear as early as 14 days after exposure, irrespective of clinical illness (Plotkin, 1973). Alford et al (1975) has described that the ratio of inapparent to apparent infection in adults is 1%. Therefore, a history of exposure to rubella on the occurrence of rash demands serologic investigation.
Toxoplasmosis:

As in rubella, maternal infection is the major pre-requisite to fetal involvement with toxoplasma infection (Kimball et al., 1971 and Desmonts et al., 1974). Fetal invasion can occur at any stage of gestation but chances increase with the advancing gestation. Throughout pregnancy, it occurs in only 40% of maternal infections. Mothers are always asymptomatic or nondescript. Ig M antibody persists for months or years in high percentage of infected cases.

Cytomegalovirus (CMV):

Reynolds et al., 1973 have found a very high incidence of active cytomegalovirus (CMV) infection in young pregnant woman which is mainly localized to cervix or urinary tract. Nataly transmitted CMV infection occurs in about 50% of cases which is chronic and dangerous in the early infancy.

Herpes virus hominis (HSV) :

Most of the women become immune to herpes virus hominis before reaching child bearing age (Nahmias et al., 1970). Mostly, HSV infection occurs secondary to gonorrhoea or cytomegalovirus infection. Herpes virus infection is considered dangerous at or near term. Clinically, it can be
diagnosed by typical vesicular lesions that occur in only one third of cases (Nahmias et al, 1971). Diagnosis is confirmed by cytologic or virologic examination. In 50% of women, infection is asymptomatic and in the remainder non specific signs and symptoms like pelvic pain and cervical inflammation are present (Nahmias et al, 1972).

**Syphilis:**

Primary, secondary, or reinfection syphilis are all dangerous to the fetus. There is an inherent protection from fetal involvement in the first 20 weeks of gestation. Tendency for invasion increases as the gestation advances. In general, high VDRL titres (Spirling, 1971) reflect active infection. The absorbed fluorescent treponemal antibody (FTA-ABS) test is specific for Treponema pallidum infection.

Persistence of infection postnatally has been the feature of certain intrauterine infections like cytomegalovirus and rubella where the organisms continue to survive and replicate in tissue for months and years. In infected infants virus can be excreted from multiple sites like pharynx, urine, conjunctiva etc. and is detectable in CSF, bone marrow or circulating blood cells (Alford et al, 1964).
The great majority of infants born with chronic perinatal infection will be missed during the early months of life because they lack signs or symptoms. Over 95% of newborns are asymptomatic, while Starr et al (1968) and Kumar et al (1973) found toxoplasmosis in 75%, rubella in 65% and syphilis in 50% cases.

Fieldman (1968) had determined from a serological survey that 70%, 10%, 90%, 40% women of child bearing age were susceptible to toxoplasmosis, rubella, syphilis and cytomegalovirus respectively.

Failure to thrive, psychomotor retardation and visual defects may follow toxoplasma, rubella, and cytomegalovirus infections (Berenberg et al, 1970).

Toxoplasma gondii or cytomegalovirus infections cause no congenital deformity. Some have obvious multiorgan involvement and in others only a single organ is involved (Peckhan, 1972). Intrauterine rubella infection may produce typical congenital rubella syndrome or may lead to hearing defect, as being the only manifestation.

Townsend et al (1975) demonstrated that in some intrauterine infections like rubella, cytomegalovirus, progressive tissue destruction occurred e.g. progressive encephalitis seen in rubella.
DIAGNOSIS OF IUGR:

The most important procedure for detecting IUGR in vitro is the identification of women who is at risk to deliver a growth retarded fetus. The neonatal diagnosis of infection acquired in utero, nataly and postnatally is inherently difficult. Asymptomatic or clinically indistinguishable infection in the pregnant women can produce fetus involvement and the resultant neonatal infections may be clinically in-apparent (Phillips et al, 1965). The author opined that fulminant forms of perinatal infection are often accompanied by indistinctive findings, therefore, methods adjunctive to clinical means were needed for early detection of infectious disease in a neonate.

Normally in pregnancy, the human placenta does not actively transport gamma M (γM) or immune gamma A (γA) globulins from maternal to fetal serum and reduced levels of these are maintained in late gestation and neonatal period. However, Alford and his co-workers (1965) demonstrated increased quantities of γM and γA globulins in umbilical cord serum. McCracken et al (1965), Sieber et al (1966) and Stiehm et al (1966) have demonstrated raised levels of immunoglobulin after intrauterine infection caused by herpes simplex virus, cytomegalovirus suggesting the importance of immunoglobulins in the diagnosis of intrauterine infection.
Specific γM antibody may be produced in utero and in the immediate neonatal period after many types of infections (Hodes et al, 1966). If detectable, presence of γM and γA globulins generally denotes primary response to antigen. This phenomenon helps in the diagnosis of a variety of infections in the newborn period (Alford et al, 1966) which are clinically inapparent. Demonstration of Ig M (above 19.5 mg/100 ml) in the cord blood or in the blood sample of a neonate is taken as an indication of excessive intrauterine antigenic stimulation and serves as a non specific monitor of intrauterine infection (Alford, 1971).

IMMUNOGLOBULINS

Immunoglobulins (Ig) consists of a heterogenous group of proteins (Globulin) whose main function is to act as antibody. The concept of immunoglobulins has been extended to include a group of structurally related proteins (Myeloma protein macroglobulin) produced in several proliferative disorders of plasma cells and lymphocytes. Electrophoresis separates the serum protein into four major fractions namely albumin and three distinct globulins (alpha, β, γ). The γ globulins were first designated by Tiselius in 1937 as a distinct groups of serum proteins having electrophoretic mobility. 'γ' globulin have an isoelectric point of 7.3
are the most slow in their movement towards anode (during electrophoresis) because they have a very low negative charge. Kunkel et al (1951) and Pitman (1953) showed that multiple myeloma protein in the serum of patients with multiple myeloma belonged to 'γ' globulin fraction of serum proteins.

Heidelberger and Pederson (1937) were the first to separate immunoglobulin (Ig) by size (The 1gS fraction and 7S fraction). The larger fraction was named as "Ig macro" or "Ig M" and the smaller "Ig Gamma" or "Ig G", a reflection of its electrophoretic mobility.

Porter (1959) was able to clear the immunoglobulins into two fragments, separable by ion-exchange chromatography. One fraction retained the capability to react with the immunogen and was called the antigen binding fraction, or Fab. The other crystallized upon standing and was called the crystallizable fraction or Fc.

Later studies in Porter's Laboratory (Fleischman et al, 1963) delineated the relationship between the chain structure and the proteolytic fragments, and a general model for Ig structure was proposed. In general, each molecule consists of two larger polypeptide chains referred to as heavy chains (H-chains) and two identical smaller ones, referred to as light chains (L-chains). These poly-
peptide chains are held together by disulfide bonds and
by non-covalent bonds which are primarily hydrophobic.
The heavy and light polypeptide chains are synthesized
on separate ribosomes, assembled in the cell and secreted
as intact molecule.

Five immunoglobulins (Ig G, Ig A, Ig M, Ig D
and Ig E) are recognized on the basis of structural diffe-
rences of their heavy chains including the amino acid
sequence and length of polypeptide chains.

**Immunoglobulin G (Ig G):**

Synthesis of Ig G occurs at 11th week of gesta-
tion (Coonchi et al, 1969 and McCracken et al, 1971). The
most abundant of classes of immunoglobulins is Ig G. It
has a molecular weight of 150,000 daltons. Ig G is the
only immunoglobulin that crosses the placenta and thereby
provide maternal antibodies to neonate. Mean serum concen-
tration of Ig G in cord blood of a new born baby is usually
in the range of 740-1650 mg% (Hardy et al, 1969), which
consists mostly of maternal antibodies and falls to a level
of about 200-600 mg% by about 6 months of age. As the infant
is exposed to antigenic environment, Ig G levels gradually
starts increasing reaching to adult level i.e. 800-1200 mg%
by about 4 years of age.
**Immunoglobulin A (Ig A):**

Ig A is the next most abundant immunoglobulin in serum. The Ig A comprises about 10% of the γ globulin in human serum. It is the predominant immunoglobulin in mucous secretions, viz, oral, nasal, bronchial and intestinal secretions, tears, milk. Ig A synthesis is virtually undetectable in the fetus and does not become substantial for several months after birth. Serum level of Ig A is 0-3.9 mg% (Malik et al, 1977) at birth and gradually increase to about 25-75 mg% by 2 years of age. Adult levels (150-300 mg%) are reached in adolescence. Most of the Ig A exocrine secretions appear to be locally synthesized in plasma cells in the submucosa or regional nodes. Molecular weight of serum Ig A is 160,000 daltons and of secretory Ig A is 370,000 daltons.

**Immunoglobulin M (Ig M):**

Ig M is the largest of the polymeric immunoglobulins, usually being a pentamer of the H₂L₂ structure with one J chain. Molecular weight of Ig M is 900,000 daltons. Ig M is typically the immunoglobulin produced as a result of primary response to antigenic challenge. Ig M is the main immunoglobulin produced by the fetus, and the amount
formed is usually small. However, when there is fetal infection, substantial Ig M response may occur. Serum concentration of Ig M in newborn is about 1.6-31 mg% (Hardy et al, 1969), which rapidly increases to adult level of 50-150 mg% by about 1 year of age. The Ig M level in serum is between 5 and 10% of the total antibody protein.

**Immunoglobulin D (Ig D):**

Recently Rowe and Fahey (1965) discovered a unique globulin Ig D, having a molecular weight of 180,000 daltons. It is found mainly in the intravascular space and on resting B cells as a cell surface immunoglobulin. It is easily degraded by proteolytic enzymes and heat. The function of free Ig D in blood is not clearly known. But, Ig D on B cells may in association with monomeric Ig M, play an important role in the binding of antigen to B cells. Ig D comprises about 3 mg% of normal serum.

**Immunoglobulin E (Ig E):**

The distinctive biologic feature of Ig E is its role in the immediate allergic or hypersensitivity reactions. Ig E has a molecular weight of 190,000 daltons and binds to basophils and mast cells. When a specific antigen
combines with the antigen binding site on Ig E histamine, serotonin and slow reacting substance of anaphylaxis (SR/SA) are released from these cells. The bulk of body pool of Ig E is bound to basophils and mast cells. Serum concentration of this immunoglobulin is extremely low.

A quantitative gel diffusion technique with antibody incorporated into the agar was referred to by Feinberg in 1957. This approach was further developed by Mancini et al in 1964 and has been utilized in commercially supplied immunochemical equipment.

Fahey, J.L. et al in 1965 did quantitative determination of serum immunoglobulins while using agar plates.

McCracken et al in 1965 estimated immunoglobulin concentration in eight mothers who had given birth to newborn infants suffering from congenital cytomegalic inclusion disease (CID) with clinical signs of microcephaly, hepatosplenomegaly, jaundice and retinal changes. Simultaneously, neutralizing antibodies were studied by standard technique and complement fixing antibodies by standard microtechnique. Results were compared with normal. They found raised Ig M level in all the babies while complement fixing antibodies were detected in the sera of 6 out of 8 of the cytomegalic inclusion disease infants and neutralizing antibodies were found in 7 out of 8 such infants.
Alford et al in 1967 used a correlative immunologic, microbiologic, and clinical approach in the diagnosis of acute and chronic infections of newborn infants. For this study, they took 222 samples of umbilical cord serum and 210 samples of different materials from 58 infants, for virologic examination. In 156 serum samples gamma M globulin values were more than 20 mg per 100 ml while in 12 serum samples, the level was less than 20 mg%. Analysis performed on 65 serums, showed that gamma A globulin level was in excess of 25 mg%. Clinical status of these infants was assessed viz-a-viz gamma M globulin levels. High levels were believed to be due to the infection and were correlated with diagnosis established in the majority of cases by serial clinical or microbiological examinations performed at varying intervals for a period of a year. During the month after the beginning of symptoms, the serum gamma M globulin serially increased with the initial elevations first detected approximately a week after the beginning of symptoms. Same was true in the case of gamma A globulin. The rate of formation of these immunoglobulins in serum from infected patient was independent of age or birth weight as observed by the authors.

Gitlin and Biasucci in 1968 detected immunoglobulin M (Ig M) synthesis in in-vitro cultures of human embryonic spleens as early as 10.5 weeks of gestation.
Hardy et al in 1969 made some preliminary observations from a survey of cord blood immunoglobulin levels in 2,600 infants and established their relationship to race, sex, birth weight and clinical status. They found that both G and M immunoglobulins were independent of sex. High Ig G and low Ig M in Negro's in comparison to Caucasian neonates were also observed. Ig G level was related to the birth weight, with the small infants manifesting lower levels. Lowest level of Ig M was related to the perinatal deaths and severe congenital malformations as observed by the authors. 8 out of 17 neonates had levels below 1.6 mg% in this category. The results of this study were consistent with those of Alford et al (1967). Authors have confirmed a previously reported relationship between high levels of Ig M i.e. above 30 mg% and congenital infections in 5% consecutively born infants.

Usher, Maclean and Yerushalmy (1969) have shown that perinatal mortality increased 8 fold in IUGR. It accounted for 25% of the total perinatal mortality rate. These cases were also subjected to intrapartum and neonatal asphyxia (Low et al, 1978), hypoglycemia, polycythemia (Burd, 1971), meconium aspiration, pulmonary hemorrhage, disorders of temperature regulation and congenital malformation (Low
et al, 1978). Also, long term morbidity in the form of impaired motor and cognitive function, lower IQ and neurological abnormalities increased up to 20% in IUGR infants (Werner, 1970).

Chandra et al in 1970 observed that Ig M and other large molecular weight proteins could be detected at birth but their concentration in the serum of healthy neonates seldom exceeded 20% of the adult levels. In the first few weeks of postnatal life, raised Ig M concentration, with or without significant change in Ig G was an indication of perinatal infection such as rubella, syphilis, toxoplasmosis or cytomegalovirus, as the authors have opined.

Fitzhardinge and Stevens (1971) have shown 40% of IUGR infants to have difficulty in school. Authors found twenty percent of still births to be growth retarded. Earlier the insult, longer the exposure, poorer the prognosis were some other observations made by these authors.

Lichtig et al in 1971 worked on cord blood levels of Ig M in Latin American neonates and found elevated cord Ig M levels in 47.8% neonates.

Fitzhardinge et al in 1972 studied the longitudinal growth pattern of 96 fullterm small-for-date infants,
followed until 4 years of age. Serial measurements of weight, height and head circumference were compared with the stuart growth percentiles. They found that by 4 years of age, mean weight and height of these infants fell between 10th and 25th percentile, with 35% children remaining below the 3rd percentile and only 8% going above the 50th percentile norm. Increase in head circumference paralleled linear growth. Bone age showed a high positive correlation with height age. There was no difference in 6 year old's height or weight, whether a severely intrauterine retarded baby or his less affected counterparts. The growth pattern of SFD children was similar to the normal children with a high velocity of growth occurring in the first 6 months of life.

Cham et al in 1972 estimated serum immunoglobulin G, A, M in 800 healthy children of various age groups from birth to adolescence by using the method of Mancini et al (1965). The accuracy and reproducibility of the method had been tested by calculating coefficient of variation and the values were Ig G 5.9%, Ig A 6.8% and Ig M 5.7%. They have proved that at the time of birth, the major protein of immunoglobulins was constituted by Ig G which was placentally transferred from the mother. Ig A and Ig M was synthesized by the fetus only when there was a stimulus for immunologic reaction,
such as perinatal infection. The immunoglobulin level in the first year showed a relatively low mean Ig G concentration i.e. 75% of the standard but comparatively higher Ig A (50.6%) and Ig M (83.7%) values were found by the authors. After the age of one year, level of all the three immunoglobulins was fairly high.

Alford et al in 1975 has explained the importance of demonstrating elevated Ig M level, as a nonspecific monitor to characterize newborns with high probability of intrauterine infection. The causative maternal infection could often be asymptomatic CMV and or toxoplasma. There could also be other unnoticed infections like rubella, syphilis or herpesvirus etc.

There is a report by Behar et al. (1975) which explains the meaning of elevated Ig M level in cord serum. Authors suggest that it was not always due to the infection, but some at least could be caused by another stimulus viz., damage to placental vessels. Also, raised Ig M could be due the sample getting inadvertently contaminated by maternal blood. Mata (1976) got raised level of Ig M in only 15% of the newborns whose blood samples were collected properly.

Raghavan et al (1976) studied serum proteins with special reference to immunoglobulins in 15 healthy neonatal
maternal pairs. The total proteins obtained in the cord sera samples were $6.43 \pm 0.43$ g percent as compared to $7.30 \pm 0.41$ g percent in maternal sera while Ig M levels were $11.38 \pm 6.76$ IU/ml in cord sera compared to maternal levels of $366.7 \pm 226$ IU/ml. The levels of Ig A in cord sera were from 0 to 3.92 IU/ml as compared to maternal levels of $140.5 \pm 114.4$ IU/ml and were detected only in 5 out of 15 patients. This supports the view of Gitlin et al (1959) that Ig M and Ig A do not traverse the placental barrier, hence the low values are obtained in healthy newborns.

Raghavan et al in 1976 compared the serum protein immunoglobulin levels between premature and fullterm Indian neonates. The total serum proteins in the two groups were $6.50 \pm 0.50$ gm% and $6.43 \pm 0.43$ gm% respectively. The two groups did not show any significant difference. Similar was the observation with regard to Ig M, Ig G values of $170.8 \pm 99.1$ IU/ml obtained in the premature neonates were significantly lower when compared to the values of $253.6 \pm 137.6$ IU/ml observed in fullterm ($p < 0.025$).

Bhaskaram et al (1977) studied cell mediated immunity and circulating level of immunoglobulins in 75 fullterm newborn babies. The results showed that though the immunoglobulin level was not altered, cell mediated immunity was significantly depressed in infants with birth weight less than 2500 g and that this could result in lowered resistance to infection.
Lars et al (1978) analysed the relationship between immunoglobulin level and the gestational age, birth weight and sex in 176 normal infants. They have noticed increased levels of Ig M and Ig G with the increasing gestational age for those weighing more than 2000 g.

Singh et al (1978) assessed the humoral and cellular immune status at birth in 20 term appropriate for gestation babies, 12 preterm babies and 24 term babies with intrauterine growth retardation. The preterm babies showed significantly lower level of Ig G which supported the study of Malaviya et al, 1976. However, the cellular immune response was satisfactory in preterm babies when compared to normal. Infants with severe IUGR, on the other hand, showed marked impairment of both humoral and cell mediated immune response.

Low et al (1978) published a preliminary report of prospective follow-up study of 88 IUGR babies (with characteristic clinical features) and a control group of 97 babies with normal weight. IUGR group had a phase of accelerated growth during 3 months following delivery although they continued to be smaller than the babies of control group at 12 months of age. No major neurologic abnormalities were observed during the neonatal period and at 12 months of age. IUGR babies with lowest birth weight had lower mental and physical development indices at 12 months of age.
Kaur et al in 1979, assessed the immunological response of low birth weight babies from birth to 6 months of age and their results were consistent with the previous studies.

It is now becoming increasingly apparent that growth retarded babies do not form a single homogeneous group, but are perhaps made up of several groups differing in their aetiology, body proportions and later outcome. Bhatia et al in 1984 studied 41 full term intrauterine growth retarded (IUGR) babies of different aetiology, viz., maternal undernutrition (12), small maternal size (12), toxaemia of pregnancy (9) and idiopathic (8) for assessment of growth pattern during the first 9 months of life, and compared them with 18 fullterm and 11 preterm babies. The IUGR babies of undernourished mothers had lowest mean for weight, crown heel length and skull circumference. The babies of small sized mothers suffered most in crown heel length followed by weight. The head growth was not affected in these babies. The IUGR babies of mothers with toxaemia of pregnancy demonstrated a catch-up growth for all the three parameters, while the IUGR babies of idiopathic group showed a spurt in weight gain around 3 to 6 months and a similar spurt for crown heel length and head circumference was observed between 6 to 9 months of age. The preterm AGA babies also demonstrated a catch-up growth for weight, crown heal length and circumference.
Seth et al (1985) studied 231 newborn infants with birth weight 2 kg or less and analyzed the presence of antibodies against rubella, cytomegalovirus, toxoplasma, syphilis and hepatitis B surface antigen. Simultaneously Ig G, Ig M and Ig A were also estimated. They found that as many as 2% fetuses were infected in utero. Upto 10% of infants were detected to have infection at the time of delivery or within the first few months after delivery. None of the cord samples had Ig M levels more than or equal to 20 mg%, as reported by author.

Immunological system in man is known to develop largely before birth. Intrauterine nutritional deprivation is bound to affect the immunocompetence of such a neonate. Bharadwaj et al (1987) evaluated the immunological effects of intrauterine growth retardation in 45 fullterm neonates with birth weight less than 2.5 kg and compared them with 10 fullterm neonates weighing more than 2.5 kg at birth. In 4.44 percent of growth retarded babies mean cord serum Ig M level was elevated while Ig G was significantly low as compare to that of the control group indicating some kind of intrauterine infection. Chaturvedi et al (1989) worked on 35 full term intrauterine growth retarded singleton babies weighing less than 2.5 kg and their mother. They also took 10 control patients weighing more than 3 kg for their investigation.
In the study population both mean cord serum Ig M (26.8 mg%) and mean maternal serum Ig M levels (142.42 mg%) were raised as compared to the mean cord serum Ig M (13.76 mg%) and mean maternal serum Ig M (100.16 mg%) of the control group. Cord serum Ig M levels exceeding 20 mg% and 30 mg% were found in 51.43 and 22.8% fullterm IUGR neonates respectively. Among the control neonates only 20.0% had levels exceeding 20 mg% and none had levels above 30 mg%, suggesting possible intrauterine antigenic challenge in higher proportion of IUGR babies.

Bhatia et al (1990) has established a relationship between the development of IUGR and the maternal etiology in 41 full term IUGR babies after comparing them with 18 full term and 12 preterm babies. The IUGR babies of undernourished mothers had maximum retardation in head circumference. The IUGR babies of small sized mothers had head circumference equal to that of fullterm appropriate-for-gestational age babies while IUGR babies of toxemic mothers and idiopathic group were very close to the full term babies at nine month of post natal age. Only 19.5 percent of IUGR babies had delayed development as compared to 16.7 percent of preterm and 5.6 percent of fullterm appropriate-for-gestational age babies.
MATERIAL AND METHODS
MATERIAL AND METHODS

The present study was carried out in the Department of Paediatrics, in active collaboration with the Department of Obstetrics and Gynaecology, Maharani Laxmi Bai Medical College, Jhansi. Babies delivered in the labour room of Obstetrics and Gynaecology department, between May, 1990 to June, 1991 were included in the study.

SELECTION OF CASES

Study Group:

This consisted of 24 live newborn babies delivered by normal vaginal route. They were born at full term gestation; were singleton; had birth weight not exceeding 2500 grams. Babies born to mothers with short stature (height below 145 cm and weight below 40 Kg), gross anaemia (Haemoglobin \( \leq 8 \) gm% by Sahli's method), edema, hypertension (blood pressure exceeding 140 mmHg systolic and or 90 mmHg diastolic), congestive cardiac failure, evidence of perinatal stress, metabolic disease and blood group incompatibility were excluded. Also, babies born to mothers with a history of febrile episode in the third trimester and those having early rupture of membranes were excluded from the study.
Control Group:

This consisted of 10 healthy live newborn babies delivered by normal vaginal route. They were born at full term gestation; were singleton; had birth weight 3000 gms or more.

Obstetrical History:

In each case, history of last menstrual period was recorded when the mother was sure of it. Gestational age was calculated in complete weeks from the first day of last menstrual period up to the time of delivery.

Natal and Postnatal History:

History was taken to record the mode of delivery. The other points noted were duration of labour, per vaginal bleeding, meconium staining of liquor, cry and activity of the child after birth including cyanosis; all to rule out any evidence of perinatal stress. All cases presenting with perinatal stress were excluded from the study.

Examination of Newborn:

Apgar scoring of the child was done at 1 minute and after 5 minutes following birth to detect any evidence of birth anoxia. Cases having Apgar score less than 7 were excluded from the study group.
Thorough clinical examination was done in each case to exclude the possibility of congenital infection or anomaly. Anthropometric measurements viz, head circumference, chest circumference, crown-heel length were recorded in the proforma. Birth weight of the newborn was recorded within one hour of the delivery.

Assessment of gestational age was done by using the physical and neurological characteristics laid down by Dubowitz et al (1970). The neurological characteristics were scored from 0-5, while eleven physical characteristics were scored from 0-4 in a pre-designed proforma and conversion of total score into gestational age was done by using the formula (Dubowitz et al, 1970).

\[
\text{Estimated period of gestation} = R \times 0.2642 + 24.5950 \\
\text{(in weeks)}
\]

Where \( R \) represent the total score.

In the case of discrepancy found between gestational age calculated by Dubowitz criteria and that calculation from the history of menstrual period, the case was dropped from the study.

**Collection of blood sample**:

For the estimation of Ig M, blood (5 ml) was collected from the cut end of umbilical cord, from the placental side, in the clean glass tube, with due precautions to avoid haemolysis and contamination with maternal blood. All glassware
used in the study was thoroughly sterilized, washed with distilled water and dried in hot air oven.

Blood sample was allowed to clot at room temperature. After 2-4 hours, serum was separated using a pippette and then serum was centrifuged at 1000 rpm for 15-20 minutes. After centrifugation 2 ml of clear serum at the top of sample was transferred to another dried vial. All samples were stored at -20°C in a deep freezer. Similarly, a venous blood sample of the mothers was taken and serum was separated and stored in the deep freezer. The samples were analyzed for Ig M at a later date.

**Method of Estimation:**

Serum immunoglobulin Ig M was determined by using single radial Immunodiffusion method (Mancini et al, 1965). For this commercially prepared Immunodiffusion plates were used. The principle being that specific antibody against human immunoglobulin is incorporated into a buffered agarose medium and uniformly spread on a glass plate. The test serum containing the human immunoglobulin (antigen) is placed in a well prepared in the antibody agarose plate. When agarose contains sufficient amount of antibody, there is a free diffusion of antigen from the well. Reference serum containing known amount of immunoglobulin (antigen) is run concurrently with unknown specimens. Since the immunoglobulin concentration
in the serum is related to the area of precipitin zone, this can be directly read from the table of the reference value or from the calibration curve.

(i) Reagents and Material used:

1. Immuno diffusion plates
2. Reference serum
3. Auto pippette
4. Measuring scale for measuring the diameter of precipitin ring.

(ii) Preparation of Test Sera and Reference Serum Dilutions:

For obtaining accurate results, the reference serum and test serum were diluted, using 0.85% normal saline as shown in Table - I.

<table>
<thead>
<tr>
<th>Dilution required for</th>
<th>Reference Serum</th>
<th>Test Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>3/4 part Reference Serum and 1/4 part Normal saline</td>
<td>1/2 part Reference Serum and 1/2 part Normal saline</td>
</tr>
<tr>
<td>75%</td>
<td>1/2 part Reference Serum and 1/2 part Normal saline</td>
<td>1/4 part Reference Serum and 1/4 part Normal saline</td>
</tr>
<tr>
<td>50%</td>
<td>1/4 part Reference Serum and 1/4 part Normal saline</td>
<td>1/2 part Test Serum and 1/2 part Normal saline</td>
</tr>
<tr>
<td>25%</td>
<td>1/4 part Reference Serum and 1/4 part Normal saline</td>
<td>1/2 part Test Serum and 1/2 part Normal saline</td>
</tr>
<tr>
<td>50%</td>
<td>1/2 part Reference Serum and 1/2 part Normal saline</td>
<td>1/2 part Test Serum and 1/2 part Normal saline</td>
</tr>
</tbody>
</table>

TABLE - I
(iii) Procedure:

(1) Lid of the Immuno-diffusion plate (having 16 wells) was carefully removed. Each of the first four wells was filled with 5 microlitres of the reference serum of different dilutions (100%, 75%, 50%, 25%) and the remaining wells were filled with 5 microlitres of the test sera appropriately diluted as mentioned in the Table - I.

(2) Lid of the plate was then replaced and the same was incubated at room temperature (25-30°C) for 72 hours.

(3) After incubation, the diameter of each precipitin ring was measured accurately (upto 0.1 mm accuracy) with the help of a measuring scale.

(4) The Ig M concentration corresponding to the measured diameter of precipitin ring was read directly from the calibration curve.

(iv) Calibration Curve:

Calibration curve was drawn using squared diameter of the precipitin rings obtained from various dilutions (25%, 50%, 75% and 100%) of the reference serum. On a centimeter graph paper, the squared diameter of precipitin rings were plotted on the vertical axis (Y-axis) against the corresponding known immunoglobulin concentrations (mg per 100 ml)
on the horizontal axis (X-axis). Using 4 points of square of diameter against dilutions of reference serum concentration, a straight line of best fit was drawn. The concentration of particular immunoglobulin in the test serum was determined by reading the concentration against the point representing the square of the diameter of the respective ring of precipitate.