A DISSERTATION ON

ETIO PATHOLOGICAL STUDY OF ANEMIA IN PRESCHOOL CHILDREN (3 TO 6 YEARS)

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CERTIFICATE

This is to certify that this dissertation entitled "ETIOPATHOLOGICAL STUDY OF ANEMIA IN PRESCHOOL CHILDREN (3 TO 6 YEARS)" submitted by DR.G. MUGUNTHAN to the faculty of Pediatrics, The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of the requirement of the award of M.D. Degree Branch VII (Pediatric Medicine) is a bonafide research work carried out by him under our direct supervision and guidance.

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DECLARATION

I, Dr. G.MUGUNTHAN solemnly declare that the dissertation titled "ETIOPATHOLOGICAL STUDY OF ANEMIA IN PRESCHOOL CHILDREN (3 TO 6 YEARS)" has been prepared by me.

This is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of the requirement for the award of M.D., degree Examination (Pediatric Medicine) to be held in FEBRUARY 2006.

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CONTENTS

INTRODUCTION

Pediatric Hematology is a sub speciality of Pediatrics and a sine qua non of the modern teaching institutions.

Among the hematological disorders, Anemia is the commonest entity of multiple aetiologies. The meaning of the Greek word "Anemia" is without blood.

It is a major world health problem and is an important cause of morbidity and mortality much of which can be preventable. Man has only partially adapted to the rapid growth in human population and environmental changes and Anemia is one of the effects of these factors. Thus still the most common cause of Anemia in children is related to nutritional deficiency especially iron deficiency. Initially man depended mostly on animal food, and as he learned agricultural practices, the contribution of animal food reduced drastically to less than 5%. This profoundly affected the bio available Iron and folate resulting in nutritional deficiency Anemia.

Anemia is defined as the reduction of RBC volume or Hemoglobin concentration below the range of values for a particular age and sex.

The detection and diagnosis of Anemia are frequently the focus of attention in the care of patients because accurate quantification and rational analysis of problem is must.

Anemia is not a diagnosis in itself like fever but merely is an objective sign of the presence of disease. The correct diagnostic terminology for a child with Anemia requires the detection of etiology, pathology and pathogenesis of the Anemia.

A systematic approach through proper history, physical examination and relevant investigations are very essential to diagnose the various causes of Anemia.

Our aim is mainly to study the Etiology, pathology of Anemia and use of various laboratory investigations in the evaluation of Anemia in preschool children(3 to 6 years of age),since this group of patients forms one of the major risk group to develop Anemia.

REVIEW OF LITERATURE

HISTORICAL ASPECTS:

The basic concepts of hematology came largely from internal medicine and from the experimental sciences. Every pediatrician, particularly haematologist, must remember the names such as Ehrlich, Metchnikoff, Landsteiner, Chauffard, Downey, Minot, Castle, Whipple and Wintrobe who have pioneered in hematology.

Traditionally, the history of Anemia began in 1889 with Von Jaksch's report on the condition that bears his name which he designated as pseudo leucaemica infantum [8,9].

In 1889, Hayem, known for his invention of Hayem's solution to study the blood corpuscles, described the blood picture of newborn in one of the oldest haematological text (Du Sang et de Ses Alterations Anatomiques) [23] .

In 1891,Hayem and his compatriot Luzet described Anemia pseudo leucaemica infantum as Anemia of infancy par excellence [30].

In 1909, H. Flesch a German author wrote, "Differences in the heamoglobin values of different observers according to the ages of

children are so considerable that one is really in no position to give definite normal figures."

In 1919, Winifred Ashby measured the normal life span of R.B.C's of adult using the differential agglutination [22].

More lasting and far more important was the contribution made to pediatric hematology by Thomas.B. Cooley in 1925 when he salvaged a distinct entity now known as thalassemia [10].

In 1927, Heinrich Baar wrote, "The Hemoglobin content suffers more than the RBC count" in Anemia [2].

Increased number of nuclear segments in neutrophils in megaloblastic Anemia was described during 1923-1927. Minot and Murphy in 1928 described the conversion of megaloblastic bone marrow to normoblastic bone marrow and correction of Anemia by treatment with liver.

George Guest conducted meticulous studies between 1932 and 1942 with regards to Hb levels, RBC, Packed cell volumes of a large group of infants and young children and showed convincingly that a fall in the mean corpuscular hemoglobin (MCV) in the presence of seemingly adequate Hb levels could be normalised by administration

of Iron and was the first person to observe iron deficiency Anemia of infancy.

In 1944, blackfan and dimond's Atlas of the blood in children was published, which used wintrobe's classification and described the principal Anemia of infancy under the title "iron deficiency Anemia" [20]

In 1949, Linus Pauling, Harvey Itano and their co-workers identified sickle Hemoglobin as a discrete protein separable by electrophoresis from normal haemogobin.

In the same year, James V. Neel's study of genetics of sickle cell Anemia and sickle cell variant established the former as the homozygous and the latter as heterozygous state for sickling gene. This became the fountainhead of a veritable flood of investigations leading to the discovery of the Hemoglobinopathies.

In 1950, Philip Sturgeon who created hematology service at the children hospital of Los Angeles studied the bone marrow examination in Infants. Later it has become the diagnostic procedure in hematology.

In 1956, Carson and his associates reported deficiency of glucose-6-phosphate dehydrogenase deficiency in primaquine sensitive erythrocytes.

In 1959, Ingram and Stretton postulated two classes of thalassemia, α and β thalassemia, corresponding to α and β chain variants of the Hemoglobinopathies.

PREVALENCE OF ANEMIA IN PRESCHOOL CHILDREN:

Various studies have been carried out world wide in the field of Anemia. Most of such studies were conducted in tropical countries where Nutritional Anemia, particularly iron deficiency is a major cause of morbidity and mortality especially in growing children [19].

A recent study of Sarada Sidhu (1996) on prevalence of Anemia among scheduled caste preschool children, reported a prevalence rate of 55%. [40]

As per ICMR study (V.P. Choundhry et al in 1995), it has been estimated that over 60% of rural children between 1 to 3 years have Anemia and 40% of children between 3 to 6 years are anaemic and iron deficiency was proved to be the commonest cause. [16]

Williams K. Simmons et al in 1982 did a survey of anemia status in preschool children, pregnant and lactating women in Jamaica. He reported a prevalence rate of 61.9 % in children between 0 to 5 years of age using a cut off value of 11.0 g % for Hemoglobin. Iron deficiency Anemia was found to be the commonest cause. [45]

K. Ahmad et al (Bangladesh, 1980) reported a prevalence rate of 82% between age groups 0-4 years. (used 11g% as cut of value).

A study conducted in Indonesia by Soemantro et al (1979) in children between 6 months to 5 years reported a prevalence rate of 37.8 to 73%.

A prevalence of 60% was reported in children less than 4 years in a study conducted in 331 Filipino children between 9 months to 7 years by Marzan et al. A strong association was found between Anemia and nutritional status.

Simmons et al in (1982) reported a prevalence rate of 67.8% in children between 0-5 years in Middle Cairos island.

PHYSIOLOGY :

Normal red blood cells are biconcave disc having a mean diameter of about 7.8 micrometer .The shape of red blood cells can change remarkably as the cells pass through capillaries.

GENESIS OF RED BLOOD CELLS :

Red blood cells are derived from pluripotential hemopoietic stem cells in the bone marrow. The development of RBC passed through the stages of proerythroblast, basophilic, polychromatophilic, orthochromatic erythroblasts, reticulocytes, mature RBC.

DEFINITION OF ANEMIA :

There are various definitions for Anemia. A functional definition of Anemia is a state in which, the circulating red cell mass is insufficient to meet the oxygen requirement of the tissues.

G.C. De Gruchy defined Anemia as, a reduction in the concentration of Hemoglobin in the peripheral blood below the normal for age and sex of the patient.[21]

Maxwell M. Wintrobe described Anemia as decrease in concentration of oxygen carrying substance in a certain volume of blood. [32]

A working or clinically useful definition is reduction in concentration of Hb per unit volume of blood of two standard deviations below the normal for that population, age and sex. [20]

Such a precise definition is essential because the symptoms of Anemia are often non-specific and can be misinterpreted as symptoms of Emotional, Respiratory or Cardiovascular disorder and the reference values vary for different groups of population.

WHO definition of Anemia : [44]

Hemoglobin value less than 11 gm % is called Anemia in preschool children. This was taken as cut off value in this study.

	Severity of Anemia	Hb level
1.	Mild Anemia	10 – 10.9 gm %
2.	Moderate Anemia	7 - 9.9 gm %
3.	Severe Anemia	< 7.0 gm %

CLASSIFICATION OF ANEMIAS : [20]

The best approach for providing an understanding of the multiple disorders capable of producing Anemia is to separate the cause of anemia into two categories of functional disturbances.

These two functional categories are

1. Disorders of effective red cell production in which the net rate of red cell production is depressed. This can be due to disorders of the erythrocyte maturation in which erythropoiesis is largely ineffective or to an absolute failure of erythropoiesis. In the former the marrow contains many erythroblasts that die in situ before reaching the reticulocyte stage. In the latter there is absolute erythroblastopenia.
2. Disorders in which rapid erythrocyte destruction or red cell loss are primarily responsible for the anemia.

PHYSIOLOGICAL CLASSIFICATION OF ANEMIA : [20]

The following table classifies the Anemias, most commonly encountered in infancy and childhood, into three categories of functional disturbances.

A. DISORDERS OF RED CELL PRODUCTION IN WHICH THE RATE OF RED CELL PRODUCTION IS LESS THAN EXPECTED FOR THE DEGREE OF ANEMIA :

1. MARROW FAILURE

a). Aplstic Anemia

1) congenital
2) acquired

b) Pure red cell aplasia

1) Congenital :
   i) Diamond Blackfan syndrome
   ii) Aase's syndrome
2) Acquired :
   i) Transient erythroblastopenia of childhood.

c) Marrow replacement :

Malignancies
Osteopetrosis
Myelofibrosis

2. IMPAIRED ERYTHROPOIETIN PRODUCTION :

a. Chronic renal disease

b. Hypothyroidism, Hypopituitarism

c. Chronic Inflammation

d. Protein Energy Malnutrition

e) Hemoglobin mutants with decreased affinity for O_2

B. DISODERS OF ERYTHROID MATURATION AND INEFFECTIVE ERYTHROPOIESIS :

1. ABNORMALITIES OF CYTOPLASMIC MATURATION

a) Iron deficiency

b) Thalassemia syndrome

c) Sideroblastic anemias

d)) Lead poisoning

2. ABNORMALITIES OF NUCLEAR MATURATION :

a) Vitamin B 12 deficiency

b) Folic acid deficiency

c) Thiamine – Responsive megaloblastic anemia

d) Hereditary abnormalities in folate metabolism

C HEMOLYTIC ANEMIA

1. Defects of hemoglobin

a) Structural mutants

b) Synthetic mutants (Thalassemia syndrome)

2. Defects of red cell membrane(Hereditary Spherocytosis)
3. Defects of red cell metabolism
4. Antibody mediated
5. Mechanical injury to the erythrocyte
6. Thermal injury to the erythrocyte
7. Oxidant induced red cell injury
8. Infectious agent induced RBC injury
9. Paroxysmal nocturnal hemoglobinuria

MORPHOLOGICAL CLASSIFICATION OF ANEMIA :

Depending upon the Hematocrit, Hemoglobin and RBC indices, Anemia is classified as follows.

HYPOCHROMIC MICROCYTIC ANEMIA*:(MCV less than 75 fl)*

1) Iron deficiency
2) Copper deficiency
3) Thalassemia
4) Sideroblastic Anemia
5) Chronic Lead and Aluminium toxicity
6) Congenital atransferrinemia

MACROCYTIC ANEMIA : (MCV MORE THAN 100 FL).

With Megaloblastic bone marrow:

1) B 12 deficiency
2) Folic acid deficiency
3) Lesch – Nyhan syndrome
4) Hereditary Orotic aciduria
5) Thiamine responsive Anemia

Without Megaloblastic bone marrow:

1) Diamond Blackfan syndrome
2) Hypothyroidism
3) Liver disease
4) Aplastic Anemia

NORMOCYTIC ANEMIA :

1) Anemia of chronic disease
2) Chronic Infections
3) Connective tissue disorders
4) Malignancy
5) Uremia
6) Hemolytic anemia

CLASSIFICATION OF ANEMIA BASED ON

RED BLOOD CELL MEAN CORPUSCULAR VOLUME (MCV) AND HETEROGENEITY (RED CELL DISTRIBUTION WIDTH R.D.W.) [5,20]

Microcytic homogenous : (MCV low, RDW normal)

Heterozygous thalassemia

Anemia of chronic disease

Microcytic heterogeneous : (MCV low, RDW high)

Iron deficiency

HBS-β thalassemia

RBC fragmentation

Hemoglobin H

Normocytic Homogenous : (MCV normal, RDW normal)

Anemia of chronic disease

Chronic liver disease

Chronic lymphocytic Leukemia

Chronic myelocytic Leukemia

Hemorrhage

Hereditary spherocytosis

Normocytic Heterogenous : (MCV Normal, RDW high)

Mixed deficiency

Early iron deficiency

Myelofibrosis

Sideroblastic anemia

Macrocytic homogeneous : (MCV high, RDW normal)

Aplastic Anemia

PreLeukemia

Macrocytic heterogeneous :(MCV high, RDW high)

Folate deficiency

B 12 deficiency

Cold agglutinin

Immune Hemolytic Anemia

DIAGNOSIS OF ANEMIA：

Diagnosis is made on the basis of

History

Physical examination

Laboratory investigations

Historical clues in evaluation of Anemia

1) Age :

Iron deficiency Anemia is rare in terms infants before 6 months of age, in the absence of blood loss. Sickle cell Anemia and beta thalassemia appear in between 4-8 months of age.

2) Family History and genetic considerations:

X – linked recessive: Glucose 6 Phosphate Dehydrogenase deficiency

Autosomal dominant –Hereditary Spherocytosis

Autosomal recessive – Sickle cell Anemia , Fanconi's Anemia

Family member with history of early cholecystectomy or splenectomy. (Hemolytic anemia)

Ethnicity (Thalassemia in Mediterranean origin),

3) Race :

White Race – Beta thalassemia

Black and oriental – alpha thalassemia

Black – Sickle Cell Anemia

4) Nutrition :

Cow's milk – iron deficiency

Strict vegetarian – B 12 deficiency

Goat's Milk – Folate deficiency

Pica – Iron deficiency

5) Drugs :

G 6 PD deficiency (Primaquine)

Immune mediated Hemolysis

Bone marrow suppression

Phenytoin increases the folate requirement

6) Diarrhea :

Malabsorption – Iron, B 12 and Vit. E deficiency
Inflammatory bowel diseases
Milk protein allergy – due to blood loss
Intestinal resection : B 12 deficiency

7) H/o Chronic Liver disease or Renal Disease

8) Worm infestation : Hook worm, round worm.

Physical findings in evaluation of Anemia

System	Observation	Significance
Skin	Hyper pigmentation	Fanconi's Anemia, Dyskeratosis congenita.
	Café au lait spots	Fanconi's Anemia.
	Vitiligo	Vit. B 12 deficiency.
	Partial oculocutaneous Albinism	Chediak – Higashi syndrome.
	Jaundice	Hemolysis.
	Petechiae and Purpura	Bone marrow infiltration, Autoimmune Hemolysis with autoimmune Thrombocytopenia.
	Erythematous rash	Hemolytic uremic syndrome, Parvovirus B 19 and Epstein Barr virus infection.
	Butterfly rash	SLE.
Head	Frontal bossing	Thalassemia major, Severe iron deficiency Anemia, Chronic subdural haematoma.
	Microcephaly	Fanconi's Anemia.
Eyes	Microphthalmia	Fanconi's Anemia, Sickle Cell Disease.
	Retinopathy,Optic atrophy	Osteopetrosis.
	Blocked lacrimal gland	Dyskeratosis congenital.
	K.F. Ring	Wilson's disease.
Ears	Deafness	Osteopetrosis.
Mouth	Glossitis	B 12 and iron deficiency.
	Angular stomatitis	Iron deficiency.
	Cleft lip	Diamond- Blackfan syndrome.
	Pigmentation	Peutz-Jeghers syndrome. (intestinal blood loss)
	Telangiectasis	Osler-weber-rendu syndrome. (blood loss)
	Leucoplakia	Dyskeratosis congenita.

Chest	Shield chest or widely spaced nipples Murmurs	Diamond – Blackfan syndrome. Endocarditis.
Abdomen	Hepatomegaly Splenomegaly Nephromegaly or Absent kidney	Hemolysis, Infiltration, Tumour Haemangioma, Cholecystitis. Hemolysis, Sickle cell disease, Thalassemia, Malaria, Lymphoma, E.B. Virus, Portal Hypertension Fanconi's Anemia.
Extremities	Absent thumbs Triphalangeal thumb Spoon nails (koilonychia) Beau lines Mee's lines (nails) Dystrophic nails	Fanconi's Anemia. Diamond Blackfan syndrome. Iron deficiency. Heavy metal intoxication, Severe illness. Heavy metal intoxication, severe illness and Sickle cell Anemia. Dyskeratosis congenita.
Rectal	Haemorrhoids Heme positive stool	Portal hypertension. Intestinal Hemorrhage.
Nervous system	Irritable, Apathy, Peripheral neuropathy, Dementia . Ataxia (Posterior column involvement). Stroke.	Iron deficiency, B1 and B12 deficiency and Lead poisoning. Deficiency of vitamin B 12 and E B 12 deficiency, Sickle cell Anemia, Paroxysmal nocturnal Hemoglobinuria.
Small stature		Fanconi's Anemia.

INVESTIGATIONS FOR DIAGNOSIS OF ANEMIA:

There are variety of investigations for Anemia.

Method Of Blood Sample Collection: Blood sample is collected by venipuncture into the tubes containing anticoagulant.Most commonly used anticoagulants are Tripotassium or Disodium salts of Ethylene Diamine Tetra Acetic acid (EDTA),Trisodium Citrate , Heparin.[27]

AUTOMATED CELL COUNTER : [20]

They have advantages of greater precision and reproducibility and the capacity for completing a large number of measurements quickly. Two general principles are used in most of the popular cell counters.

A. Electrical impedence principle

B. Light scatter principle

A. Electrical impedence principle :

It is used in coulter counter. With this technique cells passing through an aperture cause changes in electrical resistance that are counted as voltage pulses which are proportional to cell volume. The electrical pulses are amplified and are counted during the time an

accurately measured volume of suspension is drawn through the aperture. These devices can directly measure the hemoglobin, MCV and calculate the hematocrit from MCV and RBC count. They also calculate MCH, MCHC, RDW. Coulter counter was used in this study.

B. Light Scatter Principle :

With this flow cytometric technique, RBC's first undergo isovolumetric sphering and then MCV and MCHC are measured.

Hemoglobin :

Hemoglobin is an intensely colored protein, which allows its measurement by a variety of colorimetric and spectrophotometric techniques. Hemoglobin is found in the blood in a variety of forms including oxyhemoglobin,carboxyhemoglobin, methemoglobin and othe minor components. These may be converted to a single stable compound, cyanmethemoglobin, by mixing blood with Drabkin solution, which contains potassium ferricyanide and potassium cyanide. The Absorbance of the cyanmethemoglobin is measured in a spectrophotometer at 540 nm and hemoglobin concentration determined.

Hematocrit (HCT) :

It is the proportion of the volume of the blood sample that is occupied by RBCs. It may be determined manually by centrifugation of the blood at a given speed and time in a glass tube. Now a days it is easily performed by automated cell counter.

HCT value of less than 34% is taken as cut off value for Anemia.

Red cell Distribution Width (RDW) : [5,20]

It is the Quantitative measure of RBC anisocytosis. The RDW will rise when the variation in cell size is greater than usual. It is a coefficient of width variation in RBC size. It is measured by automated cell counter.

Visual histogram of distribution of RBC size indicates how much variation there is in the size of the RBC. High RDW correlates with RBC size variations. Normal value of RDW is 11.5 to 14.5%. (moazon CM et al 1987) [33] . Increased RDW is an early finding in iron deficiency Anemia and most megaloblastic Anemia but not, in heterozygous thalassemia. RDW is very useful in differentiating between Iron deficiency Anemia and Thalassemia

minor [4]. In iron deficiency, RDW may be abnormal even before MCV falls below normal. In the first stage of Iron depletion, this is the only other parameter which is abnormal in addition to serum ferritin.

Patton WN, et al (1991) in their study on changes in Red cell volume and Heamoglobin concentration during phlebotomy induced iron deficiency and iron repletion, concluded that an elevated Red cell Distribution width was found to be the earliest hematologic manifestation of iron deficiency. [37]

Van zeben D et al (1990) in his study on evaluation of microcytosis using serum ferritin and Red cell Distribution width concluded that Red cell Distribution width was more sensitive in screening for iron deficiency than other parameters and that a low Mean Corpuscular volume with an associated increased Red cell Distribution Width strongly suggested presence of iron deficiency Anemia.

$$\text{RDW} = \frac{\text{Standard deviation of MCV}}{\text{Mean MCV}} \times 100$$

The RDW-CV is a ratio calculated as the width of the histogram at one standard deviation divided by MCV while the RDW-SD represents the width of the distribution curve at the 20% frequency level. The RDW may also be useful in monitoring the results of hematinic therapy for iron deficiency or megaloblastic Anemia.

RED CELL INDICES : [5,20]

Mean corpuscular volume : (MCV)

The average volume of the red cells is a useful red cell index that is used in classification of Anemias. The MCV is usually measured directly with automated instruments, but may also be calculated from the RBC count and hematocrit by means of the following formula.

$$\text{Mean corpuscular volume (average volume of the cells)} = \frac{\text{Hematocrit (L/L)x 1000}}{\text{Red blood cell count } (\text{x}10^{12}/\text{L})}$$

MCV is measured in femtolitres (fl or 10^{-15} litre). Mean value of MCV in preschool children is 81 fl. MCV less than 75 fl is taken as microcytic anemia.

Mean corpuscular hemoglobin :(MCH)

It is a measure of Hemoglobin content per red cell. It may be calculated by automated cell counters or manually by the following formula.

$$\text{Mean corpuscular Hemoglobin (average wt of Hb in red cells)} = \frac{\text{Hemoglobin (gm/dl)}}{\text{Red blood cell Count } (\times 10^{12}/\text{L})}$$

MCH is expressed in picograms (pg, or 10^{-12} g). MCH reflects the mass of heamoglobin. In Anemias, where hemoglobin synthesis is impaired ,hemoglobin mass per red cell decreases, leading to decreased MCH. The mean value of MCH in preschool children is 27 pg. MCH less than 24 pg is taken as hypochromic anemia.

Mean corpuscular Hemoglobin concentration (MCHC)

It is calculated by the following formula

$$\text{Mean corpuscular Hemoglobin concentration (average concentration of Hb in red cell)} = \frac{\text{Hemoglobin(gm/dl)}}{\text{Hematocrit (L/L)}}$$

MCHC is expressed in gms of Hemoglobin per dl of packed

RBCs or percentage. Mean value of MCHC is 34%. MCHC less than 31% is significant.

The Mean Corpuscular Hemoglobin concentration (MCHC) is the least useful of the indices and it is the least sensitive of the parameters in the diagnosis of iron deficiency Anemia (Dallman et al). [15,16]

Mentzer index : It is used to differentiate Iron deficiency Anemia and Thalassemia trait. [27]

$$\text{Mentzer Index} = \frac{\text{MCV in fl}}{\text{RBC in million/cu mm}}$$

If the value is more than 13, it is Iron deficiency Anemia. If it is less than 13, it is thalassemia trait.

EXAMINATION OF THE PERIPHERAL SMEAR :

Examination of peripheral blood smear is a diagnostic maneuver of over riding importance. It is atleast equal in importance and complementary to the machine generated hematologic data. It confirms the RBC size categorization of the coulter counter and permits recognition of many variations in size and shape that are frequently signposts of the types of hemolytic Anemia .

Diagnostic importance of Peripheral Blood Smear Examination in Childhood(Etiology wise)

No.	Etiology	Red cell morphology
1.	Aplastic, hypoplastic and aregenerative Anemia	Normochromic, normocytic RBC.
2.	Acquired Hemolytic Anemia	Moderate spherocytosis, marked Polychromasia, and Reticulocytosis.
3.	Hereditary spherocytosis	Spherocytes, Polychromasia, Reticulocytosis.
4.	Hereditary enzymopathies	Normocytic Normochromic RBC, occasionally Macrocytosis, Polychromasia Anisocytosis, Poikilocytosis, Spherocytes, Reticulocytes.
5.	Sickle cell Anemia	Sickle cells, Target cells, Anisocytosis, occasionally nucleated RBC, Reticulocytosis.
6.	Thalassemia major	Hypochromic microcytes, Poikilocytes, anisocytes and Reticulocytes, Target cells.
7	Thalassemia minor	Hypochromic microcytes, basophilic stippling, target and oval cells.
8.	Iron deficiency	Microcytic and hypochromic Anemia, Aniso poikilocytosis.
9.	Megaloblastic Anemia	Macrocytic, Normochromic, Anisopoikilocytosis, Reticulocyte count is low, nucleated RBC. Neutrophils are large with hyper segmented nuclei, more than 5% of cells have five or more nuclear segments

Diagnostic importance of Peripheral Blood Smear Examination and its Interpretation(Pathology wise)

No.	Red cell Morphology	Interpretation
1.	Normocytic Normochromic	Acute Hemolysis, Anemia of chronic disease, Malignancy, Recent blood loss.
2.	Hypochromic microcytic	Iron deficiency, Thalassemia, Lead poisoning, Vit.B6 deficiency, Sideroblastic anaemic, Chronic inflammation.
3.	Dimorphic Anemia	Iron deficiency , Folic acid def., B12 def .
3.	Macrocytic	Normal newborn, Folate or Vit. B12 deficiency
4.	Target cells	Thalassemia, Hb C, E and S, liver disease, Abetalipoproteinaemia, Post splenectomy
5.	Basophilic Stippling	Hemolytic Anemia, Iron deficiency Anemia, Thalassemia, Lead poisoning.
6.	Heinz bodies	Normal newborn, Hexose monophosphate shunt defect.
7.	Howell-Jolly bodies	Spleen hypofunction or absence, Megaloblastic Anemia
8.	Tear drop cells	Bonemarrow infiltration (Leukoerythroblastosis)
9.	Spherocytes	ABO incompatibility, G6 PD deficiency or other Hemolytic Anemia, Hereditary spherocytosis, Hypophosphatemia
10	Schistocytes	Thalassemia, Micro angiopathy (DIC)
11	Nucleated RBC	Normal for first several days, Hemolytic Anemia, Acute blood loss
12.	Stomatocytes	Hereditary stomatocytosis, liver disease.
13.	Siderocytes	Sideroblastic Anemia

SERUM FERRITIN

Seventy five percent of total body iron is present as Hemoglobin, myoglobin and heme enzymes. Remaining 25% of iron is stored as storage proteins ferritin and hemosiderin. Ferritin is water soluble and comprises the major portion of iron stores which is present in all tissues. Ferritin has a protein moiety, apoferritin and a polynuclear iron core which contains 25% of iron. [14,20]

Ferritin is normally present in the serum but in such small quantities that it was undetectable prior to the development of immunoassays. Under most circumstances the concentration of serum ferritin is roughly proportional to the abundance of storage iron. Thus the serum ferritin is the only blood determination that is helpful in evaluation of iron status within the normal range as well as in the diagnosis of iron deficiency. [13,18] High values in newborn infants reflect the abundant iron stores that exists at birth. Values fall rapidly during early infancy and remain low throughout later infancy and childhood [42]. At all ages, serum ferritin value of less than 10 ng per ml in children indicates depletion of iron stores [28]. It

is measured by Enzyme Linked Immuno Sorbent Assay or Radio Immuno Assay.

SERUM IRON AND IRON BINDING CAPACITY

Almost all of the iron in the serum is bound to the iron binding protein transferrin. A disadvantage of the serum iron is the large biologic variability compared with the other laboratory tests. A low value (less than 30 ug per dl or 5.4 umol per L) is most likely to represent iron deficiency. [25,26]

The TIBC is less subject to biologic variations than the serum iron, but its analytic error is greater than that of serum iron. The normal range for the TIBC is 250 to 400 ug per dL. The transferrin saturation is calculated by dividing the concentration of serum iron and TIBC. In infants and children the corresponding value ranges between 12 and 16 percent depending upon the age.

RETICULOCYTE COUNT :

Reticulocytes are 1-2 days old RBC containing aggregates of ribosomes which are demonstrated by supra vital stains.

Absolute reticulocyte count is a better index of total production of young cells. It is calculated by multiplying the percentage of reticulocytes with RBC count. The upper limit of normal Reticulocyte count is 1,00,000 / microlitre. (Normal reticulocyte count is 50,000 to 1,00,000)

TESTS FOR HEMOLYSIS :

Indirect bilirubin, LDH, Plasma haptoglobin, hemopexin may be useful.

TEST FOR RBC SURVIVAL TIME :

RBCs tagged with the radio isotope Na_2^{51} CrO_4 are used to measure the half life of RBCs

HEMOGLOBIN ELECTROPHORESIS :

It is the movement of charged particles through an electrolyte when subjected to electric field. Cellulose acetate strips or Agar gel electrophoresis are very useful in hemoglobin electrophoresis. Hb F and Hb A2 are elevated in thalassemia major and intermedia.

BONE MARROW ASPIRATION CYTOLOGY STUDY :

It is used to differentiate between various types of leukemia and to assess iron stores.

X – RAY EXAMINATION :

Congenital Hypoplastic Anemia (Diamond – Blackfan syndrome)

Retarded bone maturation, Triphalangeal thumb, extra thumb, and hypoplasia of middle phalanx of fifth finger.

Fanconi's Anemia :

Upper extremities : Hypoplasia or aplasia of radial elements (Thumb, first metacarpals, radial carpal, radius), hypoplasia of middle phalanges, clinodactyly of fifth finger.

Hereditary Spherocytosis : Generalized marrow hyperplasia.

Sickle cell Anemia :

Marrow hyperplasia, Hand foot syndrome , Osteo myelitis of long bones, infarction, aseptic necrosis of vertebral column and Osteoporosis.

Thalassemia major and intermedia :

Severe manifestations of marrow hyperplasia, localized radiolucencies, skeletal dwarfism, premature fusion of epiphysis, (Humerus, femur, tibia, fibula), Severe hair-on-end changes, Widening of Diploe spaces of skull, thinning of outer table, coarse trabecular pattern in medulla, inhibition of pneumatization of maxillary sinuses, maxillary overbite, ocular hypertelorism, and malocclusion, notching of margins, bulbous extension of posterior rib ends and demineralisation of vertebrae, pathological fractures.

Leukemia :

Destruction of spongiosa, erosion of cortex, periosteal elevation, osteoporosis, Moth eaten appearance, cystic rarefaction, transverse zones of diminished density in metaphysis.

AIMS OF THE STUDY

1) To study the Etiology and pathology of Anemia in preschool children (3 to 6 years), admitted in Institute of Child Health and Research Centre ,Government Rajaji Hospital ,Madurai.

2) To analyse the Red cell Distribution Width value in various types of Anemia.

3) Diagnostic correlation between Red cell Distribution Width value and Red Blood Cell morphology in Peripheral smear.

4) To analyse the importance of various red cell indices in the diagnosis of various types of Anemia and their correlation with RBC morphology in peripheral smear.

MATERIALS AND METHODS

Study Population :

Children attending inpatient services at Institute of child Health and Research Centre, Government Rajaji Hospital, Madurai.

Study Group :

A total number of 100 cases of Anemia were studied. Our cases consist of in-patients in the age group of 3-6 years (preschool children) in the Institute of Child Health and Research Centre , Government Rajaji Hospital, Madurai, who were admitted for evaluation of Anemia. This hospital serves as a referral centre for most of South Tamilnadu.

Study Period :

January 2004 to June 2005

Study Design :

Observational Study

Collaborating Department :

Department of Pathology, Government Rajaji Hospital, Madurai.

Inclusion Criteria :

The criteria used in the selection of children was those whose Hemoglobin less than 11 gm % based on WHO recommendation in the age group of 3-6 years.

10.0 – 10.9 g / dl	-	mild Anemia
7 - 9.9 g / dl	-	moderate Anemia
< 7 g / dl	-	severe Anemia

Exclusion Criteria :

We have excluded the cases, who were already diagnosed elsewhere, who were on treatment prior to admission to hospital, cases of Anemia due to chronic infections and chronic renal failure, chronic liver disease and also the children whose age is away from 3-6 years.

Ethics committee approval was obtained.

Methods :

All the 100 cases of Anemia were studied by taking detailed history and thorough clinical examination with meticulous care and the findings were recorded in the predesigned proforma annexed in the last few pages. The cases were evaluated with the following investigations.

1. Automated Blood cell counter:

One ml of blood was collected by venepuncture into bottles containing Ethylene Diamine Tetra Acetic acid (EDTA) solution and transported immediately to laboratory.

In this study, **COULTER^{R} Ac.T. DIFF ^{TM} Analyzer, SUN Diagnostics,** type of automated cell counter was used. This coulter counter uses the principle of electrical impedence. It requires 12 microlitres of blood and requires no manual assistance for suction of blood and loading. The following parameters were measured in this cell counter.

a) Hemoglobin

b) Hematocrit

c) MCV (Mean corpuscular volume)

d) MCH (Mean corpuscular Hemoglobin)

e) MCHC (Mean corpuscular Hemoglobin concentration)

f) RDW (Red cell distribution width)

g) RBC count

h) Total WBC count

i) Platelet count

2. Peripheral Blood smear:

Blood smears were prepared on glass slides by wedge method. A drop of capillary blood is placed in the middle of the slide about 1 to 2 cm from one end. A second spreading slide is placed at 30 to 45^{o} angle and moved forward. This smear is air dried, stained with Leishman's or Wright's stain, and examined under the microscope.

3. Reticulocyte count, osmotic fragility

4. Serum Ferritin:

Serum Ferritin was estimated by Solid phase Enzyme linked Immuno sorbant assay (ELISA) using **Genix EIA** Ferritin Kits of **Genix Technology, Vancouver, Canada.** This principle used the specific monoclonal antiferritin antibodies.

5. Hemoglobin Electrophoresis

In this study, **Agarose Gel Electrophoresis** was used.2 ml of blood added with EDTA solution was used.

6. Bone marrow Aspiration Cytology

Bone marrow is semi fluid and easily aspirated through a 18 gauge size bone marrow needle. It is usually taken from posterior

iliac crest. The aspirated material is made into smears and stained with Leishman's or Wright's stains and examined under the microscope.

7. Tests for Hemolysis (Indirect Bilirubin)
8. Stool – ova, cyst
9. Skull x ray
10. Serum protein

All the above investigations except Red cell indices, RDW, Serum ferritin, Hb Electrophoresis were done here. Red cell indices, RDW were done for all 100 patients at Madurai Kidney centre, Madurai. Serum Ferritin was done for only 10 patients, Hb electrophoresis for only 10 patients at Meenakshi Mission Hospital, Madurai, for want of facility. Serum levels of Folic acid and vitamin B 12 were not measured because of non availability.

Statistical Analysis :

Computer analysis of data utilizing the software – Epidemiological Information Package – 2002 (Epi Info 2002) – developed by the Centers for Disease Control and Prevention, Atlanta for World Health Organization.

Range, Mean, Standard deviation and 'p' values were calculated using this package.

Mean Values and Lower limits of the various parameters used in this study for preschool children (3-6 years)

Developmental changes affect hematocrit and various red cell indices [27]. Hence, normal values for different age groups is important. The cut off value of red cell indices used in this study are according to the definition ie. 2 SD below the normal mean for that age and sex. (F.A.Oski 1998, Dallman). [20]

S.No.	Parameters	Mean value	Lower limit (< 2SD)
1.	Hemoglobin	12.5gm %	11.0 gm %
2.	Hematocrit	37 %	34 %
3.	MCV	81 fl	75 fl
4.	MCH	27 pg	24 pg
5.	MCHC	34%	31 %

6). RDW : Normal 11.5 % - 14.5%. More than 14.5% is taken as elevated RDW.

7). Serum ferritin < 10 ng / ml is taken as the cut off value.

OBSERVATION , ANALYSIS and RESULTS

TABLE – 1

Incidence of Anemia in relation to Sex

Sex	No.of case	%
Male	52	52
Female	48	48

Of 100 children, 52were males,48 were females.

There did not seem to be significant difference in the incidence of Anemia among both sex. ($p > 0.05$)

TABLE - 2

Severity of Anemia

S.No.	Degree of Anemia	Severe (<7 gm%)	Moderate (7-9.9gm%)	Mild (10 – 10.9gm%)
1.	Number of cases	42	38	20

Of the above analysis, 42 cases of severe Anemia, 38 cases of moderate Anemia, 20 cases of mild Anemia were documented. Since this is a hospital based study, number of severe Anemia cases is more.

The mean value of Hb in this study is **6.98 (± 2.65) gm%**

TABLE - 3

Relationship between Sex and Severity of Anemia

Degree of Anemia	SEX		Total
	Male	Female	
Mild (10-10.9 gm%)	11	9	20
Moderate (7-9.9 gm%)	21	17	38
Severe (< 7 gm %)	20	22	42
Total	52	48	100

There is no statistical difference between Sex and Severity of Anemia. 'p' = 0.4392 (not significant).

TABLE - 4

Etiology of Anemia

Etiology	Number of cases	Percentage
1.Nutritional(Iron)Deficiency Anemia	83	83%
2. Acute Lymphoblastic leukemia	8	8%
3. Thalassemia	6	6%
4. Refractory Anemia (Myelodysplastic syndrome)	2	2%
5. Non Hodgkin's Lymphoma	1	1%
Total	100	100%

From the above analysis, Nutritional deficiency Anemia is the most important cause constituting about 83%. Acute Lymphoblastic Leukemia comprises of 8%, Thalassemia is responsible for 6%, refractory Anemia of about 2% and Non Hodgkin's Lymphoma of 1%.

TABLE - 5

Relationship between Etiology and Sex

Etiology	SEX	
	Male (52)	Female (48)
1.Nutritional (Iron) Deficiency Anemia (83)	40	43
2. Acute Lymphoblastic leukemia (8)	5	3
3. Thalassemia (6)	5	1
4. Refractory Anemia (2)	1	1
5. Non Hodgkin Lymphoma (1)	1	-

p value is 0.6129 (not significant)

There is no statistical significance between etiology and sex.

From the above analysis, among the 83 cases of nutritional deficiency Anemia, 40 cases were male children, 43 cases were female children. Among the 8 cases of ALL, 5 cases were male children, 3 cases were female children.

TABLE - 6

Relationship between Etiology and severity of Anemia

Etiology	Severity of Anemia					
	Mild		Moderate		Severe	
	No.	%	No.	%	No.	%
Nutritional (Iron) deficiency Anemia (83)	20	24.1	38	44.6	25	31.3
Acute Lymphoblastic Leukemia (8)	-	-	-	-	8	100
Thalassemia (6)	-	-	-	-	6	100
Refractory Anemia (2)	-	-	-	-	2	100
Non Hodgkin's lymphoma (1)	-	-	-	-	1	100

'p' = 0.0031 (significant)

There is a statistical significance between the Etiology and severity of Anemia in this study.

From the above analysis, all the 17 cases of non-nutritional deficiency Anemia like ALL, Thalassemia, Refractory Anemia, Non Hodgkin's Lymphoma had severe degree of Anemia.

Of the nutritional Anemia, 25 cases had severe Anemia, 38 cases had moderate degree of Anemia, 20 cases had mild Anemia.

TABLE - 7

Relationship of Nutritional deficiency Anemia

With Protein Energy Malnutrition (PEM)

Grading of P.E.M	Grade 1 (71-80%)	Grade 2 (61-70%)	Grade 3 (51-60%)	Grade 4 (< 50%)	Normal Nutrition (>80%)	Total
No. of cases	25	31	8	6	13	83

According to Indian Academy of Pediatrics classification, 70 out of 83 cases (84.3 %) of nutritional deficiency Anemia had PEM. Grade I and II PEM was found in 56 children, Grade III and IV PEM found in 14 children. And still 13 children with nutritional deficiency Anemia were having normal nutrition. This indicates importance of micro nutrient deficiency in normal nutrition and lesser Grades of Protein Energy Malnutrition.

Of the 83 cases of iron deficiency Anemia, Pica was found in 40 cases, Ankylostoma ova was found in 7 cases, Ascariasis ova found in 1 case, Entaemoeba histolytica cyst found in 5 cases.

TABLE –8

Relationship between Severity of Nutritional deficiency Anemia and Protein Energy Malnutrition.

Severity of Anemia	Normal > 80%	Grade I 71-80%	Grade II (61-70%)	Grade III (51-60%)	**Grade IV** (< 50%)	Total
Mild (10-10.9 gm%)	8	8	4	-	-	20
Moderate (7-9.9 gm%)	5	13	20	-	-	38
Severe (< 7 gm%)	-	4	7	8	6	25
Total	13	25	31	8	6	83

From the above analysis, 12 out of 20 (60%) cases of mild degree Anemia due to Nutritional (Iron) deficiency had first and second Grade PEM, 33 out of 38 (86.8 %) cases of moderate degree Anemia due to Nutritional (Iron) deficiency had First and Second Grade of PEM. Where as 11 out of 25 cases (44 %) of severe degree Anemia had First and Second Grade of PEM. 14 cases of severe Anemia had Third and Fourth Grade of PEM.

Still 13 patients (8 mild, 5 moderate Anemia) had normal nutrition.

TABLE-9

Relationship between Diet history and Nutritional deficiency anemia

Diet	Number of cases(83)	Percentage
Cerebral based	83	100
Occasional Non vegetarian food once / week	45	54.2
Tea / Coffee	74	89.1
Greens	42	50.7

The diet was cereal based in all 83 cases (100%) of nutritional deficiency Anemia. 45 children (54.2%) used to take non vegetarian food occasionally (once in week). 74 children (89.1%) used to take tea / coffee daily. Only 42 children (50.7%) were taking greens daily or once in two days. 17 children (20.5%) had normal protein and calorie intake. 66 cases(79.5%) had significant calorie gap and protein gap.

TABLE -10

Relationship between socio economic status of the parents and Etiology of Anemia

	Class V(51)	Class IV(43)	Class III(6)
Nutritional (Iron) deficiency Anemia (83)	45	36	2
Acute Lymphoblastic Leukemia (8)	3	2	3
Thalassemia (6)	2	3	1
Refractory Anemia(2)	1	1	1
Non Hodgkin's lymphoma(1)	-	1	-

Socio economic status of the parents of the children were analysed according to modified Kuppuswamy scale. In this study 51 cases(51%) were in class V (lower), 43 cases (43%) were in class IV (upper lower), 6 cases (6%) were in class III (Lower middle).

TABLE - 11

Peripheral Blood smear (Pathological) Evaluation of Anemia :

Type of Anemia	No.of cases	%
Microcytic, hypochromic	44	44%
Dimorphic	40	40%
Normocytic, Normochromic	15	15%
Macrocytic	1	1%
Total	100	100%

From the above chart, 44 cases had microcytic, hypochromic picture in their peripheral blood smear, 40 cases had Dimorphic picture (Both microcytes and normocytes), 15 cases had normo chromic normocytic, 1 case had macrocytic picture. Commonest types of Anemia in peripheral smear in this study were Hypochromic, microcytic (44%) and Dimorphic (40%).

TABLE 12

Relationship between Pathology and Sex

Pathology	SEX	
	Male (52)	Female (48)
Microcytic, hypochromic (44)	30	14
Dimorphic (40)	13	27
Normocytic, Normochromic (15)	8	7
Macrocytic (2)	1	1

p value is 0.0087 (significant)

There is a statistical significance between Pathology and Sex.

From the above analysis, among the 44 cases of hypo chromic and microcytic Anemias, 30 cases were male children, 14 cases were female children. Among 40 cases of dimorphic Anemia, 13 cases were male children, 27 cases were female children.

TABLE - 13

Relationship between Pathology and severity of Anemia

Pathology	Severity of Anemia					
	Mild		Moderate		Severe	
	No.	%	No.	%	No.	%
Microcytic Hypochromic (44)	11	25	16	36.4	17	38.6
Dimorphic (40)	7	17.5	20	50	13	32.5
Normocytic normochromic (15)	2	13.3	2	13.3	11	73.4
Macrocytic(1)	-	-	-	-	1	100

'p' = 0.094 (Not significant)

There is no statistical significance between pathology and severity of aneamia.

From the above analysis, 17 cases (38.6%) of microcytic, hypochromic, 13 cases (32.5%) of Dimorphic, 11 cases (73.4%) of normocytic normochromic Anemia had severe degree of Anemia.

16 cases (36.4%) of microcytic hypochromic, 20 cases (50%) of Dimorphic Anemia had moderate degree of Anemia.

TABLE - 14

Relationship between Etiology and Pathology

Etiology	Pathology							
	Microcytic Hypochromic (44)		Dimorphic (40)		Normocytic Normo chromic (15)		Macro cytic (1)	
	No.	%	No.	%	No.	%	No.	%
Nutritional deficiency Anemia (83)	40	48.2	39	47	3	3.6	1	1.2
Acute Lymphoblastic Leukemia (8)	1	12.5	-	-	7	87.5	-	-
Thalassemia (6)	2	33.3	1	16.7	3	50	-	-
Refractory Anemia (2)	1	50	-	-	1	50	-	-
Non Hodgkin's lymphoma (1)	-	-	-	-	1	100	-	-

'p' = 0.0001 (significant)

There is statistical significance between the Etiology and pathology in this study.

From the above analysis, of the 83 cases of Iron deficiency Anemias, 40 cases (48.2 %) had hypochromic microcytic picture, 39 cases (47%) had dimorphic picture, 3 cases (3.6%) had normochromic, normocytic picture.

7 cases (87.5%) of Acute Lymphoblastic Anemia had normochromic normocytic picture.

So the commonest types of peripheral smear picture in iron deficiency Anemia were Hypochromic, microcytic and Dimorphic Anemia.

All the 39 cases of Dimorphic Anemia due to Nutritional deficiency were later turned out to be microcytic according to automated cell counter stressing the subjectivity of peripheral smear reporting and importance of automated cell counter.

In addition to above findings, target cells and tear drop cells seen in 9 cases of which 6 cases were thalassemia, 3 were nutritional deficiency Anemia.

RED CELL DISTRIBUTION WIDTH (RDW)

Red cell distribution width was measured in all the 100 children using automated cell counter. Those children with RDW values more than 14.5% were considered elevated which usually occurs in Iron deficiency Anemia. Totally 80 children had elevated RDW.

Mean RDW observed in this study was **20.1 (±5.6)%**

TABLE - 15

Relationship between Etiology and RDW

Etiology	Normal RDW (11.5%-14.5%) (n=20)		Elevated RDW (> 14.5%) (n=80)	
	No.	**%**	**No.**	**%**
Nutritional (Iron) Deficiency Anemia (83)	4	4.8	79	95.2
Acute Lymphoblastic Leukemia (8)	8	100	-	-
Thalassemia (6)	6	100	-	-
Refractory Anemia (2)	1	50	1	50
Non Hodgkin's lymphoma (1)	1	100	-	-

'p'=0.0001 (significant)

There is a statistical significance between Etiology and RDW in this study

From the above analysis, 79 out of 83 cases (95.2%) of nutritional deficiency Anemia had elevated RDW which is statistically significant. All the six thalassemia (100%) cases and eight ALL(100 %) cases had normal RDW.

TABLE - 16

Relationship between pathology and RDW

Pathology	Normal RDW (11.5% -14.5%) (n=20)		Elevated RDW (> 14.5%) (n=80)	
	No.	%	No.	%
Microcytic Hypochromic (44)	4	9.1	40	90.9
Dimorphic (40)	1	2.5	39	97.5
Normocytic normochromic (15)	15	100	-	-
Macrocytic(1)	-	-	1	100

'p'=0.0001 (significant)

There is a statistical significance between Pathology and RDW

RDW was elevated in 40 out 44 cases (90.9%) of Hypochromic microcytic Anemia which is statistically significant.

RDW was elevated in 39 cases, out of 40 cases (97.5%)of dimorphic Anemia which is also statistically significant.

RDW was normal in 15 out of 15 cases of normocytic nomochromic Anemia(100%) .

RED CELL INDICES :

For all the 100 children included in this study, Hematocrit, MCV, MCH, MCHC were done using automated cell counter.

The cut off values for Hematocrit and Red cell indices were used according to the recommendation by F.A. Oski which is 2SD below from the mean value for that particular age group.

Hematocrit (HCT) :

Accordingly, using the cut off value of 34% for HCT, all the 100 children had reduced HCT. Mean value of HCT observed in this study was **21.5 (± 6.8) %.**

Mean Corpuscular Volume (MCV):

Using the cut off value of 75 femto litres (75fl) for MCV, 80 children had reduced MCV,20 children had normal MCV.

Mean value of MCV observed in this study was **61.5 (± 11.4) fl.**

TABLE - 17

Relationship between Etiology and MCV

Etiology	Normal MCV (≥75fl)		Decrease in MCV (<75 fl)	
	No.	%	No.	%
Nutritional Anemia (83)	6	7.2	77	92.8
Acute Lymphoblastic Leukemia (8)	6	75	2	25
Thalassemia (6)	5	83.3	1	16.7
Refractory Anemia (2)	2	100	-	-
Non Hodgkin's lymphoma (1)	1	100	-	-

'p'=0.0001 (significant)

There is a statistical significance between Etiology and MCV in this study

From the above analysis, 77 out of 83 cases (92.8%) of Nutritional deficiency Anemia had reduced MCV only 6 cases (7.2%) had normal MCV.

6 out of 8 cases (75%) of ALL had normal MCV.

TABLE - 18

Relationship between pathology and MCV

Pathology	Normal MCV (≥75fl)		Decrease in MCV (<75fl)	
	No.	%	No.	%
Microcytic Hypochromic (44)	4	9.1	40	90.9
Dimorphic (40)	2	5	38	95
Normocytic normochromic (15)	14	93.3	1	6.7
Macrocytic(1)	-	-	1	100

'p'=0.0001 (significant)

There is a statistical significance between pathology and MCV in this study

From the above table, 40 out of 44 cases (90.9%) of microcytic hypochromic Anemia had reduced MCV.

38 out of 40 cases (95%) of dimorphic Anemia had reduced MCV. So they are microcytic in nature. It stresses the importance of automated cell counter in assessing the size of cell and also the subjectivity of peripheral smear reporting.

14 cases out of 15 cases (93.3%) of normocytic, normo chromic Anemia had normal MCV.

Mean Corpuscular Hemoglobin (MCH) :

Using cut off value of 24 pg for MCH to diagnose hypochromia, 78 children had low levels. 22 children had normal levels. Mean value observed in this study was **20 (± 5.2) pg.**

TABLE - 19

Relationship between Etiology and MCH

Etiology	Normal MCH (≥24pg)		Decrease in MCH (<24pg)	
	No.	%	No.	%
Nutritional (Iron) deficiency Anemia (83)	8	9.6	75	90.4
Acute Lymphoblastic Leukemia (8)	7	87.5	1	12.5
Thalassemia (6)	4	66.7	2	33.3
Refractory Anemia (2)	2	100	-	-
Non Hodgkin's lymphoma (1)	1	100	-	-

'p'=0.0001 (significant)

There is a statistical significance between Etiology and MCH in this study

From the above table, 75 out of 83 cases (90.4%) of nutritional (Iron) deficiency Anemia had reduced MCH. 8 out of 83 cases (9.6%) had normal MCH. 7 out of 8 cases (87.5%) of ALL had normal MCH.

TABLE - 20

Relationship between pathology and MCH

Pathology	Normal MCH (≥24pg)		Decrease in MCH (<24pg)	
	No.	%	No.	%
Microcytic Hypochromic (44)	4	9.1	40	90.9
Dimorphic (40)	3	7.5	37	92.5
Normocytic normochromic (15)	15	100	-	-
Macrocytic(1)	-	-	1	100

'p'=0.0001 (significant)

There is a statistical significance between Pathology and MCH. 40 out of 44 cases (90.9%) of microcytic hypochromic Anemia had reduced MCH.

37 out of 40 cases (92.5%) of Dimorphic Anemia had reduced MCH. All the 15 cases (100%) of Normochromic, normocytic Anemia had normal MCH.

Mean Corpuscular Hemoglobin Concentration (MCHC) :

Using the cut off value of 31% for MCHC, out of 100 children, only 34 children had low MCHC values. Of which, 31 children belonged to Iron deficiency Anemia group. MCHC was found to be the least sensitive of all the indices in diagnosing Iron deficiency Anemia.The mean value observed was **32.3 (± 4.5)%.**

Mentzer's Index :

Out of 83 cases of Iron deficiency Anemia, 74 cases had mentzer index more than 13.

Serum Ferritin :

Out of 100 children included in this study, Serum ferritin level was estimated only in 10 patients using the ELISA technique. Due to non-availability of ELISA kit in our hospital it could not be done in all patients.

All the 10 children who had hypochromic, microcytic Anemia, high RDW, Low MCV, Low MCH and Low MCHC were selected randomly to prove Iron deficiency. All the 10 cases had low ferritin

value (< 10 ng/ml), which confirmed Iron deficiency stage. The mean value was **8.13(±0.56) µg/L.**

Bone marrow examination :

It was done in 11 patients. 8 patients had ALL (L1-five cases, L2- 3 cases,) 2 patients had refractory Anemia(Myelo dysplastic syndrome stage I), 1 patient had Non Hodgkin Lymphoma

Lymph node Biopsy :

It was done in one patient which showed Non Hodgkin's lymphoma

Hemoglobin electrophoresis :

It was done in 10 patients. Six patients diagnosed to have thalassemia (4 – Thalassemia major, 2- thalassemia intermedia), 4 patients had normal study.

TABLE - 21

Mean Value of various Parameters obtained in this study

Parameter	Range	Mean	S.D.
Age	3 – 6	4.11	0.99
Hb (g/dl)	1.7 – 10.9	6.98	2.65
HCT (%)	6.3 – 34.8	21.5	6.8
RDW (%)	11.7 – 32.2	20.1	5.6
MCV (fl)	50.1 – 90	61.5	11.4
MCH (pg)	13.1 – 33.8	20	5.2
MCHC (%)	25.2 – 38.8	32.3	4.5
RBC (million /cu mm)	1 – 4.8	3.1	1.1
Platelet Count (lakhs / cu mm)	0.25 – 10.3	3.45	1.72
Sr. Ferritin (µg/L)	7.2 – 9.4	8.13	0.56

TABLE – 22

Mean Values of various parameters in relation to Etiology

Parameters	Etiology				
	Nutritional Anemia (83)	**Acute Lymphoblastic Leukemia (8)**	**Thalassemia (6)**	**Refractory Anemia (2)**	**Non Hodgkin's Lymphoma (1)**
Age					
Mean	4.05	4.63	4	4.25	5
S.D.	0.97	1.09	1.1	1.06	-
'p'	0.6136 (Not Significant)				
Sex					
Males	40 (48.2)	5 (62.5)	5 (83.3)	1 (50)	1 (100)
Females	43 (51.8)	3 (37.5)	1 (16.7)	1 (20)	- (0)
'p'	0.6129 (Not Significant)				
Hb					
Mean	7.32	5.65	4.93	4.9	5.4
S.D.	2.71	1.88	1.49	0.71	-
'p'	0.0001 (Significant)				
HCT					
Mean	22.37	18.36	15.43	17.4	18.7
S.D.	6.94	3.89	4.2	1.4	-
'p'	0.0001(Significant)				
RDW					
Mean	21.5	12.99	12.9	13.9	12.5
S.D.	5.1	0.7	0.7	3.1	-
'p'	0.0016(Significant)				
MCV					
Mean	58.3	76.4	78.1	76.4	80.2
S.D.	8.9	11.9	6.2	0.2	-
'p'	0.0001(Significant)				
MCH					
Mean	18.75	27.76	24.1	25.9	24.1
S.D.	4.53	3.33	3.74	0.7	-
'p'	0.0001(Significant)				
MCHC					
Mean	31.83	35.83	32.75	34.25	32.2
S.D.	4.66	2.79	3.61	3.04	-
'p'	0.0001(Significant)				
RBC					
Mean	3.39	1.69	1.52	2.05	2.4
S.D.	0.91	0.55	0.38	0.07	-
'p'	0.0191 (Significant)				
Platelet count					
Mean	3.87	0.52	2.55	2.1	0.7
S.D.	1.52	0.2	0.83	0.57	-
'p'	0.0001(Significant)				

There is a statistical significance between various red cell indices and the Etiology. ($p < 0.05$)

TABLE - 23

Mean values of parameters in relation to severity of anemia

	Mild		Moderate		Severe		'p'
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	
Hb	10.59	0.34	7.98	0.84	4.34	1.38	0.0001 (s)
HCT	30.56	1.28	23.92	2.43	14.98	3.95	0.0001 (S)
MCV	69.27	4.9	56.91	9.47	62.01	13.07	0.0004(S)
MCH	24.25	3.09	19.12	3.94	18.75	5.88	0.0001(S)
MCHC	35.6	1.92	32.75	4.86	30.22	4.08	0.0001(S)
RDW	16.09	1.11	22.1	5.68	20.11	5.93	0.0008(S)

There is a statistical significance between the various red cell indices and severity of Anemia. All the parameters tend to decrease with increasing severity of Anemia. The mean Hb values were 10.59 ($\pm$ 0.34) g % for mild, 7.98 ($\pm$0.84) g% for moderate, 4.34 ($\pm$1.38)g% for severe degree of Anemia.

TABLE - 24

Mean values of parameters in relation to sex

	Male		Female		'p'
	Mean	S.D.	Mean	S.D.	
Hb	6.74	2.66	7.23	2.65	0.3317 (NS)
HCT	21.09	7.11	21.94	6.43	0.494(NS)
MCV	62.33	11.64	60.65	11.13	0.8548(NS)
MCH	20.13	5.15	19.85	5.22	0.6786(NS)
MCHC	31.96	5.12	32.58	3.83	0.6089(NS)
RDW	19.75	5.93	20.39	5.31	0.5028(NS)

There is no statistical significance between various red cell indices and the sex.

TABLE - 25

Mean values of parameters in relation to PEM

	Grade I		Grade II		Grade III		Grade IV		'p'
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Hb	8.74	1.84	7.13	1.73	3.44	1.17	2.93	1.19	0.0001(S)
HCT	25.77	4.7	22.33	4.48	12.4	3.4	10.72	3.49	0.0001(S)
MCV	64.41	11.45	55.69	7.87	52.29	1.37	51.82	1.41	0.0009(S)
MCH	21.95	5.02	18.17	4.13	13.76	0.72	13.85	0.58	0.0001(S)
MCHC	34.07	7.91	32.01	3.23	27.1	1.94	26.8	1.54	0.0001(S)
RDW	19.1	4.64	23.51	5.58	24.4	1.14	24.35	1.9	0.0001(S)

There is a statistical significance between the severity of PEM and various red cell indices.

DISCUSSION

1. Etiopathological study of Anemia, usefulness of automated cell counter, RDW, various red cell indices and their significance in diagnosing various types of Anemia in 100 preschool children admitted with Anemia were analysed and discussed here.

2. In the present study of Anemia, the incidence of Anemia is more common in male (52%) than female children (48%). But this is not statistically significant. This is in accordance with the fact that sex plays important role in determining prevalence only in older age group.

3. Regarding the Etiology of Anemia, the commonest is Nutritional (Iron) deficiency Anemia. Its contribution to childhood Anemia is 83% in this study.

According to ICMR study (V.P. Choudry et al in 1995), Iron deficiency was the commonest cause of Anemia in preschool children. [16]

William K. Simmons et al in 1982's survey in preschool children found Iron deficiency was the

commonest cause of Anemia [45]. Where as Angom Bisharda et al[2] reported 60% in the year of 1996. [1]

The remaining 17% cases in this study constituted Acute Lymphoblastic Leukemia, Thalassemia, Refractory Anemia (MDS), Non Hodgkin's Lymphoma.

4. Prevalence of PICA in Nutritional (iron) deficiency Anemia was 40% in this study . It is not clear which is the primary cause. Associated socio economic problems such as neglect, child abuse may be responsible for pica (Crosby et al study on food pica and iron deficiency Anemia 1971)

5. As for parasitic infestation, in this study, 13 children had parasites in their stool of which 7 cases had Ankylostoma ova, 1 case had Ascariasis ova, 5 cases Entaemoeba histolytica cysts.

   K. Ahmad et al in his study reported that 90% of the Anemia children had helminths. In another study from Bolivia, 79% had Ascariasis, 12% had hookworm.

The low prevalence of parasitism in our study may be because of wide spread use of anti helminthic drugs by health officials and poor detection rate.

6. Nutritional Status

Protein Energy malnutrition was found in 70 cases out of 83 cases of Nutritional (iron) deficiency Anemia (84.3%). Grade I and II PEM was found in 56 children. Grade III and IV PEM found in 14 children. 13 children with Nutritional (iron) deficiency Anemia had normal nutrition stressing the importance of micronutrient deficiency in apparently healthy children. The severity of Anemia increases when the grade of the PEM worsens. This is plausible, because calorie deficient children are likely to be deficient in other micro nutrients like iron.

A study conducted by Morzon et al in Filipino children between 9 months to 7 years found a strong association between Anemia and nutritional status.

The study of Anemia & under nutrition among pre school children by Shally Awasthi et al, had also shown direct relationship between Anemia and malnutrition [41].

Regarding diet in this study, all 83 children with nutritional deficiency Anemia had cereal based diet. 45 children used to take non vegetarian food (meat) occasionally though it is one of best source of Iron. 74 children (89.1%) used to take coffee/ tea daily which is known to reduce Iron absorption from food. Only 42 children (50.7%) were taking greens daily. 66 cases (79.5%) had significant calorie gap and protein gap.

7. Socio economic Status :

In this study, 51 cases were in class V (lower), 43 cases were in class IV (upper lower), 6 cases were in class III (lower middle). Among nutritional deficiency Anemia cases, 45 were in class V, 36 were in class IV, 2 were in class III according to modified Kuppuswamy scale.

8. Degree of Anemia :

In our study, 42 % cases had severe Anemia, 38% had moderate Anemia and 20% had mild Anemia.

Out of 42 cases of severe Anemia, 25 cases had Nutritional (Iron) def. Anemia, remaining 17 cases had ALL, thalassemia, refractory Anemia, Non Hodgkin's Lymphoma.

The Mean Hemoglobin value was 6.98 (± 2.65)gm %. Lowest Hemoglobin found in this study was 1.7 gm%.

9. Pathological type of Anemia :

In the present study, 44 cases (44%) had hypochromic, microcytic, 40 cases (40%) had dimorphic Anemia, 15 cases (15%) had normocytic normochromic Anemia, 1 case had macrocytic Anemia.

Out of 44 cases of microcytic hypochromic Anemia, 11 cases (25%) had mild, 16 cases (36.4%) had moderate, 17 cases (38.6%) had severe degree of Anemia.

Out of 40 cases of dimorphic Anemia, 7 cases (17.5%) had mild, 20 cases (50%) had moderate, 13 cases (32.5%) had severe Anemia.

Out of 15 cases of normocytic normocromic Anemia, 11 cases (73.4%) had severe Anemia.

10. 79 cases out of 83 cases of Nutritional (Iron) deficiency Anemia had hypochromic, microcytic (40) or Dimorphic (39) picture in peripheral smear. Among 8 cases of ALL, 7 had normochromic, normocytic picture. Among 6 cases of thalassemia, 3 had normocytic, normochromic picture.

11. RDW is a measure of anisocytosis and the normal values range from 11.5% to 14.5%. Considering the values above 14.5% as high RDW, 80 out of 100 children had elevated RDW. The mean RDW observed in this study was 20.1 ($\pm$ 5.6)%.

RDW was elevated in 40 out of 44 cases of Hypochromic, microcytic Anemias, 39 out of 40 cases of Dimorphic Anemia.

RDW was elevated in 79 out of 83 cases of Nutritional (Iron) Deficiency Anemia. RDW was normal in ALL, thalassemia, NHL.

RDW is considered as an early indicator to get elevated in Iron deficiency Anemia (F.A. oski and Bessman) in addition to Serum ferritin.

Similar observations were made by many other people. Patton WN et al (1991) in their study on changes in RBC volume and Hemoglobin concentration during phlebotomy induced iron deficiency and iron repletion concluded that an elevated RDW was found to be earlier indicator of Iron deficiency. [37]

Van zeben D et al (1990) in his study concluded that RDW was more sensitive in screening for iron deficiency than any other parameters and that low MCV and high RDW strongly suggested Iron deficiency Anemia.

Mahu JL et al (1990) in his study on usefulness of RDW in association with various redcell indices showed best correlation between RDW and various redcell indices in Iron Deficiency Anemia [31].

12. In our study, all the 100 children had reduced Hematocrit < 34%. The mean heamatocrit value is 21.5 ($\pm$ 6.8)%

13. In our study, 77 out of 83 cases (92.8%) of Nutritional (Iron) deficiency Anemia had low MCV (<75fl) 6 cases of Iron deficiency Anemia had normal MCV.

38 out of 39 cases (95%) of Dimorphic Anemia diagnosed by peripheral smear had low MCV, stressing the importance of automated cell counter in measuring the size of Red blood cells. The mean value of MCV in this study 61.5 (± 11.4) fl.

14. In our study, Low MCH values obtained in 78 children. 90.4% of Nutritional (Iron) deficiency Anemia had low MCH. 37 out of 39 cases (92.5%) of Dimorphic Anemia had low MCH. The Mean value of MCH in this study 20 (± 5.2)pg

15. In our study Hematocrit and MCV, MCH were very useful in diagnosing Nutritional (Iron) deficiency Anemia to a greater Grade of accuracy. According to the diagnostic scheme proposed by Dallman et al (1977) using MCV as a starting point, Low MCV with Anemia is almost always Iron deficiency Anemia.

Herschko C et al (1981) in his study showed that there is high correlation co-efficient between MCV, MCH, Hb and Iron deficiency Anemia. 97% of Iron deficiency Anemia could be identified by MCH and MCV [24].

In another study by Piedras J et al (1981), MCH and MCV showed increased sensitivity in diagnosing Iron deficiency Anemia [38] .

16. MCHC was found to be least sensitive indicator of Iron deficiency Anemia since it showed great deviation from other parameters.

17. In Iron deficiency Anemia and heterozygous B thalassemia, peripheral blood smear is often microcytic hypochromic. Unless Hb electrophoresis is done, the diagnosis cannot be arrived at. But Hb electrophoresis is costly, time consuming, and not available in all centres, but RDW can easily differentiate Iron deficiency Anemia from thalassemia. RDW is normal in heterozygous thalassemia.

18. Serum Ferritin was done only in 10 children for want of facility. In all the 10 cases, S. Ferritin was less than 10 ng/ml. Mean value is 8.13 ($\pm$ 0.56) ng/ml

    Ferritin is the most sensitive parameter of iron status and it serves as a good index of iron stores (Peter K Dall man).[13] Reduction of ferritin is the first stage of Iron deficiency when

other parameters except RDW were normal. (RDW is the other parameter which is useful in detecting Iron deficiency in the first stage)

But the estimation of is costly and not done in all centres and cumbersome, results are delayed.

In the study of evaluation of serum ferritin as an index of iron stores by David A. Lipschitz et al (1974) geometric mean value of 4 ng / ml was found [28].

In another study on the diagnosis of Iron deficiency Anemia in children using serum ferritin by Martin A. Simies, et al (1974), ferritin promised to be a useful tool in the evaluation of iron stores in the body.

19. Hb electrophoresis done in 10 patients. 6 patients had thalassemia (4- Thalassemia major, 2 thalassemia intermedia)
20. Bone marrow examination done in 11 patients. 8 patients had Acute Lymphoblastic Leukemia, 2 patients had refractory Anemia (Myelodysplastic syndrome Stage), 1 patient had Non Hodgkin's Lymphoma.

CONCLUSION

1. The most common cause of Anemia in preschool children is Nutritional (Iron) deficiency Anemia (83%).
2. Hypochromic microcytic (44%) and Dimorphic Anemia (40%) were the commonest findings in peripheral smear
3. Most of the children (80%) had moderate to severe Anemia
4. There was a good correlation between Iron deficiency Anemia and protein energy malnutrition. Anemia becomes severe with severe PEM.
5. There was a good correlation between MCV, MCH, RDW with the Etiology and pathology.
6. MCHC is the least sensitive parameter in diagnosing Iron deficiency anemia.
7. Red cell distribution width done with modern automated blood cell counters is an useful parameter to distinguish between iron deficiency Anemia and other types of Anemia.

8. Measurement of serum ferritin is costly, time consuming and cumber some though it is confirmatory. RDW overcomes all the above problems.
9. RDW is very helpful to decide the need for Hb electrophroresis in children with peripheral blood smear picture of hypochromic, microcytic Anemia.
10. RDW, various red cell indices, peripheral blood smear findings and clinical features are very important to diagnose various types of Anemia.
11. Other investigations like Bone marrow aspiration study, Lymph node biopsy, Hemoglobin Electrophoresis may be needed in some occasions.

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ETIOPATHOLOGICAL STUDY OF ANEMIA
IN PRE SCHOOL CHILDREN
(3 TO 6 YEARS OF AGE)

CASE SHEET PROFORMA

Name : Sex : Age :

Address :

Nationality / Religion :

Date of Admission :

Date of Discharge : Diagnosis :

C/o.
- Breathlessness Y/N
- Pallor Y/N
- Oedema Y/N
- Bleeding spots Y/N
-

H/o Present Illness :H/O Easy fatiguability Y/N, lack of concentration Y/N
H/O Fever Y/N, irritability Y/N
H/o reduced activities Y/N, lassitude Y/N
H/O Passing worms in stools Y/N , Pica Y/N
H/O Bleeding Y/N, chronic diarrhoea Y/N
H/O drug intake Y/N, prolonged vomiting Y/N

H/o. Past Illness : H/O Pica Y/N , passing worms in stools Y/N

Antenatal H/O

Natal H/O

Postnatal H/O

Development H/o

Family H/o

Immunisation H/O

Socio- Economic H/o

Contact H/O

General Examination :

Anemia	Y/N	Jaundice	Y/N
Cyanosis	Y/N	Clubbing	Y/N
Pedal Edema	Y/N	Lymphadenopathy	Y/N
Koilonychia	Y/N	Glossitis	Y/N
Cheilitis	Y/N	Frontal bossing	Y/N
Hemolytic facies	Y/N		

Vital Signs :	Pulse	R.R.
	B.P.	Temp.

Systemic Examination :

ABDOMEN:

C.V.S. :

R.S. :

C.N.S. :

INVESTIGATIONS

1. Automated complete hemogram
    a) Hemoglobin
    b) Hematocrit
    c) MCV (Mean corpuscular volume)
    d) MCH (Mean corpuscular Hemoglobin)
    e) MCHC (Mean corpuscular Hemoglobin concentration)
    f) RDW (Red cell distribution width)
    g) RBC count
    h) Total WBC count
    i) Platelet count

2. Peripheral Smear
3. Reticulocyte count, osmotic fragility
4. Stool – Ova and Cysts
5. Bone Marrow studies
6. Serum bilirubin
7. Serum proteins
8. Skull x ray
9. Serum ferritin level
10. Hb electrophoresis

LIST OF ABBREVIATIONS

ALL	-	ACUTE LYMPHOBLASTIC LEUKEMIA
CV	-	CO-EFFICIENT OF VARIATION
DA	-	DIMORPHIC ANAEMIA
DIC	-	DISSEMENATED INTRAVASCULAR COAGULATION
dl	-	DECILITRE
fl	-	FEMTO LITRE
G6PD	-	GLUCOSE 6 PHOSPHATE DEHYDROGENASE
Hb	-	HEMOGLOBIN
HC,MC	-	HYPOCHROMIC, MICROCYTIC
HCT	-	HEMATOCRIT
MC	-	MACROCYTIC
MCH	-	MEAN CORPUSCULAR HEMOGLOBIN
MCHC	-	MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION
MCV	-	MEAN CORPUSCULAR VOLUME
MDS	-	MYELODYSPLASTIC SYNDROME
NC, NH	-	NORMOCHROMIC, NORMOCYTIC
NDA	-	NUTRITIONAL (IRON) DEFICIENCY ANEMIA
ng	-	NANO GRAM ; µg - MICRO GRAM
NHL	-	NON HODGKIN'S LYMPHOMA
PEM	-	PROTEIN ENERGY MALNUTRITION
pg	-	PICOGRAM
RA	-	REFRACTORY ANEMIA
RBC	-	RED BLOOD CELL
RDW	-	RED CELL DISTRIBUTION WIDTH
SD	-	STANDARD DEVIATION
SLE	-	SYSTEMIC LUPUS ERYTHEMATOSUS
TI	-	THALASSEMIA INTERMEDIA
TM	-	THALASSEMIA MAJOR
WBC	-	WHITE BLOOD CELL
WHO	-	WORLD HEALTH ORGANISATION

MASTER CHART

S. No.	Name	Age (years)	Sex	Hb (gm%)	HCT (%)	RDW (%)	MCV (fl)	MCH (pg)	MCHC (%)	RBC (million / cumm)	Platelet (lakh/cumm)	Peripheral smear study	S. Ferritin ng/ml	Hb electropho	Bone Marrow cytology	Etiology
1.	Arun Pandiyan	3	M	2.7	10.7	23.4	53.5	13.5	25.3	2.00	5.46	HC, MC	-	-	-	NDA
2.	Nithya	3 ¼	F	1.7	6.3	24.1	51.8	14.0	27.0	1.21	3.45	HC, MC	-	-	-	NDA
3.	Naveen	5 ¼	M	7.1	20.4	20.4	53.1	18.5	34.9	3.84	44.83	HC, MC	-	-	-	NDA
4.	Chinnammal	3 ½	F	8.5	24.3	20.5	52.6	18.3	34.9	2.8	3.3	HC, MC	-	-	-	NDA
5	Karthik	4	M	10.4	30.0	16.7	64.5	23	34.4	4.2	2.7	HC, MC	-	-	-	NDA
6	Saranya	3 ½	F	7.4	24.2	32.2	51.1	15.7	30.7	4.74	4.68	HC, MC	-	-	-	NDA
7	Kalaivani	6	F	7.8	23.2	18.0	54.5	18.4	33.8	4.26	6.14	D A	-	-	-	NDA
8	Poongodi	4	F	10.9	30.5	15.9	67.2	24.1	35.8	4.4	3.2	D A	-	-	-	NDA
9	Muthupandi	6	M	10.2	30.7	17.4	81.8	29.7	36.4	3.75	3.38	HC, MC	-	-	-	NDA
10	Shajahan	3	M	2.9	10.2	23.4	50.7	14.6	28.8	2.01	2.49	HC, MC	8.6	-	-	NDA
11	Damodharan	4	M	5.3	18.2	25.7	51.6	15.0	29.0	3.53	5.40	HC, MC	8.0	-	-	NDA
12	Uma Mageswari	3	F	5.2	17.0	28.2	51.2	14.4	28.4	3.60	2.28	D A	7.8	-	-	NDA

13	Arun pandy	3	M	4.1	14.5	26.8	58.6	16.5	28.2	2.47	10.3	HC, MC	8.2	-	-	NDA
14	Sandhya	5	F	10.9	30.5	15.9	67.2	22.6	35.8	3.9	2.8	HC, MC	7.6	-	-	NDA
15	Sridharan	3	M	7.1	20.4	20.4	53.1	18.5	34.9	3.84	4.83	D A	-	N	-	NDA
16	Monika	5	F	7.4	24.2	32.2	51.1	15.7	30.7	3.7	3.4	MC H C	-	-	-	NDA
17	Ramya	4	F	10.0	29.5	17.5	72.4	29.3	37.3	3.75	3.85	HC, MC	-	-	-	NDA
18	Sathyan	5	M	10.7	30.1	16.8	73.6	30.6	38.8	3.82	4.26	MC HC	-	-	-	NDA
19	Thirukumaran	3 ½	M	9.2	30.5	16.6	60.2	20.4	32	3.6	4.2	MC HC	-	-	-	NDA
20	Meenakshi sundaram	3 ½	M	9.0	30.1	24.2	52.4	22.6	31.0	1.3	2.6	MC HC	-	-	-	NDA
21	Suresh	4	M	9.1	25.5	17.2	68.3	20.4	35.8	3.73	4.73	MC HC	-	-	-	NDA
22	Vaitheeswari	3 ½	F	2.7	10.7	23.4	53.5	13.5	25.3	2.00	5.46	D A	-	N	-	NDA
23	Karthik	5	M	10.9	30.5	15.9	67.2	21.1	35.8	2.4	3.4	HC MC	-	-	-	NDA
24	Pandimeena	6	F	9.8	26.8	12.6	76.8	28.1	36.6	3.48	3.65	NC NC	-	-	-	NDA
25	Rizwana	6	F	9.6	26.7	25.1	81.8	29.5	36.0	3.26	4.51	D A	-	-	-	NDA
26	Vignesh	5	M	10.4	30.8	13.7	76.6	28.4	37.1	4.02	3.86	NC, NC	-	-	-	NDA
27	Ajay	5	M	2.7	9.7	24.4	53.5	13.5	25.3	1.6	4.5	HC, MC	7.8	-	-	NDA
28	Sridharan	3 ½	M	7.1	21.4	19.4	53.4	18.5	34.9	3.84	4.38	DA	-	-	-	NDA
29	Eswaran	3	M	7.4	24.2	32.2	51.1	15.7	30.7	4.74	4.68	D A	-	-	-	NDA
30	Sanjay	3 ½	M	5.3	18.2	25.7	51.6	15	29.0	3.53	5.40	HC, MC	7.2	-	-	NDA

31	Dhinakaran	4	M	8.0	24.4	26.6	52.0	17.0	32.6	4.69	7.99	D A	-	-	-	NDA
32	Sheik mohamed	4 ½	M	10.3	31.3	15.7	69.6	23.2	36.2	4.49	5.06	HC, MC	-	-	-	NDA
33	Meega	4 ½	F	7.8	23.2	18.0	54.5	18.4	33.8	4.26	6.14	D A	-	-	-	NDA
34	Kaliraj	4	M	10.9	30.5	15.9	67.2	23.0	35.8	4.5	1.83	MC, HC	-	-	-	NDA
35	Fathima	4	F	7.7	24.5	21.9	54.5	16.8	28	2.4	43.0	HC, MC	9.4	-	-	NDA
36	Arunadevi	4 ½	F	7.1	20.4	20.4	53.1	18.5	34.9	3.84	4.38	D A	-	-	-	NDA
37	Salim	3	M	10.8	32.5	14.9	67.7	22.6	36.4	4.8	4.6	HC, MC	-	-	-	NDA
38	Muthu	3	M	10.9	34.8	16.1	65.2	20.1	37	3.7	4.0	HC, MC	-	-	-	NDA
39	Vijayaraj	4	M	9.4	25.8	14.8	73.6	23.6	36.2	3.51	3.2	D A	-	-	-	NDA
40	Muthuselvam	4	M	4.7	16.1	24.7	50.7	14.9	29.4	3.18	1.08	D A	-	-	-	NDA
41	Sivakumar	3 ½	M	10.9	30.5	15.9	67.2	22.1	35.8	4.01	2.8	D A	-	-	-	NDA
42	Sanjay	3 ¾	M	7.4	24.2	32.2	51.1	15.7	30.7	4.74	4.68	HC, MC	-	-	-	NDA
43	Vignesh	4	M	2.4	9.4	23.6	50.2	13.1	26.1	1.87	2.66	D A	-	N		NDA
44	Balasubramanian	4	M	7.1	19.8	21.2	53.1	18.5	34.9	3.84	4.38	HC, MC	-	-	-	NDA
45	Ragunathan	3 ½	M	8.5	24.3	20.5	52.6	18.3	34.9	2.6	3.2	D A	-	-	-	NDA
46	Sivakumar	3	F	5.3	18.2	25.7	51.6	15	29.0	3.53	5.40	D A	-	-	-	NDA
47	Shalmankhan	6	M	7.4	24.8	31.2	51.1	15.7	30.7	4.74	4.68	HC, MC	-	-	-	NDA
48	Salini	3 ½	F	10.3	30.0	17.4	64.4	22.6	32	2.8	3.4	D A	-	-	-	NDA
49	Nagarajan	6	M	4.2	12.4	25.5	50.6	13.1	29.4	3.2	0.53	MC HC	8.1	-	-	NDA

50	Sandhya	5	F	9.4	25.2	14	90	33.8	37.5	2.8	0.36	NC, NC			LB-2	ALL-L2
51	Saravanan	4 ½	M	4.9	17.1	24.5	51.7	14.8	28.6	3.3	2.4	HC, MC	-	-	--	NDA
52	Kannan	3	M	2.3	8.7	25.9	50.4	13.2	28.6	1.73	2.7	HC, MC	-	N	-	NDA
53	Kavitha	5	F	5.5	17.9	29.8	51.8	15.9	30.6	3.46	1.7	D A	-	-	-	NDA
54	Muthuselvi	4	F	7.1	22.9	20.7	59.0	18.4	31.2	3.89	1.29	D A	-	-	-	NDA
55	Abirami	3	F	4.5	20	23.3	52.4	13.8	26.5	3.26	4.72	D A	9.2	-	-	NDA
56	Alagurakku	4	F	10.1	29.6	14.0	76.8	28.0	37.4	3.96	4.43	NC NC	-	-	-	NDA
57	Gauthami	5	F	9.1	26.0	15.2	71.6	23.2	35.2	3.63	2.83	MC, HC	-	-	-	NDA
58	Kalidoss	5	M	2.7	10.7	23.4	53.5	13.5	25.3	2.00	5.46	HC, MC	7.5	-	-	NDA
59	Jeeva	3	F	10.9	30.5	15.9	67.2	23.4	35.8	4.0	2.8	D A	-	-	-	NDA
60	Vairava jothi	6	F	8.5	24.3	20.5	52.6	18.3	34.9	3.1	2.4	D A	-	-	-	NDA
61	Sneha	4	F	7.1	20.4	20.4	53.1	18.5	34.9	3.84	4.83	D A	-	-	-	NDA
62	Ganesan	3	M	6.0	17.3	19.3	76.1	22.7	34.8	2.27	0.52	HC, MC	-	-	-	NDA
63	Martin	3	M	7.4	24.2	32.2	51.1	15.7	30.7	4.74	4.68	D A	-	-	-	NDA
64	Vasanthakumari	4 ½	F	2.7	10.5	23.8	53.5	13.5	25.3	2.00	5.46	D A	-	-	-	NDA
65	Jeyakumari	3	F	7.8	23.2	18.0	54.5	18.4	33.8	3.2	2.8	HC, MC	-	-	-	NDA
66	Manimala	6	F	10.2	28.2	18.0	62.4	22.0	31.0	3.1	3.3	D A	-	-	-	NDA
67	Seethalaxmi	4 ½	F	8.0	24.4	26.6	52.0	17.0	32.6	3.76	2.42	D A	-	-	-	NDA

68	Yasar arafath	4 ½	M	5.3	18.2	25.7	51.6	15.0	29.0	3.53	4.4	MC	-	-	-	NDA
69	Muthuselvi	3 ½	F	8.5	24.3	20.5	52.6	18.3	34.9	2.9	3.0	D A	-	-	-	NDA
70	Mari selvi	3	F	10.9	30.5	15.9	67.2	23.8	35.8	4.2	2.8	D A	-	-	-	NDA
71	Lokeshwari	4	F	7.1	20.4	20.4	53.1	18.5	34.9	3.84	4.83	HC, MC	-	-	-	NDA
72	Vijayalakshmi	5	F	2.7	10.7	23.4	53.5	13.5	25.3	2.00	5.46	D A	-	-	-	NDA
73	Praveen	3 ½	M	7.7	2.6	21.9	52.0	16.8	29.0	3.4	2.7	D A	-	-	-	NDA
74	Gowri	3	F	7.4	24.2	32.2	51.1	15.7	30.7	4.74	4.68	HC, MC	-	-	-	NDA
75	Nagasurya	3 ½	F	5.2	17.9	22.4	50.1	14.7	29.3	3.57	2.66	D A	-	-	-	NDA
76	Murugeswari	5	F	7.8	23.2	18.0	54.5	18.4	33.8	34.4	3.2	D A	-	-	-	NDA
77	Karuppiah	3 ¼	M	8.2	24.0	17.6	56.0	20.2	32.0	3.20	2.5	D A	-	-	-	NDA
78	Suchithra	3	F	10.3	30.0	16.7	72.8	23.0	37.1	3.26	2.74	HC, MC	-	-	-	NDA
79	Karthika	5	F	7.1	20.4	20.4	53.1	18.5	34.9	3.84	4.83	HC, MC	-	-	-	NDA
80	Kameswari	3	F	5.3	18.2	25.7	51.6	15.0	29.0	3.53	5.4	D A	-	-	-	NDA
81	Muthubarathi	3	F	8.5	24.3	20.5	52.6	18.3	34.9	2.4	2.8	D A	-	-	-	NDA
82	Jasmin banu	3	F	10.9	30.5	15.9	67.2	22.4	35.8	3.8	2.6	D A	-	-	-	NDA
83	Devadarshini	3	F	2.7	10.7	23.4	53.5	13.5	25.3	2,00	5.46	D A	-	-	-	NDA
84	Abdul rahman	5	M	5.4	18.7	12.5	80.22	24.1	32.2	2.4	0.70	NC, NC	-	-	NHL	NHL
85	Balaji	5	M	3.2	11.2	13.0	56.2	22.4	29.8	1.2	0.56	NC, NC	-	-	LB-1	ALL-1

86	Manimegalai	5 ½	F	4.6	17.4	11.8	78.4	25.6	36.4	1.8	0.8	NC, NC	-	-	LB-1	ALL L1
87	Riyas mohammed	6	M	6.2	18.7	13.5	86.3	28.7	33.2	2.17	0.38	NC, NC	-	-	LB-1	ALL L1
88	Prithivi	5	M	5.6	20.2	12.6	60.4	26.0	34.7	1.40	0.25	NC, NC	-	-	LB-1	ALL
89	Kalimuthu	3	M	4.2	17.0	12.8	77.6	28.4	34.9	1.52	0.67	NC, NC	-	-	LB-2	ALL L2
90	Surya	3	M	5.3	18.2	12.5	82.4	29.6	38.8	1.14	0.42	NC, NC	-	-	LB-1	ALL
91	Kanimozhi	4 ½	F	6.7	19.0	13.7	79.6	27.6	37.3	1.40	0.76	NC, NC	-	-	LB-2	ALL
92	Anand	5	M	5.4	18.4	16.1	76.2	26.4	32.1	2.1	2.5	HC, MC	-	-	N	RA
93	Priyadarshini	3 ½	F	4.4	16.4	11.7	76.5	25.4	36.4	2.0	1.7	NC, NC	-	-	N	RA
94	Arjun	5	M	2.8	10.2	13.5	81.6	24.8	34.4	1.1	2.4	NC, NC	-	T M	-	T M
95	Alagan	3	M	5.7	18.6	12.6	80.4	26.2	36.2	1.8	1.8	NC, NC	-	T M	-	T M
96	Rohini	5	F	3.4	10.6	13.9	84.2	27.8	35.7	2.0	1.5	NC, NC	-	TI	-	TI
97	Arunpandiyan	5	M	6.5	19.4	12.4	76.5	20.4	29.2	1.4	2.7	MC, HC	-	TM	-	TM
98	Praveen kumar	3	M	6.0	18.8	11.9	66.6	18.6	27.4	1.7	3.2	MC, HC	-	TM	-	TM
99	Karthik	3	M	5.2	15.0	12.8	79.2	26.8	33.6	1.24	3.7	D A	-	TI	-	TI
100	Lakshmi	6	F	2.7	10.7	23.4	53.5	13.5	25.3	1.4	2.8	HC, MC	-	-	-	NDA