Evaluation Of Iron Deficiency Anaemia In Children: - An In Vitro Study.

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Abstracts: Background: Anaemia or anaemia is usually defined as a decrease in the amount of red blood cells (RBCs) or the amount of haemoglobin in the blood.[1,2] It can also be defined as a lowered ability of the blood to carry oxygen. There are three main types of anaemia; due to blood loss, due to decreased red blood cell production, and due to increased red blood cell breakdown. Methodology: The material for this study was obtained from children aged 6 months to 12 years. These were the children who either attended outpatient department or were those who were admitted to paediatrics ward of our hospital. Haemoglobin percentage was estimated in all cases diagnosed clinically as anaemic. Those, whose haemoglobin level was below 10gm percent, were taken to be anaemic for the purpose of the study. Results: The incidence of anaemia was high in children below 3 years of age. The maximum incidence was between 1-3 years. In older children anaemia was less frequently seen. The mean haemoglobin values were higher in younger children as compared to older children, the difference however, was not very significant (0.4gm%). Conclusion: Primary prevention of Iron deficiency Anaemia is recommended; the role of secondary prevention through screening programs remains inconclusive but recommended by some professional organizations. Treatment of children identified with IDA includes both dietary counselling and oral iron supplementation. [Parikh A NJIRM 2015; 6(4):6-13]

Key Words: Anaemia, Children, Iron Deficiency Anaemia, Prevention.

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Introduction: Anaemia or anaemia is usually defined as a decrease in the amount of red blood cells (RBCs) or the amount of haemoglobin in the blood.[1, 2] It can also be defined as a lowered ability of the blood to carry oxygen.[3]

There are three main types of anaemia, that due to blood loss, that due to decreased red blood cell production, and that due to increased red blood cell breakdown. Diagnosis in men is based on a haemoglobin of less than 130 to 140 g/L (13 to 14 g/dL) while in women it must be less than 120 to 130 g/L (12 to 13 g/dL).[4,5] Further testing is then required to determine the cause.

Anaemia is one of the most common disorders seen in infants and children and has been a subject of great clinical interest since long. Factors playing little or no part in the production of anaemia in adults have importance at an earlier age. Rapid growth, incomplete haemopoetic adjustments, and easy susceptibility to infection in this age period are important contributory factors.

The trends in paediatric haematology closely resemble those in adult haematology, but there is in addition the special problem of blood dyscrasians of the neonatal period and early infancy. The anaemia constitutes the major section of the blood disorders of infancy and childhood. Nutritional deficiency is accepted as a major factor in the production of anaemia in infants and children in India, where low economic status of general population and diet of poor standard are mostly responsible.[6]

It is now well recognized by the health services all over the world that, iron deficiency is probably the most common cause of anaemia.[7,8] Hundreds of such cases exist for every case of megaloblastic, haemolytic and aplastic anaemia. In India its prevalence is more than 50%. In tropic upto 80% children have anaemia. The commonest variety is iron deficiency anaemia. The unusual susceptibility of the infants is due to a variety of interrelated factors inherent in this age period, which affects iron metabolism.[9,10]

Causes of anaemia in the new-born are blood loss, decreased RBC production, and increased RBC turnover. Blood loss during delivery can result from a ruptured umbilical cord, placenta previa, and abruptio placentae.[11] Maternal-foetal transfusion occurs in 50 percent of all pregnancies, but usually does not because significant loss of blood volume.[12] The patient's history eliminates most of these causes. A normal reticulocyte count confirms that the infant's bone marrow is functional. These rules out causes of decreased RBC production, including Fanconi anaemia, Diamond-Blackfan syndrome, and congenital infections. Cranial haemorrhages are often associated with birth trauma, including vacuum and forceps delivery. In particular, subgaleal bleeds can be of sufficient volume to cause shock. Physical examination findings may include mental status changes, jaundice, tachycardia or tachypnea, and increased head circumference.[12] In this patient, a computed tomography scan confirms a subgaleal haemorrhage, and the infant is transferred to a neonatal intensive care unit for transfusion and monitoring. In newborns, an elevated bilirubin level in association with anaemia suggests haemolysis. If this infant's bilirubin level had been elevated, further testing would have included a Coombs test to evaluate for isoimmunisation (asin ABO or Rh incompatibility) and a peripheral smear to evaluate for spherocytosis or other RBC membrane defects. Testing for glucose-6-phosphate dehydrogenase (G6PD) deficiency should be considered if the patient's ethnicity or family history is a risk factor.[11]

In the study done in 2010, in India the results show that more than 95 % of children, adolescent girls and pregnant women suffer from anaemia. 97.8 % mildly anaemic and 2.9 % are severely anaemic. More than half of the pregnant women in the country are mildly anaemic and 42.6 percent are moderately anaemic. The situation is the worst for adolescent girls, 27.1 percent of whom are severely anaemic. This situation can be controlled and can be prevented upto some extent when care is taken during the childhood period of the individuals. Hence the present study was undertaken with the aim to find out the prevalence of iron deficiency anaemia and related factors in children. The study pertains to clinically anaemic children and not strictly of iron deficiency states. [13]

Material and Methods: The material for this study was obtained from 100 subject children aged 6 months to 12 years. These were the children who either attended outpatient department or were those who were admitted to paediatrics ward of our hospital. Amongst out patients this study also included this children who did not come to outpatient department for any complaints but were brought for routine immunization and were clinically found to be anaemic.

Haemoglobin percentage was estimated in all cases diagnosed clinically as anaemic. Those, whose haemoglobin level was below 10gm percent, were taken to be anaemic for the purpose of the study. For the purpose of this study, following points were considered to establish and label the cases as iron deficiency anaemia. Those we did not conform to iron deficiency pattern were excluded from the study.

Cases selected, conformed to below criteria were further studied in greater detail.

1. Presence of hypochromicity and microcytosis in the peripheral smear.
2. Hemogram showing a MCV less than 74u and HCHC less than 30%
3. a) Absence of marrow hemosiderin or
   b) Serum Iron less than 60 ug and Saturation of serum iron less than 16% taken as final proof of iron deficiency.

Particular emphasis was laid on eliciting history of premature birth, low birth weight and twin birth. The physical examination was directed to a careful search for signs of anaemic like pallor, koilonychias, oedema, cardiac enlargement, haemic-murmur, presence of hepatic and splenic enlargement and lymphadenopathy. Sites of haemorrhages and signs of malnutrition and infections were carefully looked into.

The following investigations were carried out.

1. <u>Blood:</u> Serum chemistry for iron was done in 88 out of 100 individuals
   - Hemoglobin percentage of blood
   - Total and differential leukocyte count
   - Packed cell volume
   - Erythrocyte sedimentation rate
   - Peripheral blood smears for morphology of red cells and malarial parasites.
   - Reticulocyte count
   - MCV, MCH and MCHC were calculated
   - Serum iron, total iron binding capacity and percentage saturation.

2. Bone marrow and hemosiderin:
   Bone marrow estimation was done in 60 out of 100 patients
3. Other tests: These were done as and when indicated
   - Stool examination for ova and cysts and parasites
   - Routine urine examination
   - Radiological examination of chest
   - Serum protein and electrophoresis
   - Blood urea
   - Liver function tests
   - Electro cardiogram

Estimation of haemoglobin was done by using Sahli's hemoglobinometer and the readings were recorded in grams percentage.

Peripheral smear was prepared and stained by leishman's stain for typing the anaemia. Packed cell volume was determined by centrifuging, uncoagualted blood in a wintrobe's haematocrit tube, at 2500 revolutions per minute for 30 minutes, when the corpuscles were packed down to a constant volume and the volume of packed cells was then expressed as percentage of the original column of blood.

Total leukocyte count was estimated by using pipette (WBC) and neubauer counting chamber and expressed in thousands per cm^2. Differential leukocyte count was done by studying the leishman's stained peripheral smear. Erythrocyte sedimentations rate was measured by using Wintrobe's method and was expressed in mm of blood, at the end of one hour. Reticulocyte count was done by using 1% brilliant cresyl blue stain. Total red blood corpuscle count was done by using haemocytometer pipette and Neubauer counting chamber and was expressed in millions per cm^2 of blood.

Bone marrow was aspirated by puncturing the iliac crest or sternum by means of a stout trochar and cannula fitted with an adjustable stopper and a hypodermic syringe under strict aseptic precautions. The smear was prepared out of the aspirated marrow and was studied for the reaction by staining the smear by Leishman stains. Bone marrow was studied for the presence of iron stores by staining the smear by the following technique.

Fix the smear for 20 minutes in absolute menthol and air dry the smear. Prepare 20% solution of potassium Ferro cyanide and add to it drop by drop concentrated hydrochloride acid till the thick white precipitate is formed, and filter and then centrifuge the solution till a clear filtrate is obtained. Heat the filtrate to 56^{o}c in a hot water bath and then cover the smear with this filtrate for half an hour. Then wash the smear thoroughly with distilled water. Then counter stain the smear with 0.1% safranin for one minute. Then wash off the smear with distilled water and then sir dry the smear. Then the smear is examined for the tissue iron (reticulo endothelial cell iron).

Serum Iron estimation:
Two ml of serum (free of haemolysis), 2 ml. of working iron standard and 2 ml. of iron-free water (as a blank) are placed, respectively in three separate iron-free test-tube. 2 ml. of protein precipitant (Trichloracetic Acid) are added to each. The contents are mixed vigorously and allowed to stand for 5 min. the tube containing the serum is centrifuged to obtain an optically clear supernatant. 2 ml of this supernatant and 2 ml of the other mixtures are added separately in separate tube 2 ml of chromogen solution with thorough mixing. After standing for 5 min the adsorption is measured in spectrophotometer against water 535 nm.

Calculation:-

$$\text{Serum iron (mg/L)} = \frac{A^{535}\text{test} - A^{535}\text{blank}}{A^{535}\text{standard} - A^{535}\text{blank}} \times 2$$

In our haematology department serum iron level was standardized and normal range is from 60 – 100 ug%. For TIBC excess iron, as ferric chloride, is added to serum. Any iron which does not bind to transferring is absorbed on to magnesium carbonate and removed. An iron estimation is then carried out on the iron-saturated serum (N. 270-300 ug %). Serum ferritin estimation and measurement of iron absorption was not done because of lack of facility.

Results: For assessing comparative severity of anaemia in various age groups, children were grouped under two ranges of haemoglobin that is 6-8 gm% and 8-10gm% levels. The incidence of anaemia was high in children below 3 years of age. The maximum incidence was between 1-3 years. In older children anaemia was less frequently seen. The mean haemoglobin values were higher in younger children as compared to older children, the difference however, was not very significant (0.4gm%)

There were more males (66%) in the present series than females (34%). The mean Hb. And the incidence in the lower and upper Hb. ranges was almost equal in the two sexes. Thus apart from higher incidence of iron deficiency anaemia, in the male, the severity of anaemia was not different in the two sexes.

Of the 100 cases studies, 70% cases were full term with normal weight. 18 cases were premature and 9 were small for date. 3 were twins. Of the three twins 2 were twin brother and sister and the third was one of the twins. The mean Hb. of 70% full term normal babies was definitely higher than the Hb. of premature, small foe dates and twins. The premature, small for dates and twins were grossly clinically anaemic. The maximum numbers of cases (44%) in the present study were first born children. The number of 2^{nd} born and 3^{rd} born children were same i.e. 22%. Only 12% cases were 4^{th} born or over. It is difficult to comment on the incidence of birth order in anaemia since this has to be seen in the light of such an incidence in the general population to arrive at a valid conclusion. The degree of anaemia was lower in the first born than in subsequently born children. Thus the severity of anaemia appeared to increase with the increasing birth order.

The duration of breast feeding in the present series, varied from 6 months to 3 years, the largest number being fed for 1 year. The mean Hb. levels showed a decline with prolongation of breast feeding so that those subsisting on the mother's milk for over two and half years recorded the lowest level of Hb. There was an increasing incidence of anaemia with later introduction of the solid in the diet. Highest amount of anaemic cases (69%) were recorded in the children in whom the solid food were started over 2-3 years.

In the present series, those children who were not given breast milk were fed on cow milk, buffalo milk or powder milk. Of 5 cases who received cow's milk, 4 received it in undiluted form and one in diluted form. Two received buffalo milk and in both the cases the milk was diluted. Powder whole milk was given to the seven children. In five of those the milk was prepared with water beyond the recommended quantity. In two cases it was given in full strength as recommended by the manufacturers on the tin. The mean Hb. values for the undiluted cow's milk and diluted buffalo milk were 7.8gm%, for those on diluted cow's milk and full strength powder milk it was 7.5gm% and for diluted powder milk it was 7.2gm%. Thus children fed on diluted buffaloes' or undiluted cow's milk recorded higher mean Hb. levels than those on diluted cow and powder milk – both full strength and diluted.

Thirty percent cases were vegetarian by choice; sixty percent were forced vegetarian as they could not afford to buy non vegetarian articles of food. Only 10 percent cases were non vegetarians. The daily diet of cases consisted of rice/roti, with dal/one seasonal vegetable. The non-vegetarian received fish, meat or egg in very small quantity very infrequently. Fruits were taken infrequently and in most occasions it was banana. The mean Hb levels did not differ significantly in the three groups.

To assess the Hb. level in relation to nutritional statue the children's weight was employed as a criterion for the latter. This was graded according to the percentage of the expected weight. A linear relationship was observed between the nutritional statue and the mean Hb level, as the weight increase in the individuals the Hb levels also increased in the individuals.

In the present study cases were in range of 6 to 10 gm% of the haemoglobin. The mean corpuscular haemoglobin concentration (M.C.H.C) ranged between 22% to 30%. The average M.C.H.C. was 27%. The mean corpuscular volume (M.C.V.) ranged between 50-73u. the average M.C.V. was 68u. serum iron was in the range of 30 to 60 ug% with an average of 40 ug%. Percentage saturation was below 16% with an average of 14%. Total iron binding capacity was increased with an average of

400 ug%. Peripheral smears of all the patients were showing microcytic-hypochromic. Bone marrow examinations of all patients were showing normoblastic hyperplasia with absence of hemosiderin granules.

Table 1: Showing age incidence & Severity of anaemia.

Age	Cases having Hb. 6-8gm%	Cases having Hb. 8-10gm%	Total No. of cases	Mean Hb. In gm%
Below 1 year	18	16	34	8.2
1-3 years	26	24	50	8.0
3-5 years	4	4	8	8.2
Over 5 years	2	6	8	7.8

Discussion:

Age: Among infants and children suffering from iron deficiency anaemia, included in this study, the severity did not vary with age although the incidence was highest among those below the age of 3 years. It is common experience and is borne out by the works of Patel et al. Gandagule et al. that anaemia is most frequent in the first two years of life. By and large, therefore, the general incidences are comparable with the present study. The higher incidence of iron deficiency anaemia in younger children can be mainly explained by a) Poor iron content of the toddler's diet due to delayed introduction of solids and poor quality of solids, milk being the main diet, that too is often diluted, b) Higher incidence of infections due to poor resistance and increased susceptibility.[14]

Sex: Most studies carried on anaemia in children indicate preponderance of male children, Man Chand & Hanna (1962) etc. the present study reveals similar findings. The higher incidence in the male may be attributed to one or several of the following reasons. a) More rapid growth in the male than females with consequent greater demand for iron (Woodruff), b) Preferential treatment of male children in India bring to the doctor's notice more males than females, c) To add to the above reasons is the well known fact from hospital statistics that a larger number of sick children belong to the male sex. [15]

Birth statue, Birth order & Family size: In the present study 70% infants were full term with normal weight. Their mean Hb was 8.5gm%. The 18% premature of the present series had a mean Hb of 7.5gm%, while the mean Hb of 9 small for the date infants and 3 twins were 7.5gm% and 7.4gm% respectively. The mean Hb values of premature, small for date & twins were defiantly below the expected mean Hb of full term normal weight babies.[16]

Defective antenatal storage of iron is the cause of anaemia in infancy. The heavier the child at birth the greater is the amount of blood available for destruction and iron storage. It has been estimated that as much as 250-300 gms of iron may be stored as the result of blood destruction during the first few days of life.

Development of IDA in the twins may be attributed to the small size at the birth or foeto-foetal transfusion. However in our study there was no evidence of foeto-foetal or foet-materanal transfusion. In premature and small for-date infants, it is due to the more rapid rate of growth which places additional burden on the small iron stores at birth.[17]

Forty four cases of the present study were first born. Another 44% were second and third born. Only 12% cases were 4th born or over. The degree of anaemia as judged by the mean Hb level was less severe in the first born than the 2nd, 3rd and 4th born.

Diet: Failure to establish lactation deprived 14 infants of breast milk in the present series. The remaining 86 were breast fed, nearly half the number (48%) were breast fed past the first year of life. Solida were not started before two years in 69% and when introduced where poor in quality and quantity. Even those children, who ate non-vegetarian diet, took meat, fish or eggs very infrequently not more than once in a few weeks. The solids consumed were therefore, mostly of vegetable origin. Thuas dietic patients leave hardly any doubt as to the cause of anaemia. Exclusive subsistence on breast feeding until late, with delayed introduction of solids appear to account for anemia.[18]

It was observed that during meal time the young child was served with bits of food routinely cooked for adult consumption, with which he played more and of which he ate little, unattended by the older members. A general pattern of feeding in India, especially in the low-socioeconomic group as observed by various Indian workers, like Achar, Gopalan, and Rao is that of breast feeding upto the age of one & half years on an average, and inadequate feeding with top milk and solids. They further noticed that milk was often diluted and solids fed were mostly of poor nutritional value, particularly in respect of iron and protein. Roti, rice and dal were the main items of diet with a little milk added occasionally.

Infant's upto three months may be in negative iron balance since the diet contributes little to the infants haemoglobin iron during first 3-4 months of age. [19]

Nutritional Status: Children in this study were distributed into weight groups on the basis of percentage of expected body weight. A linear relationship was seen between the nutrional status as determined by this parameter, and the severity of anaemia. This relationship was fairly consistent and indicates a close relationship between weight of the child, that is his nutrional status, and the haematological status as determined by the haemoglobin level.[17]

Clinical Signs: The table shows a comparison of various clinical signs observed by different workers. The other four studies include all types of anaemia in infants and children. Since it has been established that 80-90% of all anaemia's in infants and children are due to iron deficiency, so the present series could well be compared with the works of other authors.

The lesser incidence of pallor in the present series i.e. 80% as compared to 100% in the series of Kumbhat, and patel appears to be due to the fact that 50% cases in the present study had haemoglobin value between 8 & 10 gm% and the other 50% between 6-8gm%. In other series hemoglobinvalues where much lower. The incidence of anaemia and skin changes was also lower in the present study, indicating that anaemia was comparatively less severe in the series presented here. Hepatomegaly was observed in 34% of the cases. Hepatomegaly was from 1cm to 3cm, below the costal margin and never more than 3 cm. There was no evidence of congestive cardiac failure or Hepatitis.

Incidence of koilonychias has been greater as compared to other workers and this indeed is expected since koilonychias is a feature of iron deficiency anaemia. Twelve percent of cases in the present study did not exhibit any of the signs of anaemia enumerated above. The mean haemoglobin recorded by these 12% cases was 9.0gm%. it is evident therefore, that the number & severity of clinical signs is related to severity of anemia.[20]

Haematological Parameters: The diagnosis of iron deficiency anaemia is relatively simple. Simple parameter likes age, presence of microcytosis and hypo-chromic, a mean corpuscular haemoglobin concentration less tham 30% can be used for the diagnosis of it.

The lowest haemoglobin was 6.0 gm%. The mean corpuscular haemoglobin concentration (M.C.H.C.) ranged between 22% to 30% with the average of 27 percent. In Manchanda et al. series serum iron value ranged from 18.2 mic.gm to 210 min.gm% with an average of 82.7 mic.g%. None of our cases had haemoglobin less than 6 gm% and where selectively of iron deficiency anaemia that probably explains why we could not get so low value and this type of wide range of serum iron. Total binding capacity was increased in our majority of the cases. In our series bone marrow examination revealed absence of hemosiderin granules. However we are aware of the fact that in children between six months and three years of age rarely has stainable iron in the bone marrow, even in the absence of iron deficiency.

The use of serum transferring and serum ferritin will make possible the diagnosis of iron deficiency without anaemia. Transferring saturation greater than approximately 16% will allow delivery of iron to the marrow in amounts sufficient to avoid any limitation on the normal production of haemoglobin and erythrocytes or on the ability of the marrow to respond to moderately increased demands for haemoglobin production. In our cases transferring saturation was in the range of 10% to 16% with an average of 14%.

The concentration of ferritin in serum gives a quantitative measure of the amount of storage iron in normal subjects and those with iron deficiency, or iron over load. The mean level in normal men is 69ng/ml, compared with 35 mg/ml in normal women. A concentration below 10 mg/ml is associated with a low transferring saturation and iron deficient erythropoiesis. In both sexes a serum ferritin concentration below 10 mg/ml. is associated with transferrin saturation less than 16%. A lower than normal level of serum ferritin is found only in iron deficiency state. But an iron deficient patient with low serum ferritin when put on to iron therapy shows a rapid rise of serum ferritin well above normal range in the first week of treatment. The study related to the serum ferritin could not be conducted as facilities were limited.

Conclusion: Iron deficiency Anaemia in children remains a public health problem, and certain populations of children are at particularly high risk. Iron deficiency Anaemia is associated with poor developmental outcomes in children; the impact of iron deficiency is less well understood. Laboratory investigations include haemoglobin and iron tests, such as serum ferritin. Primary prevention of Iron deficiency Anaemia is recommended; the role of secondary prevention through screening programs remains inconclusive but recommended by some professional organizations. Treatment of children identified with IDA includes both dietary counselling and oral iron supplementation.

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