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Iron Deficiency Anaemia in the Females and Children of the Northern Areas of Pakistan

A thesis submitted in the partial fulfillment of the requirement for the degree of Doctor of Philosophy

By

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Dedicated to

Ijlal, Ali, Abdullah,
Fakhra
and
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CERTIFICATE

This thesis by Fasihunnisa is accepted in its present form by the Department of Biological Sciences as satisfying the thesis requirements for the degree of Doctor of Philosophy in Biology (Reproductive Health/Nutrition).

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LIST OF ABBREVIATIONS

AKHS, P	Aga Khan Health Services, Pakistan
BMI	Body Mass Index
DHQ	District Head Quarter
DNA	Dioxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
Hb	Haemoglobin
IDA	Iron Deficiency Anaemia
INACG	International Nutritional Anaemia Consultative Group.
LHV	Leady Health Visitor
LHW	Lady Health Worker
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular volume
MUAC	Mid Upper Arm Circumference
NA	Northern Areas
nc	Normal Red Blood Cell Morphology
NNS	National Nutritional Survey
NP Females	Non- pregnant Females
NWFP	North West Frontier Province
P	Probability
PCV	Packed Cell Volumes
RDA	Recommended Daily Allowances
RBCs	Red Blood Cells
SD	Standard Deviation
SE	Standard Error
SI	Serum Iron
TIBC	Total Iron-Binding Capacity
UNU	United Nation University
WFH	Weight for Height
WHO	World Health Organization

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ABSTRACT

ABSTRACT

Iron deficiency is believed to affect 20-50% of the world's population, making it the most common nutritional deficiency in the world. Iron deficiency, anaemia or both are associated with a wide variety of adverse health outcomes, including delayed child development, lower IQ, poor school achievement, decreased active play in children, lethargy and fatigue, decreased physical work capacity, decreased work productivity in repetitive tasks, preterm birth, low birth weight, perinatal mortality, infant and young child mortality, and maternal mortality.

Many reports of investigation in Pakistan also consider IDA as the most prevalent among female and children. The present study was a community-based cross-sectional survey conducted in the Gilgit and Ghizer districts of the Northern Areas of Pakistan. A two-staged stratified sampling technique was used for selection of 606 subjects including 416 females and 190 children.

Blood was drawn from antecubital vein by means of vacuette to determine Hb, PCV, RBC count, and serum ferritin levels. Peripheral smears were also made at the spot. Physical examination was done for all respondents. Age was noted and weight, height and MUAC were measured to assess the nutritional status of the subjects. Additional information regarding food composition was elicited through the questionnaire.

The results of the study showed that 22% women were underweight and 17% were short stature. It was found that caloric consumption on average in the adult non-pregnant non-lactating females, pregnant females and lactating females were 49.61%, 46.92% and 32.31% of their daily recommended allowance. Similarly, the iron intake in these groups was 15%, 14%, and 17%, respectively. Of the total children 29.84% children of 2-12 years were underweight. There were 5.62% severely malnourished children. By calculating their caloric intake it was found that on average the children of age group 2-5 years consumed 40.69% of their recommended allowance, while children of age group 6-12 years consumed 30.3% of their recommended allowance for daily caloric intake. Children (both male and female) of age group 2-5 years were having 2.6 mg of iron per day. Female children of age group 6-12 years were consuming 2.8 mg of iron per day. Likewise, male children of age group 6-12 years were consuming 2.9 mg of iron per day. Consumption of tea was higher at high altitudes.

Using four different criteria for estimation of prevalence, it has been found that by applying the WHO method 22.20% of pregnant and 15.71% of non-pregnant female were anaemic. According to the CDC method 24% of pregnant and 17% of non-pregnant women were anaemic. Using the ±2SD method 27% of pregnant and 10.34% of non-pregnant females were considered anaemic. On the basis of serum ferritin levels 42.85% of pregnant and 38.24% of non-pregnant women were anaemic. In case of children, 21% of children

were anaemic according to WHO and CDC methods. On the basis of ±2SD 18.38% of children were anaemic. By using serum ferritin level as an index of anaemia 27.01% of children were considered anaemic.

Prevalence of IDA was also analyzed separately in female and male children. There were 11 (11.22 %) females with normal RBC morphology out of 98 female children. In the case of male children there were 16 (17.39%) children with normal RBC morphology out of 92 male children.

Owing to variations in altitude and other physiological and environmental features it is hard to record the exact figures for prevalence of anaemia in the region. This calls for more specific and in-depth studies in the Northern Areas on the model followed by Cohen and Haas in their study of pregnant women residing at high altitude in Bolivia. Such studies will not only be helpful in revising the cut-off values for more accurate estimation of anaemia in the Northern Areas but will also provide a baseline for implementation of anaemia prevention programme in the region.

INTRODUCTION

There is a limitless array of risks to human health. Many of these risks, today, "concern consumption – either too little, in the case of the poor, or too much, in the case of the better off". On the one hand there are 170 million undernourished children in poor countries, over 3 million die each year as a result and on the other hand there are more than one billion adults worldwide, who are overweight and at least 300 million who are clinically obese (WHO, 2002). Numerous studies depict contrasting pictures of disparities existing in many societies. Of the two problems, nutritional deficiencies have received much attention to determine which deficiencies are most prevalent. As a result, iron deficiency has been found to be the most common nutritional problem resulting in nutritional anaemia (Baig, 2004).

Associated with weakness and tiredness long before its cases were known, iron deficiency anaemia has now become the single most significant public health problem in both the developing and the industrialized world. There is a good evidence to show that more than three and a half billion people including children, youth and elderly persons are affected by iron deficiency or its anaemias worldwide. Prevalence studies show that iron deficiency is highest in South Asia followed by countries of Africa and other parts of the world (Hoang *et al.*, 2001; Hercberg and Dupin, 1987; Supplement, 2001).

Among different sections of the population iron deficiency anaemia is highly prevalent in women of childbearing age particularly during pregnancy and among young children. In pregnant women iron deficiency increases the risk for a preterm delivery and delivering a low birth weight baby (MMWR, 1998). In children this can cause developmental delays and behavioural disturbances. Anaemia in school children is receiving more attention because of the potential of iron deficiency adversely affecting educability and learning abilities. Iron deficiency anaemia also adversely affects work capacity and productivity. On account of costs incurred in therapeutic measures, its resultant restraint on productivity and negative

consequences on human capital formation, iron deficiency has a negative impact on the overall national development (WHO, 2001).

Despite the best efforts made by various agencies there is a general lack of awareness of iron deficiency anaemia in our part of the world. Most of the available information is either incomplete or limited to special groups and more particularly to the patients. This calls for more detailed and in-depth studies on this important area of public health concern.

Anaemia

Anaemia is defined in a variety of ways to refer to one and the same disorder associated largely with the blood. Relatively old definitions regard anaemia as a condition in which the haemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency (WHO, 1968). The study carried out by Kumar and his colleagues considers anaemia as a reduction in the oxygen transporting capacity of blood usually due to a reduction below normal limits of the total circulatory red blood cell mass (Kumar *et al.*, 1992). While according to Fauci *et al.*(1998)" a patient has an anaemia whenever the haemoglobin level or the number of red blood cells is significantly reduced". According to recent studies anaemia is present when there is a decrease in the level of haemoglobin in the blood below the reference level for the age and sex of the individual (Kumar and Clark, 2002). WHO (2001) have established cut-off points of haemoglobin (Hb) for pregnant females (11 g/dl), for non-pregnant females (12g/dl), for men (13g/dl) and for children 6-59 months (11 g/dl), for children 5-11 years (11.5 g/dl) and for children 12-14 years (12 g/dl).

Whatever may be the definitions there is a general consensus among scholars that a patient has anaemia whenever the haemoglobin level or the number of circulating red blood cells is significantly reduced. From a laboratory standpoint, the presence and severity of anaemia are easily described based on the deviation from a standard set of normal values.

Types of Anaemia

Anemias are classified according to their pathophysiologic basis i.e. whether related to diminished production or loss of red blood cells (RBCs), or according to cell size (Tierney *et al.*, 2004). An overview of the most commonly used classifications recorded by Kumar *et al.* (1992) is given below:

Classification of Anaemia according to mechanism of production of RBCs

Blood Loss

A. Acute blood loss e.g. trauma
B. Chronic blood loss e.g. lesion of gastrointestinal tract, gynaecological problem

Increased rate of destruction of RBCs

C. Intrinsic (intra-corpuscular) abnormalities (Haemolytic Anaemia)
    1. Hereditary
        a) Disorders of Red Cell membrane
        b) Red Cells enzyme deficiencies
        c) Disorders of RBC synthesis
        d) Deficient globin synthesis (Thalassemia syndrome)
        e) Structurally abnormal globin synthesis (haemoglobinopathies e.g. Sickle Cell anaemia) (Beutler, 1998)

    2. Acquired
        a) Membrane Defect e.g. Paroxysmal Nocturnal Haemoglobinuria

D. Extrinsic (extra corpuscular) abnormalities
    1. Antibody mediated

2. Mechanical trauma to Red Blood Cells (RBCs)
3. Infections: Malaria

Impaired red cell production

E. Disturbance of proliferation and differentiation of stem cells e.g. Aplastic Anaemia

F. Disturbance of proliferation and maturation of erythroblasts
   1. Defective DNA synthesis: defective or impaired utilization of vitamin B_{12}
   2. Defective Haemoglobin synthesis
      a) Deficient haem synthesis: Iron Deficiency
      b) Deficient globin synthesis: Thalassemia

Iron Deficiency and its anaemia

It is an undeniable fact that iron is the key among micronutrients including vitamins, minerals and trace elements, which are fundamental to life. Only minute quantities are needed and they are usually present in a balanced diet. Studies have shown that deficiency of micronutrients in the diet seriously impairs healthy growth and development and prevents the achievement of our full physical and intellectual potential. The most prevalent micronutrient deficiencies include iodine, Vitamin A, folate, zinc, Vitamin D and iron (Blum, 1997). Iron deficiency is reported as the most common both in the developing as well as in the industrilized world and the most severe stages of iron deficiency are associated with anaemia (WHO 2001). However, to confirm anaemia as Iron Deficiency Anaemia (IDA) at least one additional iron status indicator such as serum ferritin, transferrin saturation, mean corpuscular volume, erythrocyte protoporphyrin or serum transferrin receptor is required (Haas and Brownlie, 2001).

Iron deficiency affects more people than any other condition, constituting a public health condition of epidemic proportions. Subtler in its manifestations than, for

example, protein-energy malnutrition, it exacts the heaviest overall toll in terms of ill health, premature death and lost earnings (WHO, 2004).

Control of Normal Erythropoiesis

Normal erythropoiesis (red cell production), involves both the appropriate erythropoietin stimulation of a healthy erythroid marrow and an adequate supply of iron. Peritubular interstitial cells of the kidneys produce erythropoietin in response to lower oxygen supply. As the Hb levels falls below 10 g/dl, erythropoietin levels increase logarithmically, stimulating the erythroid marrow to proliferate and increase red blood cell production several fold. Blood loss anaemia in a patient with a healthy marrow and normal iron stores will usually generate a 2 to 3 fold increase in red cell production within 7 –10 days (Hillman, 1998).

Iron supply plays a key role in this production response. The bulk of the iron required for basal erythropoiesis is recycled from senescent red cells by the reticuloendothelial system. This iron is transported through transferrin, a plasma glycoprotein that binds two atoms of iron. The majority of iron laden transferring molecules are bond to the receptors on erythroid precursors and subsequently internalised. The iron is then released and the transferrin-receptor complex returns to the cell surface where transferrin molecules are released back to the circulation. The erythroid uses this iron in Hb synthesis and storing any excess iron as ferritin. The number of erythroid precursors and the expression of transferring receptors on the surface are directly influenced by the levels of erythropoietin stimulation. In case of iron deficiency the process of normal Hb synthesis is disrupted and result is a microcytic hypochromic anaemia (WHO, 2001).

Iron deficiency is defined as a condition in which there are no mobilizable iron stores and in which signs of a compromised supply of iron to tissues, including the erythron, are noted. The more severe stages of iron deficiency are related to anaemia. When iron-deficient erythropoiesis occurs, haemoglobin concentrations are reduced to below-optimal levels. When individual haemoglobin

levels are below two standard deviations (-2SD) of the distribution mean for haemoglobin in an otherwise normal population of the same gender and age who are living at same altitude, iron deficiency anaemia is considered to be present (WHO, 2001). In a normal population, 2.5 % of the population would be expected to be below this threshold. Hence, IDA would be considered a public health problem only when the prevalence of haemoglobin concentration exceeds 5.0% of the population. It is recognized that even without anaemia, mild to moderate iron deficiency has adverse functional consequences (WHO, 2001).

Iron Physiology and Metabolism

Iron is needed for the transport of oxygen by the blood to all organs in the body, including the brain. Essential for growth, for brain development and for physical activity, iron is a key to strength, energy and work capacity (Beard, 2001).

In the blood, iron is transported by haemoglobin. When haemoglobin is rich in oxygen it is red. When iron-deficiency becomes serious, there is less haemoglobin, less oxygen, less red colour. This is called anaemia. There are other causes for anaemia, but iron-deficiency is the most common cause. When sufficient iron is not available to the body, the consequences can be devastating to individuals, to families and to entire nations (WHO, 2004).

In the human body, iron is present in all cells and has several vital functions—as a carrier of oxygen to the tissues from the lungs in the form of Haemoglobin (Hb), as a facilitator of oxygen use and storage in the muscles as myoglobin, as a transport medium for the electron within the cells in the form of cytochromes, and as an integral part of enzyme reactions in various tissues. Too little iron can interfere with these vital functions and lead to morbidity and mortality (CDC, 1998).

Total body iron averages approximately 2.3 g in women, which is equivalent to 42-mg/kg body weight for a 55 kg women (Bothwell and Charlton, 1981). When the body has sufficient iron to meet its needs, most iron (greater than 70%) may be

classified as functional iron; the remainder is storage or transport iron. More than 80% of the functional iron in the body is found in the red blood cell mass as Hb, and the rest is found in myoglobin and cytochrome enzymes. Iron is stored primarily in the form of ferritin, but some is stored as hemosiderin. Iron is transported in the body by the protein transferring. The total amount of iron in the body is determined by intake, loss and storage of this mineral (Bothwell, 1995).

Iron Intake

Regulation of iron balance occurs mainly in the gastrointestinal tract through absorption. When the absorptive mechanism is operating normally, a person maintains functional iron and tends to established iron stores. The capacity of the body to absorb iron from the diet depends on the amount of iron in the body, the rate of red blood cell production, the amount and kind of iron in the diet, and the presence of absorption enhancers and inhibitors in the diet (CDC, 1998). The percentage of iron absorbed (i.e., iron bioavailability) can vary from less than 1% to greater than 50% (Hallberg, 1981). It has also been found that in iron-deficient persons, iron absorption is also high (Finch and Cook, 1984).

In addition, iron bioavailability also depends on dietary composition. - *Bioavailability* of food iron is strongly influenced by *enhancers* and *inhibitors* in the diet which are listed below:

Enhancers of iron absorption:

- Haem iron, present in meat, poultry, fish and seafood.
- Ascorbic acid or vitamin C, present in fruits, juices, potatoes and some other tubers, and other vegetables such as green leaves, cauliflower, and cabbage;
- Some fermented or germinated food and condiments, such as sauerkraut and soy sauce (note that cooking, fermentation, or germination of food reduces the amount of phytates).

Inhibitors of iron absorption:

- Phytates, present in cereal bran, cereal grains,

- Food with inositol content,
- Iron-binding phenolic compounds (tannins); foods that contain the most potent inhibitors resistant to the influence of enhancers include tea, coffee, cocoa, herbal infusions in general, certain spices (e.g. oregano) and some vegetables
- Calcium, particularly from milk and milk products (Blum, 1997).

Coffee and tea consumption at the time of a meal can significantly decrease iron absorption. Tea can cause iron absorption to drop by 60% and coffee can cause a 50% decrease in iron uptake. The tannins in both adversely affect iron availability (Anderson, 2004).

Mechanism of Iron Absorption

Different mechanisms participate in the absorption of heme and non-heme iron. Although iron can be absorbed along the intestinal tube, it is more effective at the duedenum level.

Ionic iron (Fe+++) is reduced to (Fe++) by chlocidric acids in the stomach. Non-heme iron is delivered to the intestinal mucosa in an ionic form and it is taken across the brush border on a carrier protein by means of a receptor mediated endocytosis. Heme iron is absorbed intact mediated by an intestinal heme receptor. Once inside the intestinal cell, the iron is released by heme oxygenase.
Ionic iron is a promotor of free radical reactions, which are toxic to living cells, so it binds to a protein in the intestinal cell. Iron binds to transferring in the intestinal cell; when transferring is saturated, the remaining iron is stored in the intestinal cell as ferritin.

Iron Turnover and Loss

Red cell formation and destruction is responsible for most iron turnover in the body. In adults, approximately 1 mg of iron is lost daily through feces and desquamated mucosal and skin cells (Green *et al.*, 1968). Women of child bearing age require

additional iron to compensate for menstrual blood loss at an average rate of 0.3-0.5 mg per day (Bothwell and Charlton, 1981) and for tissue growth during pregnancy and blood loss at delivery and postpartum, an average of 3 mg/day over 280 days' gestation (Hallberg, 1988). A minute amount of iron is also lost from physiological gastrointestinal blood loss. Pathological gastrointestinal iron loss through bleeding occurs in infants and children sensitive to cow's milk and in adults with peptic ulcers, inflammatory bowel syndrome, or bowel cancer. Hookworm infections are also associated with gastrointestinal bleeding and ultimately iron depletion (Stolzfus *et al.*, 1997).

Iron Stores

Iron is stored in the body as the soluble protein complex ferritin or the insoluble protein complex hemosiderin (Bothwell, 1995). These are present primarily in the liver, bonemarrow, spleen and skeletal muscles. Small amount of ferritin also circulates in the plasma. In healthy persons most iron is stored as ferritin (70% in men and 80% in women) and smaller amounts are stored as hemosiderin. Men store approximately 1.0-1.4 g of body iron (Dallman *et al.*, 1996), women approximately 0.2-0.4 g (Bothwell and Charlton, 1981) and children even less (Dallman, 1980).

Role of Nutrition

Adequate nutrition is essential for health and for the management of disease. Eating is intermittent, whereas energy needs are continuous. The body contains thousands of types of molecules but requires for health the intake of only a small number of organic compounds - 9 essential amino acids, 1 fatty acid, and 13 vitamins - in addition to sufficient energy, water and minerals. Most inorganic molecules in food are nutritionally essential: calcium, phosphorus, potassium, sodium, chloride and magnesium are major constituents of the body; Moderate amounts of iron and zinc are required; whereas, fluoride, copper, chromium, iodine manganese, molybdenum and selenium are required in trace amounts.

The requirement of an essential nutrient is defined as the smallest amount that maintains normal body mass, chemical composition, morphology and physiologic function and prevents any clinical and chemical sign of the corresponding deficiency state. For most essential nutrients, like iron, this value is exceedingly difficult to define even for healthy adults, and requirements may differ at different periods, as in the developing embryo, the growing child, and the lactating or pregnant women. Minimal requirement also differ among normal individuals due to a variety of environmental, genetic, hormonal and physiologic variables.

Consequently, at the clinical level the concept that nutritional standards should be based on absolute nutritional requirements has been replaced by that of the recommended daily allowances (RDA), namely, the levels of intake of essential nutrients that, on the basis of scientific knowledge, are judged by the Food and Nutrition Board of the National Research Council to be adequate to meet the nutrient needs of healthy persons (Denke and Wilson, 1998).

The nutritional assessment is designed to evaluate three aspects of overall nutrition - energy, protein and micronutrient balance - and has three components; the nutritional history, appropriate physical examination with simple anthropometric measurements and laboratory studies.

In nutritional history, weight is the first step. The history of weight gain from school life to college and after marriage can facilitate this assessment. Acute and chronic illnesses increase the demands for energy, protein and micronutrients. Patients with restricted intakes are at higher risk for nutritional deficiencies. A healthy person consuming a variety of foods is unlikely to have a dietary deficiency, but not all individuals consume a varied diet. To evaluate dietary intake, the person should be asked to list everything eaten in the past 24 hours (breakfast, lunch, dinner and snacks), and this information can be used to determine the variety and adequacy of food consumed. In this way it is also cleared whether the person is taking adequate vitamins and minerals or not.

In physical examination weight and height should be measured first. Body weight and weight for height are indices of energy balance. Imbalance between energy intake and expenditure causes weight loss or weight gain.

Assessment of Body Mass Index (BMI) and Mid Upper Arm Circumference (MUAC) has the advantage of simplicity and is useful for assessing both over and undernutrition.

Laboratory assessment of different mineral status, like iron, is more useful clinically (Denke and Wilson, 1998).

Prevalence of IDA

Iron deficiency is the most common nutritional disorder in the world. The numbers are staggering: as many as 4-5 billion people, 66-80% of the world's population, may be iron deficient; 2 billion people – over 30% of the world's population – are anaemic, mainly due to iron deficiency, and in developing countries, frequently exacerbated by malaria and worm infections (WHO, 2004).

Furthermore, iron deficiency is the only nutrient deficiency that is significantly prevalent in both developing as well as industrialized countries. It is reportedly the most common cause of anaemia both in general medical practice of clinical haematology, and is alleged to be the most common organic disorder seen in clinical medicine (WHO, 2004). The global anaemia prevalence has been estimated as 43% and 35% for young and school-age children respectively by DeMaeyer and Adiels-Tiegman in 1985. The same authors, however, reported estimates of anaemia prevalence as 51% and 38% for 0-5 and 6-14 year old children in less developed countries (*SCN News* No. 7, 1991). Nearly half of the pregnant women in the world are estimated to be anaemic: 52% in non-industrialized – as compared with 23% in industrialized countries (WHO, 1992). The following figures present a vivid account of both the industrilized and developing countries:

Table 1.1 **Estimated percentages of anaemia prevalence (1990-95) based on blood haemoglobin concentration**

	Percentage of total population affected in:	
	Industrialized countries	Non-industrialized countries
Children (0 – 4 years)	20.1	39.0
Children (5 – 14 years)	5.9	48.1
Pregnant women	22.7	52.0
All women (15 – 59 years)	10.3	42.3
Men (15 – 59 years)	4.3	30.0
Elderly (60 years or more)	12.0	45.2

Source: WHO, 2001

Statistics available for different regions show that prevalence of IDA is highest in South Asia among all the regions. For example, India is reported to have 88% of pregnant and 74% non-pregnant anaemic women (WHO, 2001). Another study in India by Kanani (WHO, 2001) showed that anaemia was present in 98% of the study population i.e. adolescent girls aged 10-18 years. Throughout Africa about 50% of pregnant and 40% of non-pregnant women are affected. In Latin America and Carribean prevalence of anaemia in pregnant and non-pregnant women are about 40% and 30%, respectively (WHO, 2001). Studies in Cote d'Ivoire and Benin estimated that iron deficiency anaemia accounted for about 50% of the anaemia observed. In the Cote d'ivoire study, the proportion of anaemic individuals with iron deficiency varied by age and sex. About 80% of the anaemic pre-school age children had iron deficiency anaemia, compared with 50% of the school-age children and women and 20% of the men. Malaria and other infections or inflammatory disorders contributed significantly to the high prevalence of anaemia, particularly in young children, but these infections and/or disorders and iron deficiency could not explain all the cases (Staubli *et al.*, 2001; Hercberg *et al.*, 1988).

In a study involving a technique called attributable risk analysis, among Zanzibari schoolchildren, 62% of the children were anaemic, 3% were severly anaemic, and 51% were iron-deficient anaemic. The authors estimated that if hookworm infection could be eradicated, the prevalence of anaemia could be reduced by as much as 25%, IDA by 35% and severe anaemia (Hb<7g/dl) by 73%. Ten percent or less of anaemia and IDA was attributable to malaria, infection with the nematode *Ascaris lumbrricoides*, or stunting (Stoltzfus *et al.*, 1997).

The situation in Pakistan is not different from most of the developing countries. Here anaemia is predominantly seen in the children and women of low socio economic class. In Pakistan iron deficiency is reported as the leading cause of anaemia in pregnancy (Hashmi *et al.*, 1973; Aziz *et al.*, 1972). According to the National Nutritional Survey Report (1985-87) published in 1988, anaemia as defined by Hb level below 10 g/dl was found in 45.2% of pregnant and lactating mothers in Pakistan.

Usually, infants are born with adequate iron stores. However, iron deficiency starts developing after six months if due attention is not given to supplementary diet. Among girls iron deficiency has been commonly observed in the preschool years and also during puberty. IDA is more common among females as compared to males. Major factors responsible for this include adolescence, pregnancy, lactation and old age. Iron deficiency can also be caused by blood loss due to chronic and recurrent infections, and some parasitic infections like hookworm, *Ascaris lumbricoides, trichuriasis, amoebiasis and schistosomiasis* (INACG, 2002).

It has also been observed that iron deficiency is most common among low socio-economic segments of the society and in the areas where foods are low in iron or of low bioavailability.

Altitude and Anaemia

A number of factors including age, gender, physiological, pathological, environmental and socio-economic conditions influence iron requirements and iron intakes. Haemoglobin concentration increases in smokers and it also increases at elevations above 1000 meters. Around 20-30 million people worldwide live at altitudes higher than 3000m, defined as high altitudes (Pawson and Jest, 1978), especially in the high plains of Ethiopia and the Tibetan plateau of the Himalayas, where the adaptation of life to high altitudes could occur without an increase in the concentration of Hb (Kolsteren and Van der Stuyft, 1994). Many studies were done to describe Hb changes with high altitude and they developed different methods to adjust Hb values accordingly (Cohan and Hass, 1999; WHO, 2001; Berger *et al.*, 1997).

On the international level the values are defined by WHO and International Nutritional Anaemia Consultative Group (INACG), on the basis of studies done on populations living at sea level. However, adaptation to living at high altitudes carries an increased blood capacity for the transportation of oxygen. Persons living at high altitudes have increased concentrations of Hb as compared to those living at sea level (Dallman *et al.*, 1980). The variation is due to the decrease in the partial pressure of oxygen at high altitudes, which induces a decrease in the absolute rate of oxygen available per unit of pulmonary surface and a reduction in the saturation of oxygen in the blood (Berger *et al.*, 1997).

The relation between the concentration of Hb and altitude was studied in the 1940s by Hurtado *et al.* (1945). They demonstrated that the curve of the increase in the concentration of Hb in relation to altitude is exponential. This work led Dallman et al. (1980) to suggest an adjustment of the reference cut-off values established at sea level to include a 4% increase in the concentration of Hb per 1000 m elevation. The work of Hurtado and colleagues was confirmed by a later study by Dirren et al. (1994) in Ecuadorian children. The curve of the increase in Hb in Ecuadorian children is parallel to the curve of Hurtado *et al.* (1945) for altitudes lower than

3000m. The Centre for Disease Control and Prevention (CDC), Pediatric and Pregnancy Nutrition Surveillance System used data from 2-5 years old children with little or no iron deficiency from clinics at 1200 to 3000 m elevation to develop a curve that describes Hb changes with altitude. The progression is curvilinear, with the increase in Hb concentration becoming steeper as altitude increases (1989). The adjustments obtained were slightly lower than those proposed by Dirren (1994).

Indicators of Iron Deficiency Anaemia

Several methods have been applied in order to assess iron deficiency in a population, showing different results in terms of identifying the true anaemic group. This is related to the variability of some of the measurements even in normal subjects, and each determination is related to different stages of iron deficiency. Haemoglobin cut off points have been one of the most frequently used criteria, but the usefulness of this method is limited due to the variability of haemoglobin values in normal subjects (Duran, 2001). Despite its limitations this method is still widely in use.

The Hb concentration is used only for the detection of anaemia. Mostly anaemias in children and females are linked to iron deficiency, the main objective for the diagnosis of anaemia is to detect individuals at high risk of deficiency of this micronutrient (Berger *et al.*, 1997). The diagnosis of anaemia requires analytical methods for the detection of Hb concentration as well as the proper cut-off values.

The definition of a cut-off value for Hb that permits the diagnosis of anaemia requires a reference population consisting only of healthy individuals free of all nutritional deficiency that could influence the concentration of Hb (Berger *et al.*, 1997).

The establishment of reference ranges for the parameters tested in any clinical laboratory is mandatory in order to interpret subsequent testing of patients and to discriminate between health and disease. It has been recognised that there

is a homeostatic mechanism in every individual. In the presence of optimum supplies of nutrients and in the absence of pathological states it sets an appropriate level of haematology parameters (Khattak *et al.*, 1991). Khattak and colleagues established their own reference ranges for haematological parameters and compared them with other countries. They found that their highly selected sample from the Northern areas of Pakistan had the haematological values comparable to those reported for normal individuals from well nourished Caucasian population.

Gilles (1981) suggested that each country should lay down its own minimal acceptable standards below which an individual is considered anaemic.

Assessment of IDA

Several clinical and laboratory indices have been used for assessing nutrition iron status. Laboratory indices are the most common methods used to assess iron nutrition status.

Hb

The prevalence of anaemia in a population is best determined by using a reliable method of measuring Hb concentration. The only methods generally recommended for use in surveys to determine the population prevalence of anaemia by haemoglobinometry are the cyanmethemoglobin method in the laboratory and the HemoCue system. The cyanmethemoglobin method for determining Hb concentration is the best laboratory method for the quantitative determination of Hb. It serves as a reference for comparison and standardization of other methods (National Committee for Clinical Laboratory Standards, 1994).

A fixed quantity of blood is diluted with a reagent (Drabkins solution) and Hb concentration is determined after a fixed time interval in an accurate, well-calibrated photometer.

The HemoCue system is a reliable quantitative method for determining Hb concentrations in field surveys (WHO, 2001), based on the cyanmethemoglobin method. This system consists of a portable, battery-operated photometer and a supply of treated disposable cuvettes in which blood is collected.

This system is uniquely suited to rapid field surveys because the one-step blood collection and Hb determination do not require the addition of liquid reagents. The HemoCue system gives satisfactory accuracy and precision when evaluated against standard laboratory methods (Jhons and Lewis, 1989).

The Hb concentration varies with age. Even when Hb is related to iron deficiency and the definition of IDA is based on Hb concentration, it alone is not a sufficient indicator.

Mean Corpuscular Volume (MCV)

Among all the red cell indices measured by electronic blood counters, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) are the two most sensitive indices of iron deficiency. Reduction in MCV occurring in parallel with anaemia is a late phenomenon in the development of iron deficiency. It depends on Hb content in the red blood cell, so a decrease in Hb is associated with a decrease in MCV.

Ferritin

The serum ferritin level is the most specific biochemical test that correlates with relative total body iron stores. A low serum ferritin levels reflects depleted iron stores and hence is a precondition for iron deficiency in the absence of infection. The generally accepted cut-off level for serum ferritin, below which iron stores were consider to be depleted, is <15 μg/l for adult females (WHO, 2001).

Even when ferritin is present within cells, a small amount circulates in plasma and permits estimation of total ferritin, has been the earliest indicator of iron

deficiency. Serum Ferritin expresses iron stores. During infancy serum ferritin concentration below 10μg/l is considered as the expression of depleted iron stores. In a study conducted by Khan *et al.* (2002) at Shaikh Zayed Medical Complex, Lahore, the lower limit for serum ferritin was set at <12 μg/l. In his study out of 85 hypochromic microcytic children, 78 (92%) were iron deficient. The serum ferritin assay is the most sensitive and specific index for the determination of iron stores. Ferritin is also an acute phase reactant protein so during infection it increases, so in such instances it is not an accurate measurement of iron deficiency.

Serum Iron, Transferrin and Transferrin Saturation

Iron deficiency results in a reduction in serum iron (SI) levels, an elevation in transferrin (total iron-binding capacity {TIBC}) levels, and hence a net reduction in transferrin saturation (i.e. SI/TIBC). However, the diurnal variation both in serum iron and transferring saturation is considerable. In addition, there is a marked overlap in these indices between normal and iron-deficient subjects. This overlap diminishes the usefulness of these indices in establishing or rejecting a diagnosis of iron deficiency. Percentages below 16% in adults and children suggested insufficient iron delivery to the haematopoietic tissues (WHO, 2001).

Serum Transferrin receptors

The measurement of serum transferrin receptors is a recent addition to the available selection of tests for iron deficiency. However, epidemiological studies have yielded limited information concerning the usefulness of this test in discriminating between iron-deficient and iron-replete subjects.

An increase in serum transferrin receptors is a sensitive response during the early development of iron deficiency. Serum transferrin receptor levels increase progressively as the supply of iron to the tissues becomes progressively more deficient (Skikne *et al.*, 1990). Major advantages of measuring serum transferrin receptors involved the facts that the assay is not significantly affected by infection or

inflammatory processes, and it does not vary with age, gender, or pregnancy (WHO, 2001; Carriaga *et al.*, 1991; Kogho, 1986).

Serum transferrin receptors reflect the number of receptors in immature red blood cells and thus the level of erythropoiesis. Serum levels of serum transferrin receptor correlate with the level of erythroid precursor proliferation and the adequacy of the iron supply to the marrow. Normal levels are 4-9 μg/l by immunoassay. Levels increase rapidly in patients with iron-deficient erythropoiesis (Hillman, 1998).

Erythrocyte Protoporphyrin

The level of red cell protoporphyrin is another sensitive indicator of iron-deficient erythropoiesis. Protoporphyrin is the molecule made in mitochondria to which iron is added to form heme. Under iron deficiency, the lack of iron determines an increase in erythrocyte protoporphyrin, which cannot combine with iron. Increased values of erythrocyte protoporphyrin indicate impaired erythropoiesis due to iron deficiency. Values greater than 100 μg/dl and 120 μg/dl have been used as cut-off points (WHO, 2001).

Protoporphyrin levels also rise in children exposed to lead. This reflects the inhibition of heme synthetase, the enzyme required for the formation of heme (Hillman, 1998).

Peripheral Smear

IDA is recognized from the combination of abnormal iron supply studies and microcytic hypochromic red blood cell morphology (Hillman, 1998). Anaemia can be broadly categorized into three major classifications according to the morphology of the erythrocytes (RBCs).

1) Microcytic hypochromic (Fig.1.1)
   *IDA
   *Alpha or beta thalassemia
   *Anaemia of chronic diseases

*Sideroblastic anaemia

2) Normocytic normochromic (Fig.1.2)
*Acute haemorrhage
*HIV-related anemia
*Hemoglobinopathies

3) Macrocytic
*Megaloblastic anaemia:B12 or folate deficiency
*Hemolytic anaemia
*Aplastic anaemia

Clinical Indices

Pallor of the conjunctiva, tongue, nail bed and palm can be used as clinical indices of iron deficiency (Duran, 2001).

Many symptoms of IDA, including weakness, lassitude, palpitations and sometimes exertional dyspnea, are common to all types of anaemia. Glossitis characterized by a reddened, swollen, smooth and shiny tongue occurs sporadically. Angular stomatitis, tenderness and erosion at the corners of the mouth, is also common. Koilonychia, or spoon-shaped nails, is another finding of IDA. Mennoragia is a common symptom in iron deficient women.

One peculiar symptom that is a quiet characteristic of iron deficiency is pica. In this condition patients develop cravings for substances like clay, starch, ice, coal etc. These items bind iron in the gastrointestinal tract and worsen the condition (Beard, 2001).

Causes of IDA

Iron deficiency comes about either as a late manifestation of prolonged negative iron balance or because of failure to meet an increased physiologic need for iron.

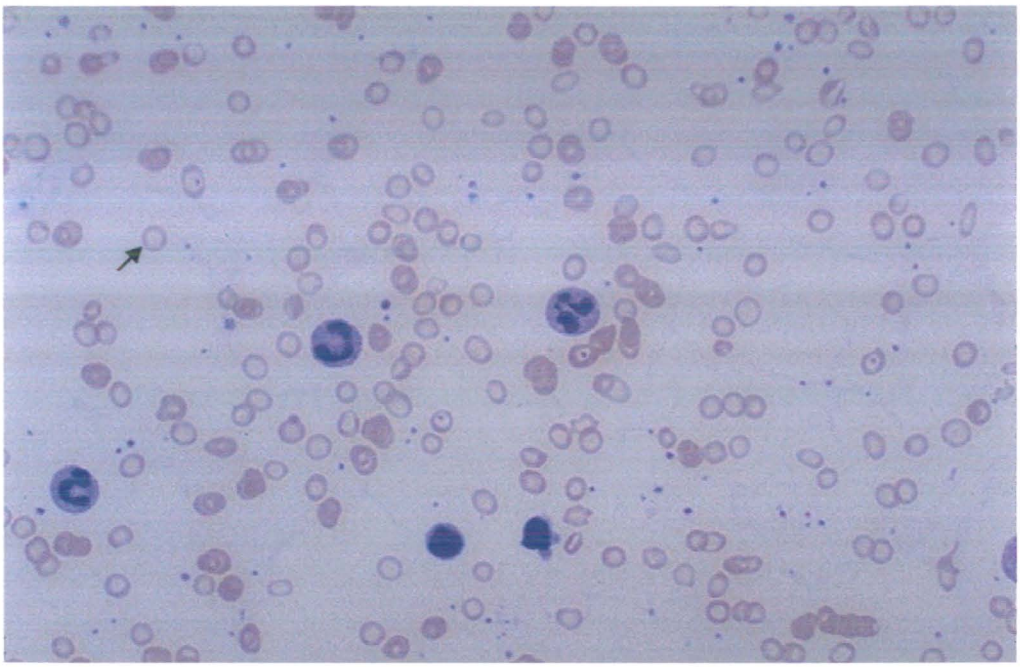


Fig. 1.1 Microcytic Hypochromic RBCs (↗)

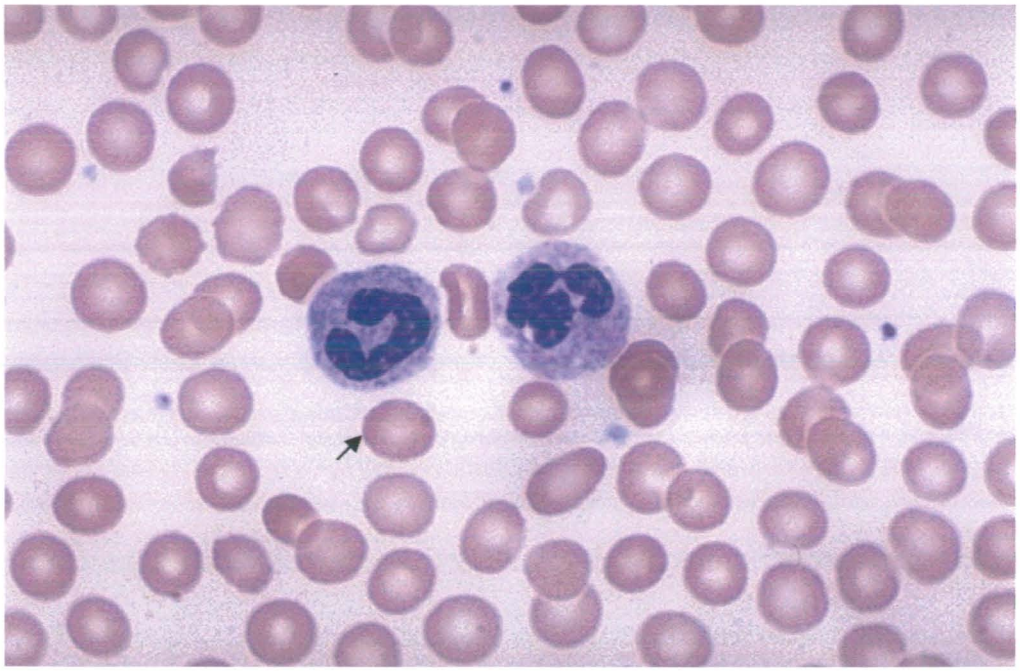


Fig. 1.2 Normochromic Normocytic RBCs (↗)

In many instances, multiple etiologic factors are involved. In Pakistan, nutritional deficiency is the most common cause of iron deficiency in infants and children. Cow's milk in first year of life and exclusive breastfeeding after4-6 months of age increase the risk of IDA (Baig, 2004). The association of a marginal diet with some source of blood loss, such as that associated with menstruation is a common combination. Another example is hookworm infection, which produces anaemia only in those whose diets are marginally adequate.

Iron deficiency occurs when the amount of dietary iron absorbed over extended periods is insufficient to meet body iron needs, including those imposed by pathologic causes (for example hookworm infection). It relates both to the actual quantity of intake and to the bioavailibility of a given intake. Bioavailability refers to the availability of a substance from the diet for use in normal metabolic processes and functions, and is influenced by both dietary factors (dietary iron contents, physiochemical form of the food, enhancers and inhibitors, food processing techniques) and host -related physiological factors (body iron stores, physiological state).

In summary the common causes of Iron Deficiency include:

1. Deficient diet
2. Decreased absorption
3. Increased requirement e.g. pregnancy, lactation etc.
4. Blood Loss:
    A. Gastrointestinal tract
    B. Menstruation
    C. Donation
    D. Trauma
5. Haemoglobinuria
6. Iron sequestration

Functional consequences of IDA

Iron deficiency Anaemia adversely affects:

1. **Cognitive Development:** In experimental animals, iron has been shown to play a key role in brain function. There is strong evidence that findings from animal studies also apply to humans. For example iron deficiency anaemia has been seen to delay psychomotor development and impaired cognitive performance in Chile (Walter *et al.*, 1983), Indonesia (Pollitt *et al.*, 1985), India (Seshadri & Gopaldas, 1989) and in many other countries. Mainly IDA results in impaired and behavioural effects in infants and young children but also it has an impact on the cognitive and behavioural performance of mothers, the mother-child interaction and the physical and neuropsychologic development of their infants (Perez *et al.*, 2001). In children some factors found to be associated with anaemia and poor cognitive development are low socio-economic status, poverty, lack of stimulation in the home, including lack of maternal warmth, poor maternal education and intelligence quotient, maternal depression, more absent fathers, low birth weight and early weaning, parasitic infections, elevated blood lead levels and undernutrition (Grantham-McGregor and Ani, 2000). In an extensive study, behaviour observations were made in a 15 minute free play situation and throughout developmental testing. Anaemic children stayed closer to their caretakers, showed less pleasure and were more wary, hesitant and easily tired.

2. **Resistance to Infection:** Morbidity from infectious disease is increased in iron-deficient populations (Hussaini, 1988) because of the adverse effect of iron deficiency on the immune system (Walter, 1986). Iron is essential for proper cell differentiation and cell growth. In addition it is a critical component for the proper enzymatic functioning of immune cells. In one of the few studies of role of iron nutrition in the development of the immune system, a delay was noted in the development of cell-mediated immunity (Kochanowski and Sherman, 1985). T-lymphocytes decreases in IDA. Neutrophils have a reduced intracellular killing ability

of pathogens in iron deficiency anaemia (Spear and Sherman, 1992). Infection is one of the main pathological risk factors for preterm labour (Allen, 2000).

3. **Work Capacity and Productivity:** Physically strenuous work requires high aerobic capacity and would be impaired by anaemia (Hass and Brownlie, 2000). A linear relationship has been found between iron deficiency and work capacity for agricultural workers in Sri Lanka (Edgerton, 1981), Kenya and a number of other countries. Chandrasekhar and Radhai Sri (2001) studied improvement in work performance of anaemic women by giving them iron rich food supplements. Another study also revealed the same results with an increase in their Hb levels (Dirk and Vatucawaqa, 2001). Compared with non-anaemic women, anaemic female workers in China were 15% less efficient in performing their work (Li, 1993).

4. **Pregnancy:** Iron Deficiency in childbearing women increases maternal mortality, prenatal and perinatal infant loss (WHO, 1975; Schorr & Hediger, 1994). The importance of adequate iron stores for pregnancy to prevent adverse birth outcomes was also studied by Cogswell and colleagues (2001). A study in Nepal showed that severe anaemia during pregnancy is associated with high risk of neonatal deaths (Dreyfuss *et al.*, 2001). IDA is one of the leading causes of Pakistan's maternal mortality rate estimated at between 300-600 deaths per 100,000 births. More than 20% of the maternal deaths are believed to be directly or indirectly related to IDA (Unicef, 1998). Iron deficiency anaemia can cause preterm labour by secreting stress hormones mainly norepinephrine and cortisol (Dallman, 1986; Milley, 1997; Campos *et al.*, 1998). Iron deficiency anaemias may contribute to increased morbidity and mortality by increasing maternal susceptibility to infection (Brabin *et al.*, 2001). Low birth weight is a strong predictor of mortality, and low birth weight babies are also at high risk of developing anaemia during infancy (Brabin *et al.*, 2001). It has recently been recognized that fetal anaemia occurs frequently in developing countries (Brabin, 1992). Post neonatal infant mortality in Malavi has also been related to fetal anaemia and low birth weight. In an infant cohurt study of 92 infants with low birth weight, 120 with fetal anaemia and low

birth weight, and 188 with neither, those with fetal anaemia and low birth weight had the poorest survival (Verhoeff, 2000). This is aggravated by infection, which further increases risk of anaemia because sick children do not absorb iron well. In Brazil, anaemic children were more likely to have had diarrhea than nonanaemic children, and diarrhea was a predictor of anaemia in a multiple regression analysis (Brabin, 2001).

5. Growth

Growth improved in iron-deficient children who were given supplementary iron in Indonesia, Kenya, and Bangladesh, as well as in the United Kingdom and the United States. Whether or not an effect of iron supplementation is observed apparently depends on local factors. These include frequency of diarrhoea and other infections, age at iron depletion and other dietary factors (WHO, 2001).

6. Endocrine and Neurotransmitters

Iron deficiency alters the production of triiodothyronine (T3) and thyroid function in general, and the production and metabolism of catecholamines and other neurotransmitters. This results in impaired temperature response to a cold environment.

In both experimental animals and human subjects, those with IDA more readily become hypothermic and have a depressed thyroid function (WHO, 2001; Martinez-Torres *et al.*, 1984; Dillman *et al.*, 1982; Dillman *et al.*, 1980). This condition may be the cause of some of the discomfort from cold felt by poorly nourished individuals at temperatures in which well-nourished persons are quite comfortable.

7. Heavy-Metal Absorption

An important consequence of iron deficiency is an apparent increased risk of heavy-metal poisoning in children. Iron-deficient individuals have an increased absorption capacity that is not specific to iron. Absorption of other divalent heavy metals,

including toxic metals such as lead and cadmium, is also increased (Massawi *et al.*, 1978).

Prevention of iron deficiency, therefore, reduces the number of children susceptible to lead poisoning. Such prevention may also help to reduce their lead burden after exposure to high levels of lead from chipped lead paints, pollution from automobile fumes (such as occurs in many cities), or other excessive exposure to lead in the environment (Andelman and Sered, 1982). Children from poor families who often have iron deficiency, are at greatest risk of developing lead poisoning. The toxicity of lead is due at least in part to a disruption of heme synthesis in neural tissues, a process abetted by iron deficiency.

Scope of the Study

Many reports of investigations in Pakistan present a dismal picture with regard to the severity of iron deficiency anaemia in the country. For instance, the study by Awan and colleagues (2004) was conducted to learn the prevalence, types and causes of anaemia in pregnant females. They found that the major reason for anaemia among them was iron deficiency. Their results showed 76% microcytic hypochromia and 32% fetal loss. Another study done by Sheikh *et al.* (2003) confirmed that iron deficiency anaemia was very common among paediatric population of his study consisting of 100 children from Jamshoro. The results of the study by Manzoor *et al.* (2003) revealed that after assessing clinical manifestations, serum iron, total iron binding capacity, percentage transferring saturation, serum ferritin levels and red cell morphology, iron deficiency was the cause of anaemia in about 60% of the sample comprising 168 children of 6-12 years in Lahore. Yaqoob and Abbasi (2002) documented in their study that the most common cause of anaemia in non-pregnant females and men was iron deficiency (43.36%) confirmed by hypochromia and microcytosis and their mean serum ferritin levels were 5.6 μg/l. The mean age of the sample was 32 years. Karim (1988) proposed that depending upon the socio-economic circumstances in our country any pregnant female having a Hb level below 10g/dl should be considered as anaemic. Ali and Zuberi (2001) made a connection between maternal education, low monthly income and childhood

anaemia. According to their results low monthly income had significant association with anaemia in children. A study by Karim and colleagues (1994) found that hypochromic microcytic anaemia had the highest percentage (63.5%). The majority (56%) were of age group 30-39 years, 37% of the age group 20-29, 2% below the age of 20 and 5% above the age of 40 years. Hassan and co-workers (1993) revealed that in addition to pregnancy itself, important contributory factors for iron deficiency were early marriage, a high number of pregnancies with shorter spacing between consecutive pregnancies, lack of meat in the diet, a high incidence of intestinal worms and a lack of iron supplementation.

As most of these studies have been carried out in hospitals of the urban areas of the country, therefore, despite their usefulness they do not provide a deep insight into the real extent. For most of these accounts, true random samples of a larger population have not been taken which make the prevalence figures more limited to special segments or groups.

There are practically no steps that have been taken to gain an in-depth knowledge of iron deficiency in the Northern Areas. This region due to its unique history; culture and high altitude presents a complete case to fill this gap. The present study is the first attempt towards a deeper understanding of the problem with a view to develop an effective prevention strategy.

Several interventions can be used to prevent and treat anaemia. Three main strategies can be applied in order to improve iron status in individuals and populations. They are:

*Dietary modification

*Food fortification

*Iron Supplementation

They tend to modify iron balance by increasing iron dose and bioavailability. Moreover, improved reproductive health (family planning to prevent early births, reduced number of births, increased birth intervals), and helminth and malaria

control can be used (Literature Review on Maternal Anaemia and Iron Supplementation, 2000).

Suppositions

This research is largely descriptive in nature, the prime aim being to determine the burden of Iron Deficiency Anaemia (IDA) in the study districts. Therefore, we are not testing a formal research model in this study, however, considering the available information on IDA and the characteristic features of the area like including remoteness and economic backwardness we assume that:

i) The prevalence of anaemia among women and children in Gilgit and Ghizer districts would not be less than the estimated national prevalence.

ii) IDA would be more common among pregnant women.

iii) There will be a positive relationship between altitude and blood haemoglobin levels as has been found on high altitude areas elsewhere in the world.

It is also clarified that any relationship between different parameters does not always equate to causation.

Objectives

The study has the following major objectives:

- Conduct a community-based study to document the prevalence of IDA in the Gilgit and Ghizer districts of the Northern Areas
- Study the correlation between high altitude and IDA
- Formulate recommendations for a prevention strategy

SUBJECTS AND METHODS

Study Area

Geographical location

The Northern Areas is the official name given to an isolated mountainous terrain in the northern extreme of Pakistan spread over an expanse of 72,496 square kilometers. It is situated between 35-37° N latitude and 74° W longitude and bounded by the Xinjiang province of People's Republic of China on the north-east, the North-West Frontier Province (NWFP) of Pakistan on the south, Kashmir on the east and Afghanistan on the west.

Amidst Himalayas, Karakorum and Hindukush, in the Northern Areas, there are a number of towering snow-clad peaks with heights varying from 1,000 meters to the world's second highest peak Godwin Austen (K-2) at 8611 meters. According to Ministry of Tourism (2003) this area has some of the longest glaciers outside the Polar region; Siachen (72 km), Hispar (61 km), Biafo (60 km), Baltoro (60km) and Batura (64 km).

Administrative Set-up

The Northern Areas (NAs) have the status of a Federally Administered Area, with the chief executive authority for the NAs vested in the Federal Minister for Kashmir Affairs, Northern Area, States and Frontier Regions. For local administration, the region is divided into five districts namely Gilgit, Diamer, Skardu, Ghizer and Ganche (Fig. 2.1, 2.2). There are 13 subdivisions and 103 union councils, as a whole.

Population

According to the 1998 census, the total population of the Northern Areas is 870,347 with an inter-censual annual growth rate of 2.4%. The population density is 12 persons per square kilometre. The urban population is 14%. The sex ratio (males per 100 females) is 109.

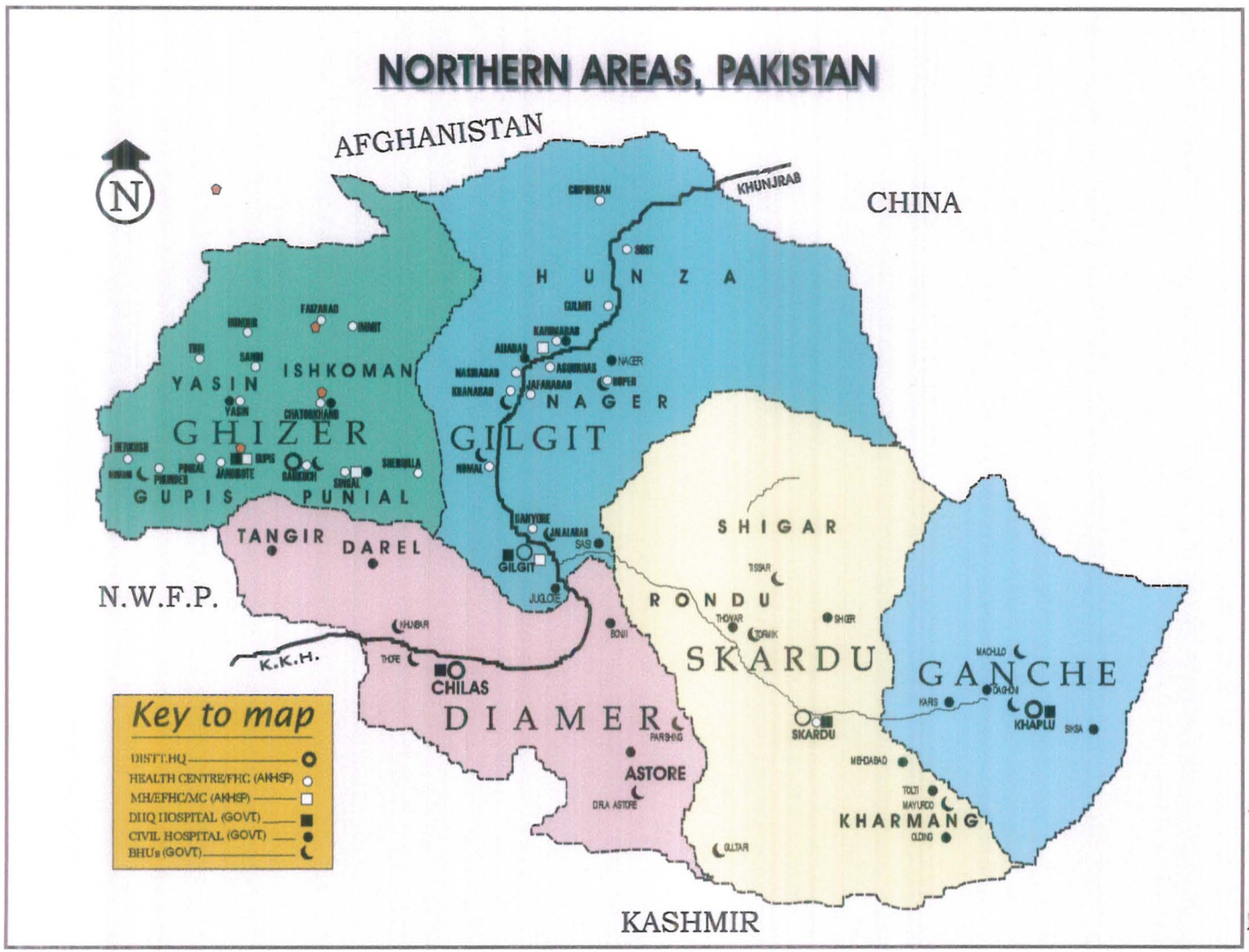


Fig. 2.1

Quaid-i-Azam University

MAP OF PAKISTAN

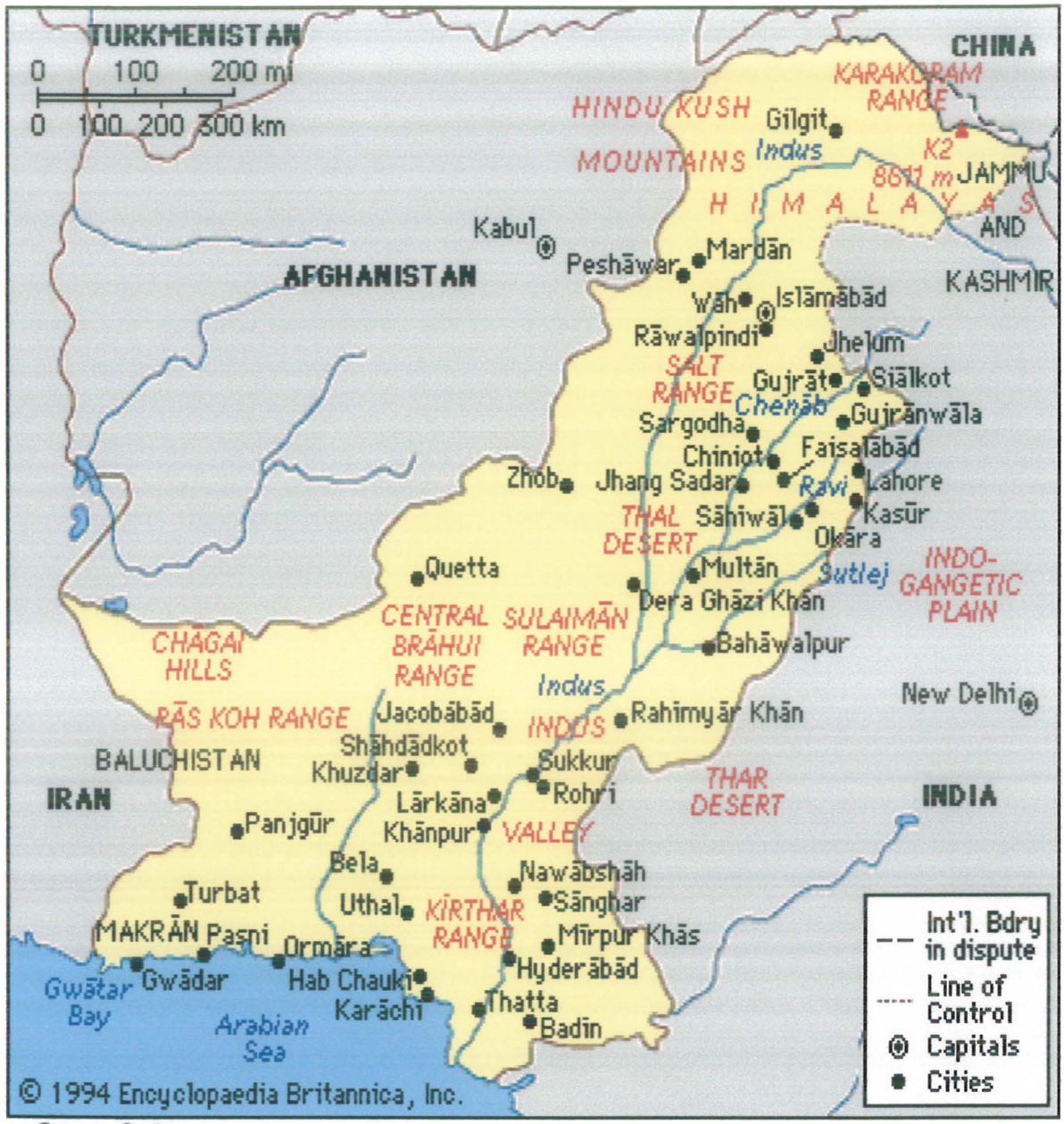


Fig. 2.2

Economy

Owing to harsh climatic conditions, topography and lack of resources the economic condition of the local population is poor compared to other parts of the country. The overall economy of the Northern Areas can be defined as mixed mountain agriculture. This type of economy is a combination of crop farming and animal husbandry with seasonal utilization of different ecological zones. The major crops are wheat and maize.

Study Design

The present study is a community – based cross sectional study carried out in the Gilgit and Ghizer districts of the Northern Areas. The total population of these two districts , according to the 1998 census, is 3,68,038; including 2,46,760 in Gilgit and 121,278 in Ghizer (67% and 33% respectively) with a total of 43,874 households (30,481 in Gilgit and 13,393 in Ghizer).

Interviews

For each respondent the methods including interviews, physical examination, blood collection and stool tests were used.

Table 2.1 **Items for which information was recorded from both women and children.**

S.no	Items
1	Name
2	Age
3	Education
4	Age at menarche
5	Marital status
6	Reproductive Status (pregnant/lactating)
7	24 hours food history
8	Daily tea consumption
9	Total monthly income
10	History of passing worms
11	Stool observation

In addition to asking questions, physical examination was carried out by a physician and laboratory investigations were also done as explained below:

Physical Examination

The following parameters were checked for each subject:

1)Temperature and Blood Pressure

The subjects within normal ranges of temperature and blood pressure were included in the study.

2)Height (cm)

3)Weight (kg)

4)Mid Upper Arm Circumference (MUAC)(cm)

5)Goiter and Spleenomegaly

In this study none of the subjects had goiter of any grade. Similarly, no subject was diagnosed for spleenomegaly.

6)Clinical signs and symptoms of anaemia includes fatigue, headaches, faintness, breathlessness, angina, palpitations, pallor, tachycardia, paleness of face and conjunctiva, spoon-shaped nails (koilonychias) and smoothness of tongue (Kumar and Clark, 2002).

Laboratory Investigations

The blood and stool samples were collected from the subjects and following investigations were done:

- Red Blood Cell count
- Hemoglobin level
- Serum Ferritin level
- Red Blood Cell Morphology (Peripheral smear)
- Packed cell volume
- Stool microscopy for parasitic ova

Sampling and Sample Size

A two-staged stratified sampling technique was adopted for the selection of the subjects. The total sample required was estimated using the formula for prevalence surveys as given below

$N=t^2 \times (pxq/d^2)$

*t=1.96 corresponding to an error risk of 5%

*p=expected prevalence (50% or 0.5)

*q=1-p (1-0.5=0.5)

*d=absolute precision (5% or 0.05)

The total subjects thus required were estimated to be more than 384. It was decided to visit around 425 households so that no responses and denial to interview would be compensated.

It was also decided that the number of households in each district will be proportionate to its population, keeping in view the proportion of urban/rural ratio.

According to population of the randomly selected villages, the number of households to be visited was determined for each village.

For a random selection all villages in each district were listed and then the required numbers of villages were selected by using lottery method. In 2nd stage, in each village, first house was randomly selected afterwards houses were selected systematically using a pre-decided number depending upon the total number of households in the village. In each house a woman of 15-55 years old was selected as the main informant of the household characteristics. In alternative households a child of 2-12 years of age was also interviewed and blood/stool samples were collected.

Before commencement of the actual fieldwork, a questionnaire was designed that was field-tested and revised accordingly. Availability of all required supplies was ensured before departure to Gilgit. A lady physician for complete medical checkup, a laboratory technician for hematological investigations and for stool examination, a lady health visitor (LHV) to assist in measuring weight and height were also accompanied. The main store for the storage of blood serum samples was established at Pathology Laboratory, District HeadQuarter Hospital (DHQ), Hospital Gilgit.

Blood Collection

Verbal consent was obtained from the respondents prior to interview. Venous blood was used for all hematological investigations. For collection of blood samples first the skin was cleaned with a 70% alcohol swab and allowed to dry before being punctured. Blood was withdrawn from the antecubital vein by means of a Vacutainer and vacutte needle attached to vacutte tube, 2 – 2.5 ml blood was taken first in red-top (plain) vacutte tube and then it was removed with purple-top test tubes i.e. coated with ethylenediamine tetra-acetic acid (EDTA). From a purple-top tube, blood was taken for packed cell volume (PCV) and red

cell count, whereas red-top tubes contained blood for haemoglobin (Hb) measurement, peripheral smears, and for the separation of serum. Serum was separated by centrifugation (Lab Centrifuge, Model Y J03-043-4000, China) at 3000 rpm for 15 minutes (at two places a hand centrifuge was used for the separation of serum because of non availability of electricity). Serum was then stored in 1.5ml microfuge tubes and preserved at -20° C until estimation.

Estimation of Hemoglobin

Hemoglobin was measured on the spot and ***HemoCue*** (HemoCue AB, Angelholm, Sweden) was used for this purpose. It is based on cyanmethemoglobin method. Sodim deoxycholate hemolyses the erythrocytes and hemoglobin is released. Sodium nitrite converts hemoglobin to methemoglobin which, together with sodium azide, gives azidemethemoglobin. The absorbance is measured at two wavelengths (570 and 880 mm) in order to compensate for turbidity in the sample. It is a battery-operated photometer with disposable cuvettes. It is a reliable quantitative method for determining hemoglobin concentrations in field surveys (Van Schenck *et al.,* 1986).

Preparation of Blood Films on Slides

With the help of a disposable pipette a single drop of blood was taken from the red top vacuette tube just after it was drawn. Then it was placed in the center line of a slide about 1 or 2 cm from one end. Immediately, a smooth edged glass slide was placed at an angle of 45° and was moved back to make contact with drop of blood and it was spread in the form of a thin film. The slide was labeled with the subject's code. Blood films were made on the spot immediately after the collection of blood. For staining, Leishman's stain was used. First the film was dried in the air, and then it was flooded with stain. After 2 minutes double the volume of water was added into the stain and the slide was stained for 5 -7 minutes. After that it was washed with buffered water for 2 -3 minutes. The back of the slide was wiped clean and dried for the reading.

The peripheral smear provides valuable information for the presence of Iron Deficiency Anaemia (IDA) regarding red cell size and color (microcytosis and hypochromasia) and also distinguish IDA from other causes of microcytosis (Baig, 2004).

The Total Red Cell count

For the estimation of red cell counting, a visual method was used. A dilution of 1: 200 of blood in formal – citrate solution was made. It was mixed by adding 20µl of blood into 4 ml of diluting fluid in a glass tube.

With great care the clean and dry Neubauer counting chamber with its cover slip in position, was filled with diluted blood. After 2 minutes the cells were counted in 4 or 8 horizontal rectangles of 1mmx0.05mm. Red cell count was finally measured by using the following formula:

$$\text{Red cell count (per l)} = \frac{\text{No of cells counted}}{\text{volume counted (µl)}} \times \text{dilution} \times 10^6$$

(Dacie and Lewis, 1991).

Thus, when the cells in 80 small squares of Neubauer chamber are counted (total volume= 0.02µl) and the blood is diluted 1:200, the red cell count will be;

$$= \frac{N}{0.02} \times 200 \text{ per µl} \quad \text{or} \quad \frac{N}{0.02} \times 200 \times 10 \text{ perl}$$

Stool Examination

After completing the interview, physical examination and blood sample collection, each respondent was given a sterile stool container and it was collected the next day. As soon as the sample arrived it was tested for (ova of) parasitic infestation. A very small amount of stool was placed on a glass slide and a drop of saline solution was added on it. With a glass rod it was mixed to make a suspension. A cover slip was added and examined under microscope.

Determination of Packed Cell Volume (PCV)

For the estimation of Packed Cell Volume (PCV) a macro-method was used (Dacie and Lewis, 1991). Wintrobe tubes, 2.5 – 3 mm in diameter and 110mm in length, calibrated at 1mm intervals to 100mm, were employed. They hold 1 ml of blood. By means of a glass pipette 1ml blood was filled in the Wintrobe's tube and centrifuged it at 3000 rpm (Lab Centrifuge, Model Y J03-043-4000, China) for 30 minutes and then readings were taken.

Indices

These included mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and these have been referred to as 'absolute' values (Dacie and Lewis, 1991).

MCV gives an account of average volume of red cells in the peripheral smear and it is calculated as:

MCV(fl)= PCV(%)/RBC(million/µl) X 10

Normal range for MCV lies between 80-100 fl and in the case of microcytosis it is less than 80fl.

MCH indicates weight of hemoglobin in red cells and is calculated as:

MCH (pg)= Hb(g/dl)/RBC(million/µl) X 10

Normal range for MCH is 27-32 pg and It is less than 27 pg in the case of hypochromia.

MCHC is the concentration of hemoglobin in a unit volume and indicates its absolute increase or decrease in red cells. It is calculated as:

MCHC (g/dl)= Hb(g/dl)/PCV(%) X 100

Normal range for MCHC lies between 32-34 g/dl, It is less than 32 g/dl in hypochromia (Baig, 2004).

Height, Weight and Mid-Upper Arm Circumference (MUAC)

In addition to other information, weight (kg), height (cm) and mid-upper arm circumference (cm), were also measured to determine the nutritional status of the subjects. Weight (kg) was recorded on Uniscales (UNICEF) specially designed for field surveys. The scale was calibrated daily using a standard 5kg weight. Height (cm) was measured using two types of instruments. For children a sliding wooden measuring board, and for adults a folding wooden measuring pole was devised in consultation with experts in the field of anthropometry at UNICEF, Islamabad. MUAC (cm) is the circumference of the left upper arm, at the mid-point between the tip of the shoulder and the tip of the elbow. It was measured using measuring tape.

For females two cut-off points were used for the estimation of undernutrition. According to the first one, the weight under 40 kg and/or height less than 150 cm showed underweight and short for stature. The second criterion was used with the weight of 45 kg and with height of 155 cm (Brough, 1993). Body Mass Index (BMI) was calculated as weight (kg)/Height $(m)^2$. For MUAC the standard value of 23 cm (for Indian women) was used for the study (Tripathy et al., 1987).

For children, the indicators of nutritional status to be used were weight-for-height, BMI and MUAC. Severe and moderate malnutrition was done on the basis of WFH (weight-for-height) Z-score. The weight for height index can be expressed either as standard deviations of the reference distribution or Z-scores or as a percentage of the reference median. Expression in Z-score has a true statistical meaning, which percentage of the mean does not have and is,

therefore, recommended (Brunet, 2002). Global and severe acute malnutrition in children are defined as follows:

Global acute malnutrition: proportion of children with a W/H index <-2 Z-scores (80%)

Severe acute malnutrition:proportion of children with a W/H index <-3 Z-scores (70%)

24-hour Dietary Recall method

A 24-hour dietary recall method, in which each subject was asked to recall all food and beverages consumed for breakfast, snacks, lunch and dinner during the previous day was used to assess the mean dietary consumption of the subjects. It was used to calculate the caloric value (k cal) and daily iron intake, using Food Composition Table for Pakistan (2001). Great care was taken but since a number of assumptions are made regarding composition of food, there is still some likelihood of getting slight ± difference during calculations.

The reference values used as Recommended Daily Allowance (RDA) are based on the 1972 Adaptation for Pakistan from UN Food and Agriculture Organization (National Nutrition Survey, 1988). The results were presented in terms of 'Average percent of Recommended intake', calculated as the mean intake for the population group divided by the group's recommended intake and expressed as percentage. Similarly, the mean intake of iron with RDA and average percent of RDA was calculated.

Estimation for Prevalence of IDA

Diagnosis of anaemia, on the basis of haemoglobin concentration, depends on a cut-off value. An individual is said to be anaemic if his/her haemoglobin concentration will be less than established cut off value. The cut off values

depend on a number of factors including altitude above sea level, sex, age, pregnancy, smoking etc. The most widely used cut-off values are those recommended by World Health Organization (2001). These values are presented in the following Table (2.2).

Table 2.2 **Haemoglobin levels below which anaemia is present**

Age or gender group	Haemoglobin (g/dl)
Children 6 months to 59 months	11.0
Children 5 – 11 years	11.5
Children 12 – 14 years	12.0
Non-pregnant women (above15 years)	12.0
Pregnant women	11.0
Men above 15 years of age)	13.0

Source: WHO (2001)

While estimating prevalence of anaemia it is important to consider the factors affecting the haemoglobin levels e.g. age, pregnancy and altitude of residence of subjects. As our subjects reside above sea level we adopted three methods to determine the prevalence of anaemia. These methods are:

1. WHO recommended adjustments
2. Adjustments recommended by Centre for Disease Control (CDC)
3. On the basis of –2 standard deviations of the mean haemoglobin concentration of the normal subjects

Another method for the estimation of prevalence of IDA was also adopted based on the serum ferritin levels of the subjects. These methods are further elaborated below:

WHO Method

As altitude from the sea level increase the partial pressure of oxygen decrease in the atmosphere. The lower partial pressure of oxygen leads to reduce oxygen saturation of blood. To compensate the diminished oxygen saturation at altitudes of more than1000 meters, haemoglobin concentration increases as an adaptive response. This is a normal physiological process. Long-term exposure to altitude (more than 1000 meters) increases the normal haemoglobin concentration in all individuals. Based on studies, WHO (2001) recommended adjustments in haemoglobin levels according to the altitude of the residence of subjects to determine the prevalence of anaemia. The WHO recommends following adjustments according to altitudes above sea level. (Table 2.3)

Table 2.3 **Altitude Adjustments in Hb according to WHO (2001)**

Altitude (metres)	*Increase in haemoglobin (g/dl)*
<1000	0
1000	+ 0.2
1500	+ 0.5
2000	+ 0.8
2500	+1.3
3000	+1.9
3500	+2.7
4000	+3.5
4500	+4.5

To estimate the prevalence of anaemia at altitude of 1000 m or more CDC recommends two ways:

- To add the correction values (the physiological increase in haemoglobin concentration due to long exposure to altitude) to the sea-level cut off values

- To subtract the adjustment from the measured haemoglobin concentration at the relevant altitude-- to the nearest 500m – to get the sea-level value

CDC Method

The CDC used data from 2 to 5 years children with little or no iron deficiency from clinics at 1200 to 3000 meters elevation to develop a curve that describes haemoglobin changes with altitude. The equation used to determine the effect of altitude is given below:

$$Hb = -0.32 \times (\text{altitude in metres} \times 0.0033) + 0.22 \times (\text{altitude in metres} \times .0033)^2$$

Table 2.4 **Hemoglobin adjustments (g/dl) for altitude (WHO, 2001)**

Altitude (m)	*Haemoglobin (g/dl)*
<1000	0
1000	0.1
1500	0.4
2000	0.7
2500	1.2
3000	1.8
3500	2.6
4000	3.4

-2SD Method

For the calculation of prevalence of anaemia in the present study sample -2SD method was followed as proposed by WHO (2001). In this method, WHO proposes "When individual haemoglobin levels are below two standard deviations (-2SD) of the distribution mean for haemoglobin in an otherwise normal population of the same gender and age who are living at the same altitude, iron deficiency anaemia is considered to be present". Based on this recommendation we determined the mean haemoglobin level of our subjects (on the basis of normal red cell morphology) and standard deviation. We subtract 2 SDs from the mean to establish the low cut-off value and labelled the population below this cut-off value as being anaemic.

Serum Ferritin Level

WHO (2001) suggests that the serum ferritin level is the most specific biochemical test that correlates with relative total body iron stores. Serum ferritin levels below 15μg/l in adult male and female population and below 12 μg/l in children of less than 15 years of age indicate depleted body iron stores. Serum ferritin levels were estimated by using EIA method.

Statistical Analysis

All values were expressed as mean ± standard error (S.E) as it shows validity of mean. Limit of significance was set at $P<0.05$. Student's *t*-tests were applied for the comparison of means. Regression analysis of variance was also applied to find out trends in relation to different variables and also, their significance from zero was calculated.

Statistical package Excel and Pad-Prism were used for data analysis.

Iron Deficiency Anaemia (IDA) in the Northern Areas of Pakistan

Questionnaire for females

Date _____

1. District _____
2. Village _____ Code _____
3. Height above sea level _____ m
4. Respondent No _____
5. Name _____
6. Present Age _____ years
7. Educational Qualification

Illiterate	☐
Primary	☐
Middle	☐
Matric	☐
FA/FSc	☐
BA/BSc	☐
MA/ MSc	☐
Any other (name)	☐

8. Height _____ cm

9. Weight _____ kg

10. MUAC _____ cm

11. Hb _____ g/dl

12. PCV _____ %

13. RBC count ____ 10^9/l

14. Red Cell Morphology

Hypochromioc Microcytic	☐
Megaloblastic	☐
Normal red cell	☐
Any other	☐

15. Color of stool (mealena)

Yellow	☐
Brown	☐
Black	☐
Any other	☐

16. Worm Infestation Yes ☐ No ☐

17. Result of stool sample _____

18. Whether goiter is present Yes ☐ No ☐

If Yes,

19. Scale of Goiter

0 ☐

1 ☐

2 ☐

20. Temperature of the body _____

21. Blood Pressure Reading _____

22. Abdominal examination _____

23. Age at Menarche _____ Years

24. Marital Status

Married ☐

Un – married ☐

25. If married whether

Pregnant ☐

Lactating ☐

Non-pregnant & Non-lactating ☐

26. Signs & Symptoms

(Mark √ whichever of the following is present)

Fatigue ☐

Weakness ☐

Pale Skin ☐

Pale Conjunctiva ☐

Unable to work properly ☐

Smooth tongue with fissures at the angles of mouth ☐

Palpitation ☐

Reduced ability to concentrate ☐

Spoon shaped nails ☐

Loss of appetite ☐

Headache ☐

27. Average monthly Income Rs_____

28. No. of Tea /day _____ cups

29. Who deserve better diet?

A boy ☐

A girl ☐

Both ☐

30. How far is the nearest healthy facility

< 5km ☐

> 5km ☐

Don't know ☐

31. What is the name of local health visitor?

Know ☐

Don't know ☐

32. Do you know what malaria is?

Yes ☐ No ☐

If Yes,

33. Do you know how malaria it spread?

Yes ☐ No ☐

34. Do you work barefoot in the fields?

Yes ☐ No ☐

35. Nutritional Assessment 24 hour Dietary Recall

Food Items	**Specification**	**Amount**
Milk Group		
Meat Group		
Vegetable Group		
Fruit Group		
Dry fruit Group		
Cereal Group		

Thank you

Iron Deficiency Anaemia (IDA) in the Northern Areas of Pakistan

Questionnaire for children

Date _____

1 District _____

2 Village _____ Code _____

3 Height above sea level _____ m

4 Respondent No _____

5 Name _____

6 Present Age _____ years

7 Educational Qualification

Illiterate ☐

Primary ☐

Middle ☐

8. Height _____ cm

9. Weight _____ kg

10. MUAC _____ cm

11. Hb _____ g/dl

12. PCV _____ %

13. RBC count ____ 10^9/l

14. Red Cell Morphology

Hypochromioc Microcytic	☐
Megaloblastic	☐
Normal red cell	☐
Any other	☐

15. Color of stool (mealena)

Yellow	☐
Brown	☐
Black	☐
Any other	☐

16. Worm Infestation Yes ☐ No ☐

17. Result of stool sample _____

18. Whether goiter is present Yes ☐ No ☐

If Yes,

19. Scale of Goiter

0	☐
1	☐
2	☐

20. Temperature of the body _____

21. Abdominal examination _____

22. Signs & Symptoms
(Mark √ whichever of the following is present)

Fatigue ☐

Weakness ☐

Pale Skin ☐

Pale Conjunctiva ☐

Unable to work properly ☐

Smooth tongue with fissures at the angles of mouth ☐

Palpitation ☐

Reduced ability to concentrate ☐

Spoon shaped nails ☐

Loss of appetite ☐

Headache ☐

23. No. of Tea /day _____ cups

24. Nutritional Assessment 24 hour Dietary Recall

Food Items	**Specification**	**Amount**
Milk Group		
Meat Group		
Vegetable Group		
Fruit Group		
Dry fruit Group		
Cereal Group		

25. Whether the three meals are taken regularly

Yes ☐ No ☐

26. Milk is taken _____ glasses/day

Thank you

RESULTS

General Information About the Subjects

Adult Females:

A total of 426 households were visited – 261 (61%) in Gilgit district and 165 (39%) in Ghizer district. There were 10 no responses in total. The number of respondents in strata females and children are summarised in Table 3.1.

General information related to family and household was collected from the female respondents. Out of the total of 416 child bearing age (CBA) women visited, 382 women provided the requested information. The findings presented below are based upon the responses of 382 women.

General Profile of the Females

The mean altitude above sea level at which these female subjects were residing was 1878±31.73 m. The mean age of the females was 28.43±0.4 years, their mean height was 154.80±0.29 cm and mean weight was 51.13±0.43 kg. The mean Mid Upper Arm Circumference (MUAC) was 22.98±0.14 cm; mean Body Mass Index (BMI) was 21.3±0.16 kg/m^2.

Clinical analysis of blood samples collected from these females included different variables as listed in Table 3.2. Mean serum ferritin level was 33.50±2.07 μg/l; mean Hb level was 13.78±0.10g/dl; mean RBC counts were 3.38±0.03x10^9/l; mean PCV was 40.20±0.48 l/l; mean MCV was 103.05±1.43 fl; mean MCH was 35.49±0.42 pg and mean of MCHC was 31.85±0.42 g/l. The mean age of menarche of all females was 14.87±0.08 years. They consumed 6.20±0.12 cups of black tea (with little milk) on average per day. Information on monthly income reveals a mean income of 7.14±0.36 thousand rupees.

Table 3.1 **Number and percentage of adult females and children respondents in Gilgit and Ghizer districts of the Northern Areas**

Category	Gilgit District		Ghizer District		Total
	Number	%	Number	%	
Female (15 to 50 yrs)	257	61	159	39	416
Children (2 to 12 yrs)	112	59	78	41	190

Table 3.2 **General profile of the female subjects under study for Iron Deficiency Anaemia**

S.No	Variable	Mean ± SE
1.	Altitude	1878.83 ± 31.73 m
2.	Age	28.43 ± 0.40 years
3.	Height	154.8 ± 0.29 cm
4.	Weight	51.13 ± 0.43 kg
5.	MUAC	22.99 ± .14 cm
6.	Serum ferritin	33.50 ± 2.07µg/l
7.	Hb	13.78 ± 0.10g/dl
8.	BMI	21.3+0.16 kg/m^2
9.	RBC count	3.38 ± 0.03x10^9/l
10	PCV	40.20 ± 0.48l/l
11.	MCV	103.05 ± 1.43fl
12.	MCH	35.49 ± 0.42pg
13.	MCHC	31.85 ± 0.42g/l
14.	Age at menarche	14.87 ± 0.08 years
15.	Tea/day	6.20 ± 0.12 cups
16.	Monthly Income (in 1000 Rs)	7.14 ± 0.36

Education

In Gilgit 35.86% of the female respondents had formal education compared to 24.86% in Ghizer district. The difference between the two percentages of educated females from these two districts was not significant (P>1.00). For this reason both districts were combined for further analysis.

Among the literate women 20.7% had education up to primary level (5 years of schooling), 20.7% up to middle level, 34.7% were matriculate, 19% were at intermediate level and only 5% were graduates.

The older women between 40-45 years of age had no formal school education. The younger females between ages 15-20 years (50.0- 68.0%) had proper formal school and college education.

Reproductive Health

Marital Status

Among women, unmarried females were 62 (16.2%), married (non-pregnant and non-lactating) were 131 (34.2%), pregnant females were 63 (16.4%) and lactating females were 126 (33.0%) in number.

Health Awareness

Ninety three percent of respondents stated that the nearest health facility is within 5 kilometre radius whereas in case of 4.7% households the nearest health facility was more than 5 kilometres and 2.4% respondents were unaware of the distance.

Ninety one percent of women knew the name of the local community-based health worker either as Lady Health Worker (LHW) or Aga Khan Health Services, Pakistan (AKHS, P) trained community health worker. Overall 19.3% of respondents, mainly educated women, knew about malaria and its mode of spread.

On inquiring that who, either male or female children, need better and more diet, 31% of women responded male children need better diet and 5% were

in favour of giving better diet to girls. According to 64% of respondents both male and female children need good diet. Gender preference for a male child exists in this society.

Sixty four percent of women frequently and 14% occasionally used to work barefoot in the fields whereas 22% used shoes in the fields.

Nutrition & Nutritional Status

Height and Weight

Females with weight under 40 kg and /or height less than 150 centimetres (4 feet 10 inches) were considered underweight and short in size. In the study population 7.33% of women were grossly underweight and 5.4% were very short. If pregnant women were excluded the proportion of underweight women increased to 8.32%. By extending the criteria for weight and height up to 45 kg and 155 cm the prevalence of underweight women extended to 22.0% and short stature to 17.14% for this study.

Body Mass Index (BMI)

The mean BMI calculated for females was 21.3±0.16 kg/m^2. Of all females 22.13% of females had BMI less than 19 kg/m^2. Most of the women (42.12%) had a body mass index from 19- 21.9 kg/m^2. The highest body mass index more than 28 kg/m^2 was observed in 5.23% women. Only a negligible number of women (0-3%) had BMI measuring less than 13 (Table 3.3).

Mid Upper-Arm Circumference (MUAC)

Using the standard value of 23.0cm as reference, 50.82% women were in this range. The mean MUAC of sample females was 23.0cm (±2.78).

Caloric Consumption

Daily caloric intake on average in adult non-pregnant non-lactating females was 1041 k cal. It was found to be 1172 k cal among pregnant females and 1000 k cal consumed by lactating females (Table 3.4). The recommended caloric intake for adult non-pregnant non-lactating females was 2100 k cal, for pregnant it was

2500 k cal and for lactating females the amount of calories recommended was 3100 k cal. By calculating their caloric intake it was found that on average the adult non-pregnant non-lactating females, pregnant females and lactating females consumed 49.61 %, 46.92 % and 32.31 % of their recommended allowance, respectively.

Table 3.3 **Percent distribution of women according to BMI (kg/m^2)**

Body Mass Index (kg/m^2)	Overall
< 13	0.3%
13 – 15.9	1.8%
16 – 18.9	20.0%
19 – 21.9	42.1%
22 – 24.9	22.9%
25 – 27.9	7.8%
28 or more	5.2%

Table 3.4 **Daily caloric intake in females with different physiological states**

Population Group	Average Intake (K cal)	Recommended Intake (K cal)	Average % of Recommended K calories consumed	Percent Under 60% of RDA
*Adult Female	1041	2100	49.6	75.0
Pregnant	1172	2500	46.9	75.4
Lactating	1000	3100	32.3	98.4

* Unmarried and non-pregnant and non-lactating married females

Iron Intake

Iron intake on average per day is given in Table 3.5. Both pregnant and lactating females were taking 4.7 mg iron per day while adult non-pregnant non-lactating females were taking 5.1 mg of iron per day. Recommended iron intake for pregnant and lactating females was 33 mg per day and it was 28 mg per day for adult non-pregnant non-lactating females. It was found that on average the adult non-pregnant non-lactating females, pregnant females and lactating females consumed 15 %, 14 % and 17 % of their recommended allowance for iron (mg), respectively.

Clinical Signs and Symptoms

Eyelid pallor was found in 55% of women. Facial pallor was detectable in 14% of females. Koilonychia (spoon shaped nails) was present in 8% of women and atrophy of lingual papillae and whitish colouration of tongue was noticed in 3.14%.

Fatigue and exhaustion was reported by 79% of female respondents, 83% of women complained of palpitation; 62% of women reported lack of concentration and forgetfulness. Headache was found in 85% of females.

Haematological Indices

Summary of the findings of indices including red cells count and packed cell volume by different states of females is given in Table 3.6. The three categories of women namely pregnant, lactating and non-pregnant and non-lactating (married, unmarried), indicated that there was no difference in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Similarly there was no appreciable difference in the mean RBC count and mean packed cell volume between the three categories. However, the mean cell volume of the lactating females (104 fl) was somewhat lower as compared to the pregnant females (111 fl).

Table 3.5 **Daily iron intake among different female groups**

Population Group	Average Intake (mg)	Recommended Intake (mg)	Average% of Recommended mg consumed	Percent Under 60% of RDA
Adult Female	5.1	28	15	99
Pregnant	4.7	33	14	100
Lactating	4.7	33	17	100

Table 3.6 **Haematological Indices of the female subjects during different physiological states**

Haematological Index	Women of Childbearing Age		
	Pregnant	Lactating	Adult* Females
RBC Count (x 10^9/l)	3.7	4.1	3.9
Packed Cell Volume (l/l)	0.407	0.421	0.418
Mean Cell Volume (fl)	111	104	108
Mean Cell Haemoglobin (pg)	36	35	36
Mean Cell Haemoglobin Concentration (g/l)	32.5	33.3	33.5

*unmarried and non pregnant and non lactating married females

Data Analysis

There were 382 females who provided detailed required information. For further analysis only these 382 female subjects were included in the study.

Age Related Changes

The age of female subjects ranges from 15 to 50 years. All females were divided into different age brackets, with an interval of 5 years. The highest representation was in two age groups from 25 to 34 years (39.2%). The lowest representation was in the age group from 45 to 50 years (3.9%) (Table3.7). The results demonstrated that the increase in age made no significant difference in the mean heights of the female respondents. Likewise, there was no appreciable change in the weights of females with increase in age. There was no significant change in MUAC of the respondents with increase in age. Mean age of menarche varies from 14.5±0.17 years to 15.33±0.33 years. The difference between the two was not significant ($t_{(75)}$=0.84; P>0.4). Mean cups of tea taken per day by the older women of mean age of 46.46±0.53 years was significantly higher than taken by the younger women of mean age of 16.9±0.19 years ($t_{(75)}$=6.44; P<0.001). Mean monthly income ranges from 5.32±1.09 rupees to 8.73±1.25 rupees. There was no significant difference in the monthly income of mean age groups of 16.9 ±0.19 years and 30.5±0.11 years ($t_{(115)}$ =1.55; P>0.10). Also, non significant difference in monthly income was observed in females with mean age 30.5±0.11 years and 46.46±0.53 years ($t_{(80)}$=2.13; P>0.05) (Table 3.8 a).

Serum ferritin levels of females also increased with age but that was not a systematic increase, it fluctuated, the highest mean serum ferritin levels (86.18±27.94μg/l) were observed in females of age between 45 to 50 years. Regression analysis of variance showed that the increase in mean serum ferritin with age was not statistically significant (b=6.506±3.22; $F_{(1,5)}$=4.07; P>0.099). There was no significant increase in the Hb levels of all categories based on the age of the respondents but a non significant increase was seen after 45 years of age (14.32±0.35 years) as compared to youngest females in the sample (15-19 years) ($t_{(75)}$=1.40; P>0.10) whose mean Hb levels were 13.7±0.27g/dl. Mean PCV in the youngest females (15-19 years) was 41.16±0.54l/l and the highest mean

was seen in the older females in the sample (45-50 years) as 43.86±1.82l/l which was statistically not a significant increase ($t_{(75)}$=1.42; P>0.10). Mean MCV (111.29±3.08fl) was the highest in the youngest females (15-19 years) which gradually decreased and in the oldest females it was 103.46±3.96 fl, but this decrease was not statistically significant ($t_{(75)}$ =1.56; P>0.10). The lowest mean MCV (99.16±3.22fl) was observed in females of age ranging from 25 to 29 years. This decrease in mean MCV in this age class was significant compared to that in age class 15-19 years ($t_{(135)}$=2.72; P<0.01). With the advance in age there was no appreciable difference in mean MCH and mean MCHC (Table 3.8 b).

Table 3.7 **Different age classes among female groups (number and percentage)**

Classes	Age in years	No	%
1	15-19 years	62	16.2
2	20-24 years	54	14.1
3	25-29 years	75	19.6
4	30-34 years	75	19.6
5	35-39 years	73	19.1
6	40-44 years	28	7.3
7	45-50 years	15	3.9

Table 3.8a **Age related changes in relation to different characteristics of adult females**

Age (yrs) Classes	Age (yrs)	Height (m)	Weight (kg)	MUAC (cm)	Age (yrs) at Menarche	Tea/day cups	Monthly Income (Rs)
15-19	16.9 ± 0.19	155.0± 0.66	49.15 ± 0.9	22.33 ± 0.32	14.5±0.17	5.38±0.22	6.62±0.55
20-24	21.38±0.21	155.6±0.74	50.65±0.93	22.37±0.38	14.96±0.21	5.56±0.28	7.25±0.64
25-29	26.13±0.14	155.27±0.65	52.12±0.95	23.13±0.31	15.14±0.19	6.13±0.26	7.35±0.80
30-34	30.50±0.11	155.5±0.79	52.99±1.02	23.53±0.33	14.55±0.18	6.63±0.28	8.73±1.25
35-39	35.41±0.11	153.46±0.58	49.72±0.99	22.74±0.29	14.89±0.20	6.87±0.31	6.30±0.75
40-44	40.14±0.08	154.51±0.93	51.68±1.58	23.95±0.51	15.33±0.33	6.75±0.50	6.39±1.11
45-50	46.46±0.53	151.97±1.40	52.6±3.63	23.96±0.86	15±0.59	8.6±0.45	5.32±1.09

Table 3.8b **Age related changes in relation to different haematological indicators of adult females**

Age (yrs) Classes	RBC Count ($x10^9$/l)	Serum ferritin (µg/l)	Hb (g/dl)	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (g/l)
15-19	3.80 ±0.07	28.11± 3. 59	13.7±0.27	41.16±0.54	111.29±3.08	36.97±1.16	33.38±0.61
20-24	3.89±0.10	29.54±4.11	13.66±0.25	40.55±1.32	104.77±1.06	34.36±1.06	31.34±1.062
25-29	3.86±0.10	31.03±3.99	14.0±0.25	40.2±1.14	99.16±3.22	35.05±1.06	32.01±1.08
30-34	3.92±0.07	30.76±4.28	13.65±0.19	39.44±1.24	100.64±3.52	35.72±0.79	30.78±1.10
35-39	3.96±0.07	36.60±4.22	13.79±0.22	40.39±0.97	102.32±2.72	35.60±0.96	32.69±0.85
40-44	3.58±0.17	30.74±6.93	13.61±0.42	37.03±2.66	100.11±7.27	35.47±1.70	29.08±2.12
45-50	4.31±0.21	86.18±27.94	14.32±0.35	43.86±1.82	103.46±3.96	33.96±1.18	32.94±1.20

Altitude Related Changes:

Altitude related changes are shown in Table 3.9 a, b. The altitude classes were categorized at an interval of 500 m. The highest number of females in the sample was from 1000-1499 m height (35.60%) and the lowest representation was from an altitude of 3000+ m (4.97%). There was not much difference in the mean age of the females with the increase in altitude. The highest mean weight (53.39±0.74 kg) was seen in the females from 1000-1499m height and the lowest mean weight was in females from 3000+ heights. It was a statistically significant decrease ($t_{(153)}$=4.59; P<0.001) compared to the former altitude class (46.18±1.39kg). There was no significant decrease in height towards higher altitude ($t_{(153)}$=1.68; P>0.05). A highly significant increase in mean cups of tea taken per day was observed at higher altitude ($t_{(153)}$=12.84; P<0.001), but in the case of monthly income there was a significant decrease at higher altitude ($t_{(153)}$=4.99; P<0.001). Mean MUAC decreased significantly in females from 3000+ altitude compared to those who were residing at 1000-1499m altitude ($t_{(153)}$=3.81; P<0.001). Similarly, mean RBC count ($t_{(153)}$=2.93; P<0.01) and mean Hb ($t_{(153)}$=4.36; P<0.001) increased significantly in the highest altitude (3000+m), compared to the lowest altitude (1000-1499m). A significant increase in mean MCHC levels of the females ($t_{(153)}$=5.87; P<0.001) was also seen in the highest altitudes compared to the lowest altitude classes. Iron intake in the lowest altitude is significantly high than in the highest class ($t_{(153)}$=10.36, P<0.001) In other parameters no appreciable change was seen with the increase in altitude.

Table 3.9a **Altitude related changes in different characteristics of adult females**

Altitude (m)	Number of subjects	Age (yrs)	Caloric Intake (k cal)	Iron intake (mg)	Height (m)	Weight. (kg)	MUAC (cm)	Age at Menarche (yrs)	Tea/day cups	Monthly Income (Rs)
1000-1499	136	28.28±0.67	1241.07±40.8	5.3±0.25	155.15±0.42	53.39±0.74	23.90±0.24	14.05±0.13	5±0.13	7.2±0.63
1500-1999	48	28.41±1.08	1384.18±57.3	8.23±0.4	155.06±0.72	50.97±1.33	22.35±0.40	14.66±0.24	6±0.20	7.28±1.18
2000-2499	131	27.91±0.72	849.33±23.1	3.49±0.18	154.95±0.58	50.55±0.68	22.65±0.21	15.5±0.12	6.34±0.18	7.56±0.57
2500-2999	48	30.06±1.25	879.39±41.9	4.48±0.7	153.88±0.80	48.425±1.11	22.58±0.36	15.76±0.17	7.91±0.44	6.95±1.12
3000+M	19	29±1.37	809.57±19.7	2.6±0.1	152.95±1.24	46.18±1.39	21.42±0.61	14.88±0.41	10.15±0.38	3.63±0.34

Table 3.9b **Altitude related changes in different haemotological indices of adult females**

Altitude (m)	RBC Count ($x10^9$/l)	Serum Ferritin (µg/l)	Hb (g/dl)	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (g/l)
1000-1499	3.70±0.066	35.28±4.10	13.28±0.19	41.04±0.69	110.49±2.91	36.55±0.86	30.69±0.70
1500-1999	3.94±0.099	37.53±7.87	14.19±0.32	42.29±0.822	107.89±1.87	36.85±1.17	33.89±0.85
2000-2499	3.99±0.07	31.61±2.59	13.82±0.13	37.87±1.11	93.10±2.56	33.39±0.58	30.80±0.87
2500-2999	4.01±0.07	31.94±4.33	14.10±0.21	41.56±0.66	103.96±1.26	35.68±0.84	34.102±0.59
3000+M	3.97±0.07	27.56±7.48	15.2±0.40	41.63±0.83	103.92±1.85	38.43±1.09	36.51±0.71

Caloric Consumption

Table 3.10 shows different classes according to caloric consumption at an interval of 500 kcal. The Table also showed means for such variables as Iron intake, serum ferritin levels, Hb levels, Tea/day, Age, Height, Weight and MUAC.

The highest percentage of subjects (51.83; n = 198) was in the category of 500 – 1000 kcal. The lowest percentage of subjects (1.05; n=4) was consuming the highest calories (>2000kcal). Regression analysis of variance shows non-significant decrease in percentage of subjects with the increase in mean caloric consumption (b= - 4.24; $F_{(1,3)}$= 0.37; P >0.58)(Fig 3.1).

The highest mean caloric consumption (2804.75±411.08 k cal) was observed in the age group of 27.42±1.53 years. The lowest mean caloric consumption (409.47±27.69 k cal) was seen in the youngest age group (25.5±3.88 years) of subjects. The difference between these two groups was highly significant ($t_{(21)}$=5.81; P<0.001). In the subjects with highest mean age (29.45±0.83 years) mean caloric consumption was quite low (1204.43±14.15 k cal). It was significantly low caloric consumption compared to the subjects consumed highest mean calories (2804.75±411.08 k cal).

Table 3.10 **Classes of caloric consumption and mean values for different variables in adult females**

S. No	Classes on the basis of caloric consumption	Mean calories (Kcal)	Iron intake (mg)	Age (yrs)	Height (m)	Weight (kg)	MUAC (cm)	Serum ferritin (µg/l)	Hb (g/dl)	Tea/day cups
1	0-<500kcal (n=19)	2804.75±411.08	2.9± 0.76	27.42±1.53	156.9±1.38	51.43±1.96	23.26±0.64	24.58±7.6	13.37±0.43	5.44±0.56
2	500-1000kcal (n=198)	1693.45±17.07	3.37± 0.17	28.37±0.58	154.62±0.44	50.26±0.55	22.68±0.17	32.05±2.17	13.92±0.13	6.68±0.18
3	1001–1500kcal (n=95)	1204.43±14.15	5.61± 0.2	29.45±0.83	154.75±0.53	51.85±0.96	23.29±0.32	40.07±5.9	13.70±0.18	5.65±0.18
4	1501–2000kcal (n=66)	802.5±7.53	8.64± 0.42	27.60±0.93	154.95±0.56	52.60±1.03	23.32±0.34	29.49±4.29	13.58±0.30	5.7±0.25
5	>2000kcal (n=4)	409.47±27.69	4.5± 0.98	25.5±3.88	152.87±2.3	51.6±4.9	24.47±1.58	58.1±39.52	13.85±0.43	6±1.08

Upside down

There was no appreciable change in mean Hb (g/dl), tea/day, height (cm), weight (kg) and MUAC (cm). A positive trend towards increase in serum ferritin levels (μg/l) had been observed with the increase in mean caloric consumption. Regression analysis of variance showed a non-significant increase in serum ferritin levels as the caloric consumption increases ($b= 6.45\pm3.02$; $F_{(1,3)}= 4.55$; $P>0.12$) (Fig 3.2). Mean iron intake appeared to be related to the mean caloric consumption. There was decrease in mean iron intake with the decrease in a mean caloric consumption. Regression analysis of variance showed non-significant decrease in mean iron intake as the mean caloric consumption decreased. ($b= - 0.85\pm0.68$; $F_{(1,3)}=1.56$; $P>0.29$) (Fig 3.3).

Prevalence of Iron Deficency Anaemia (IDA)

WHO Method

By applying WHO adjustments the prevalence of anaemia among pregnant women was found to be 22.23% and among non-pregnant it was recorded as 15.73%.

CDC Method

Applying CDC method i.e. adding the correction value calculated using the formula *[Hb = -0.32 x (altitude in meters x 0.0033) + 0.22 x (altitude in meters x 0033)^2]* the prevalence of anaemia in pregnant was 24% and in non-pregnant women it was 17%.

The details are shown in the tables 3.11 and 3.12.

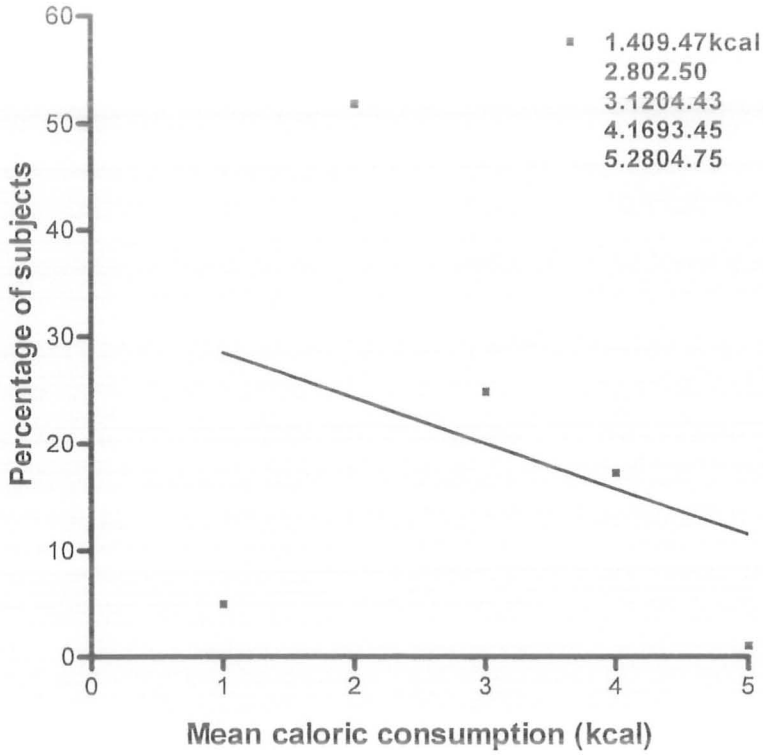


Fig.3.1 Regression analysis of variance showsdecrease in the percentage of women with the increase in mean caloric consumption (kcal)(P>0.58)

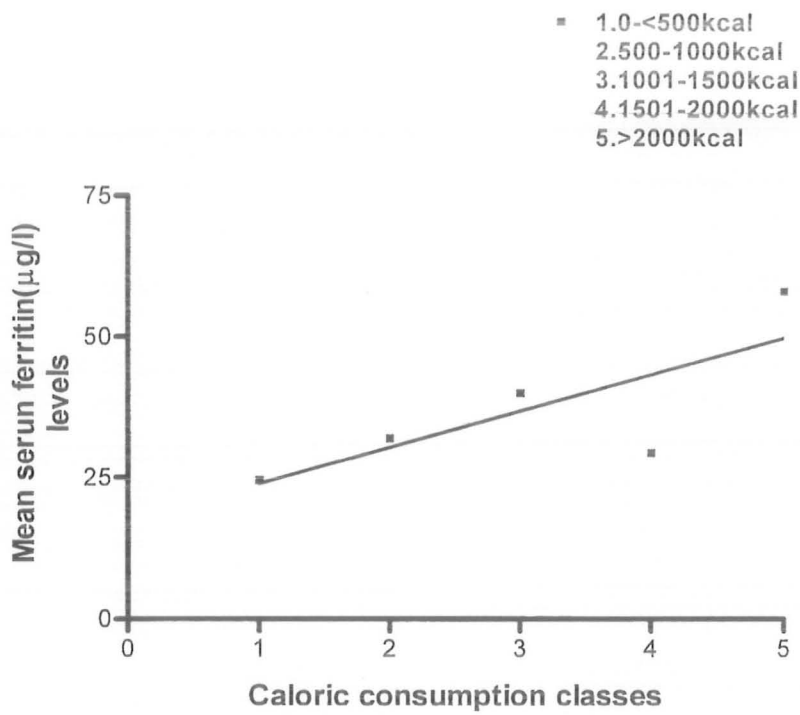


Fig.3.2 Regression analysis of variance shows increase in mean serum ferritin (µg/l) levels with the increase in caloric consumption in women (P>0.12)

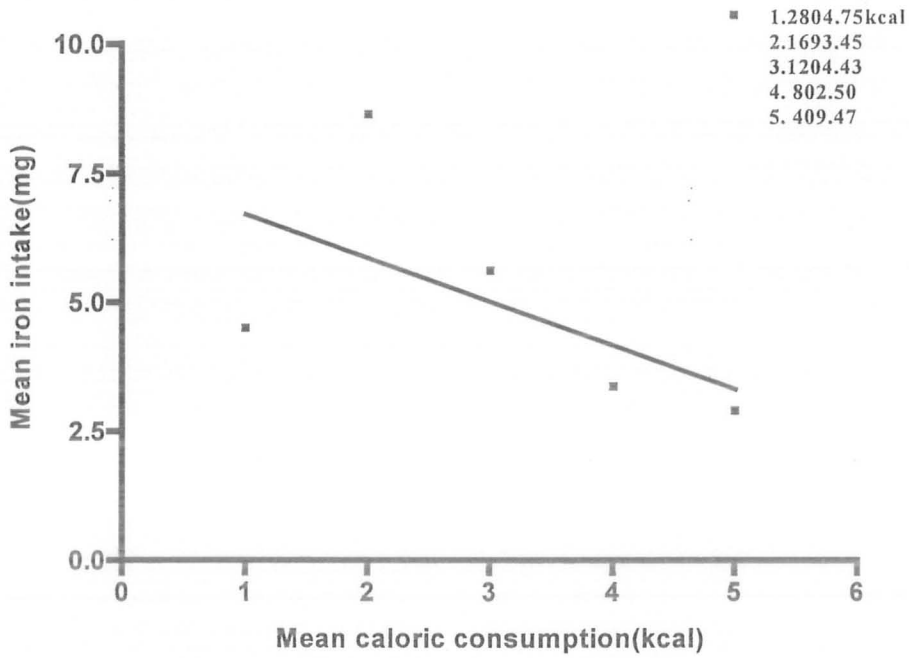


Fig.3.3.Regression analysis of variance shows decrease in mean iron intake(mg) with the decrease in mean caloric consumption(kcal) in women(P>0.29)

Table 3.11 **Prevalence of anaemia in pregnant and non-pregnant women by applying WHO method**

Female Group	Anaemic		Non-Anaemic	
	No.	%	No.	%
Pregnant (63)	14	22.2	49	77.8
Non pregnant (319)	50	15.7	269	84.3

Table 3.12 **Prevalence of anaemia in pregnant and non-pregnant women by applying CDC method**

Female Group	Anaemic		Non-Anaemic	
	No.	%	No.	%
Pregnant (63)	15	23.8	48	76.2
Non pregnant (319)	55	17.2	264	82.8

- 2SD Method

According to this method out of 382 female respondents the number of females with normochromic normocytic red cells was counted in the sample under study. There were 108 (28.27%) females who had normal red cells (normochromic normocytic). The mean Hb of the female subjects with normochromic, and normocytic RBCs was 15.13 g/dl with a standard deviation of ±1.68. From their mean Hb levels a -2SD value was calculated and it was used as a low cut-off point for the detection of IDA for the study population. The cut-off value was calculated as 11.77 g/dl. By using this 11.77 g/dl as a cut-off point of Hb for the prevalence of IDA for pregnant (n=63) and non-pregnant (n=319) females the prevalence of anaemia among pregnant women was calculated as 26.98% and in non-pregnant women it was 10.34%.

Prevalence of Iron deficiency Anaemia on the Basis of RBC Morphology (±2SD Method):

The prevalence of IDA for the whole female sample population was calculated based on RBC morphology. In 108 females with normal RBC morphology mean Hb and serum ferritin levels calculated were 15.13±0.16g/dl and 35.95±4.23μg/l, respectively. The calculations were made by applying the ±2SD formula. The low cut-off point as discussed earlier was 11.77g/dl and the high cut-off point was 18.49g/dl.

All the female respondents (n=382) were, then, divided into four groups:
those with Hb levels less than 11.77g/dl (< -2SD)
those with Hb levels between 11.77 to 15.13g/dl (-2SD)
those with Hb ranging from more than 15.13 to 18.49 g/dl (+2SD)
those with Hb levels above 18.49g/dl (>+2SD)

In the first group were 50 females (13.08%) with mean Hb levels of 10.25±0.15g/dl and mean serum ferritin levels of 16.84±3.11μg/l. Those females with less than 11.77g/dl Hb were considered as severe IDA patients.

In the second group were 271 females (70.94%) whose mean Hb levels were 13.79±0.05g/dl and serum ferritin levels were 34.82±2.4μg/l. These females were taken as patients suffering from mild IDA.

Table 3.13 **Area under curve, number of subjects, percentage of subjects with normal RBC, mean Hb (g/dl) and mean serum ferritin (μg/l)**

S.No	Area under curve Hb (g/dl)	No.	Subjects with *nc(%)	Mean Hb (g/dl)	Mean serum ferritin (μg/l)
1	<11.77	50	0(0.0)	10.25±0.15g/dl (50)	16.84±3.0
2	11.77-15.13	271	70(25.84)	13.79± 0.05g/dl (271)	34.82±2.4
3	15.13-18.5	55	34(61.81)	16.05±0.10g/dl (55)	44.00±7.16
4	>18.5	6	04(66.66)	20.78±0.6g/dl (6)	16.25±4.4

*nc= Normal Red Blood Cell Morphology

Table 3.13 a **Area under curve, number of subjects with normal RBC morphology, their mean Hb (g/dl) and mean serum ferritin (μg/l) level**

S.No	Area under curve (Hb g/dl)	Number of Subjects with *nc	Mean Hb (g/dl)	Mean serum ferritin (μg/l)
1	<11.77	0	0	0
2	11.77-15.13	70	14.32±0.08 (70)	34.68±4.29 (70)
3	15.13-18.5	34	16.05±0.12 (34)	40.97±10.15 (34)
4	>18.5	04	21.7±0.36 (4)	15.42±3.86 (4)

*nc= Normal Red Blood Cell Morphology

In the third group were 55 females (14.39%). Mean Hb levels were 16.05±0.10g/dl and mean serum ferritin level was 44.0±7.16μg/l. These were regarded as healthier females.

In the fourth group there were only 6 females (1.57%). Their mean Hb level was 20.78±0.6g/dl and serum ferritin level was 16.25±4.4μg/l. These females were considered as the healthiest females, in relation to iron deficiency anaemia.

These results showed that the lowest mean Hb (10.19±0.15 g/dl) levels were in females falling below the cut off point. There was a systematic increase in mean Hb levels in the – 2SD area under the curve (13.79±0.05g/dl); the +2SD area under the curve (16.05±0.10g /dl) and the highest mean Hb levels (20.78±0.6g /dl) were in the area above the +2SD area. In these four divisions the percentage of females with normal RBC morphology was 0 %(n=0), 25.64 %(n=70), 61.81 %(n=34) and 66.66 %(n=4).

The pattern of Hb level indicated that there was an increase in mean Hb level as we move from the –2SD towards the +2SD area under the curve. Regression analysis of variance showed that the increase in mean Hb level was highly significant as the percentage of subjects with normal RBCs increases (b= 3.38±0.32; $F_{(1,2)}$ =108.4; P= 0.0091).

The women with normal RBC morphology in the four areas under curve were analysed separately for their mean haemoglobin and serum ferritin (Table 3.13 a). These women showed higher concentrations of Hb compared to the Hb concentrations in the overall female sample.

Prevalence IDA in Relation to Different Serum Ferritin Levels:

All the females were sorted for serum ferritin levels in an ascending order. The subjects were divided into different serum ferritin level groups with an interval of 15 μg/l (WHO 2001).

Table 3.14 **Mean serum ferritin and Hb levels, number and percentage of subjects with normal RBC and number of females in each serum ferritin class**

Serum Ferritin Classes	No and % of Subjects	Mean serum ferritin (µg/l)	Mean Hb (g/dl)	No. of Subjects with Normal RBCs (%)
0-14.9	149 (39)	7.15 ±0.37	13.16 ± 0.18	33(22.14)
15-29.9	91 (23.82)	22.28 ± 0.47	14.07 ± 0.21	29(31.86)
30-44.9	60 (15.71)	35.75 ± 0.57	14.51 ± 0.19	20(33.33)
45-59.9	26 (6.81)	53.45 ± 0.76	13.95 ± 0.35	12(46.15)
60-74.9	11 (2.87)	66.17 ± 1.13	14.45 ± 0.33	4(36.36)
75-89.9	14 (3.66)	84.01 ± 0.80	14.00 ± 0.32	3(21.43)
90-104.9	11 (2.87)	97.99 ± 1.42	13.8 ± 0.54	5(45.00)
>105	20 (5.23)	159.3 ± 15.02	14.08 ± 0.27	4(20.00)

The number and percentage of anaemic females in different serum ferritin classes are given in Table 3.14. The number of anaemic subjects (based on ferritin levels) decreased as the serum ferritin level increased. When regression analysis of variance of percentage anaemic subjects on serum ferritin levels was applied, it showed that there was a highly significant decrease in anaemic females as the serum ferritin level increased ($b= -4.53\pm1.13$; $F_{(1,6)} = 16.00$; $P <0.0071$) (Fig 3.4).

Regression analysis of variance of subjects with normal red cell morphology on serum ferritin levels shows a positive trend with increasing levels of serum ferritin, but this positive trend is not significant ($b = 0.062$; $F_{(1,6)} = 0.0013$; $P >0.97$).

Prevalence of IDA in Relation to Different Altitude Levels:

The altitude categories were made as mentioned in Table 3.15. The prevalence of severe IDA was calculated in different altitude categories by applying a low cut-off point 11.77g/dl of Hb as mentioned in the prevalence of IDA on the basis of normal RBC morphology.

In the lowest mean altitude level (1168.4±5.84 m), are 136 females (35.60%) and there were 29 females (21.32%) below (– 2SD i.e., 11.77g/dl). These females suffer from severe anaemia. In these 29 females none had normal RBC morphology.

In the next higher altitude category (1689.53 ± 16.25m) are 48 females (12.56%). Here 5 females (10.41%) were in the area below 11.77g/dl Hb (-2SD) cut-off point. There was no female with normal RBC morphology.

There were 131 females (34.29%) in the mean altitude of 2225.14±9.75m. Of these females, 11(8.39%) had severe anaemia that fall in the area below the low cut off point. There were no females with normal RBC morphology.

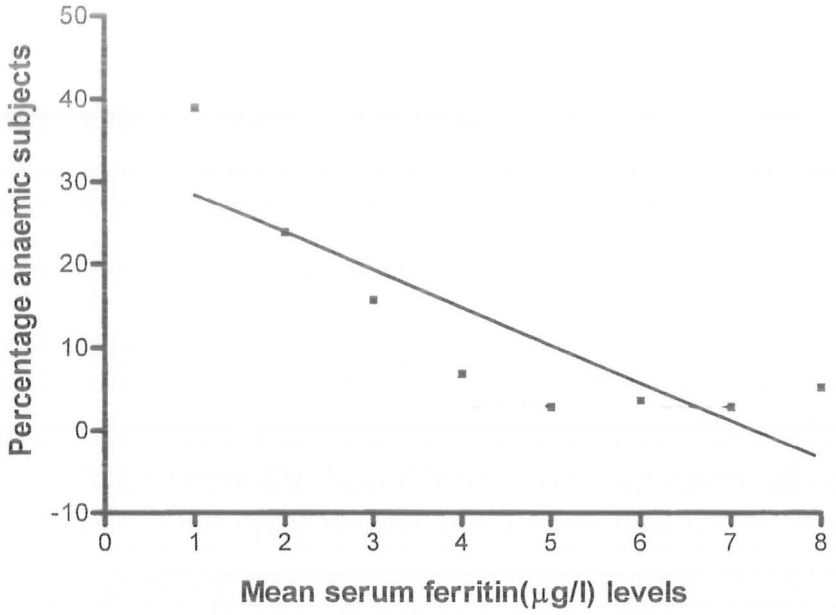


Fig.3.4 Regression analysis of variance shows decrease in anaemic women as the serum ferritin(μg/l) levels increase (P<0.0071)

At mean altitude of 2648.62±13.20 m, there were 48 females (12.56%) and of these females 4(8.3 %) suffered from severe IDA. In these anaemic females none had normal RBC morphology.

At the highest mean altitude of 3109.75±0.01m, there were only 19 females (4.97%). Of these females, only 1(5.2%) was anaemic with microcytic hypochromic red cell morphology. In all these altitude categories regression analysis of variance showed non-significant positive trends in Hb levels as the mean altitude increased ($b = 0.37 \pm 0.13$; $F_{(1,6)} = 7.5$; $P > 0.07$).

Table 3.15 **Mean altitude, mean Hb, mean serum ferritin levels, number and percentage of females below 11.77g/dl, and percentage of subjects with normal red cells in each altitude class**

Altitude class (No. of Subjects)	Altitude (m)	Hb (g/dl)	Ferritin (µg/l)	Number (%age) <11.77g/dl	Subjects with Normal RBCs%age
1000-1499(136)	1168.4±5.84	13.28±0.19	35.28+4.10	29(21.32)	0
1500-1999(48)	1689.53±16.25	14.19±0.32	37.52+7.87	5(10.41)	0
2000-2499(131)	2225.14±9.7	13.820.13	31.60+2.59	11(8.39)	0
2500-2999(48)	2648.62±13.20	14.10+0.21	31.93+4.33	4(8.3)	0
3000+(19)	3109.75±1.62	15.2+0.40	27.56+7.48	1(5.2)	0

The percentage of anaemic females decreases with the increase in altitude. Regression analysis of variance of anaemic females on altitude shows a non-significant decrease in anaemic females with the increase in mean altitude (b = -3.43±1.09; $F_{(1,3)}$ = 9.77; P >0.0522).

The lowest mean serum ferritin levels (27.56±7.48μg/l) were observed at the highest mean altitude (3109.75±1.62m). Regression analysis of variance of serum ferritin levels on altitude showed a non-significant decrease in serum ferritin levels as the mean altitude increased (b = -2.103±0.67; $F_{(1,3)}$= 9.57; P>0.0535).

Marital Status and Prevalence of Anaemia

All the female subjects were divided according to their marital status, i.e., unmarried, married, lactating and pregnant. In these females, while in a particular marital status, their mean Hb g/dl and mean serum ferritin levels μg/l were calculated to see if particular marital status has any effect on these variables. Females with normal RBC morphology and percentage anaemic female subjects were also examined (Table 3.16).

Mean Hb levels in lactating women were 13.98±0.12g/dl and mean serum ferritin levels were 30.09±2.6μg/l. There were 10 (7.9%) anaemic females (Hb levels<11.77 g/dl), none of the anemic females have normochromic normocytic red cells. There were 44 females (34%) anaemic on the basis of serum ferritin levels (serum ferritin levels less then 15μg/l).

In married females (n=131) mean Hb level was 14.03±0.17g/dl and serum ferritin level was 42.7±4.7μg/l. There were 14 (10.68%) females anaemic on the basis of Hb levels (<11.77 g/dl) and all these had hypochromic microcytic red cell morphology and 46 (35%) were anaemic based on serum ferritin levels (<15.0μg/l).

In unmarried females (n=62) mean Hb was 13.72± 0.25g/dl and mean serum ferritin levels were 33.02±4.32μg/l. There were 9 females (14.51%) who

had Hb levels below 11.77g/dl (lower cut-off point). None of them had normal red cell morphology. They were categorized as severe anaemic patients. Regarding serum ferritin levels, females with less than 15μg/l serum ferritin (WHO, 2001) were 32(51%) in number and these were considered as anaemic based on serum ferritin levels.

In pregnant females mean Hb levels were 12.90±0.32g/dl and that of serum ferritin were 21.63±3.21μg/l. There were 17(26.98%) anaemic females based on Hb levels and none had normochromic normocytic red blood cells. There were 27 (42.85%) females who were anaemic on ferritin basis (<15μg/l). The details are given in Table 3.16 (Fig 3.5, 3.5a).

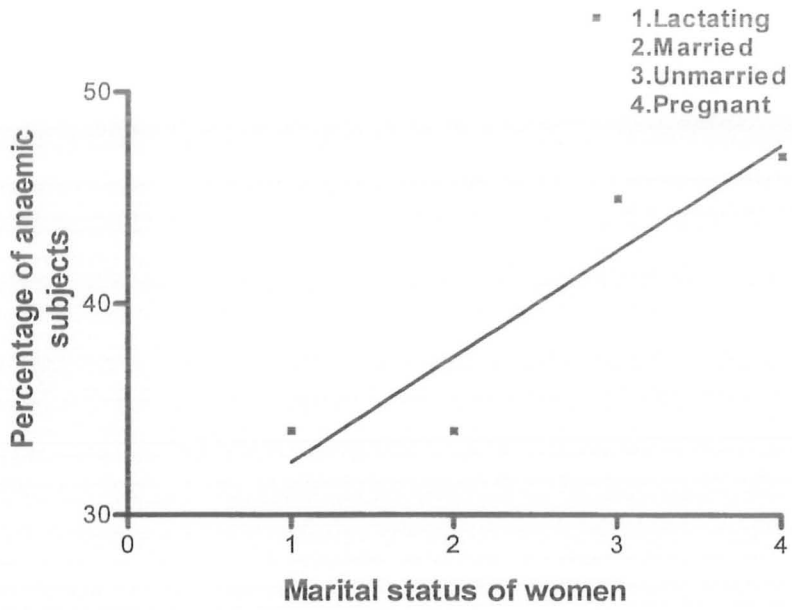
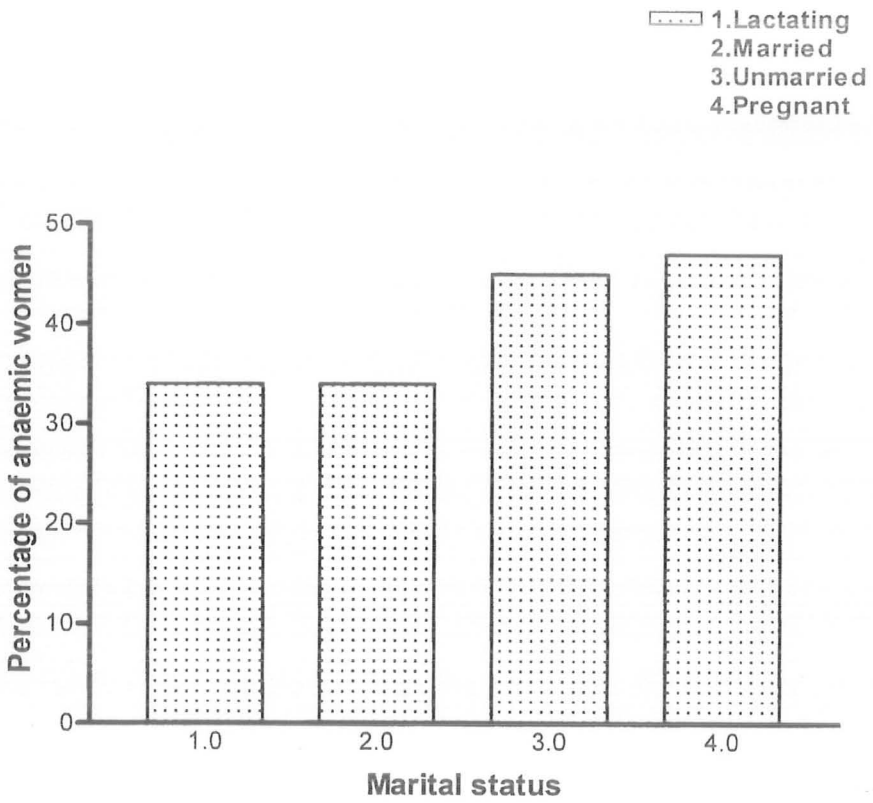


Fig.3.5 Regression analysis of variance shows percentage increase of anaemic women in different marital status based on serum ferritin (μg/l) levels(P>0.306.)



(a)

Fig.3.5 Percentage increase of anaemic women in different marital status based on serum ferritin (μg/l) levels

Table 3.16 **Marital status/physiological state, mean Hb, mean serum ferritin level and prevalence of IDA based on Hb and serum ferritin levels**

Marital status/ physiological state (No of subjects)	Hb levels (g/dl)	Serum ferritin levels (µg/l)	%age of anaemia Hb level <11.77 (g/dl) (No of subjects)	%age of anaemia Serum ferritinlevel <15 (µg/l) (No of subjects)
Lactating (126)	13.98±0.12	30.09±2.6	7.9 (10)	34.00 (44)
Married (131)	14.03±0.17	42.7±4.7	10.68 (14)	35.00 (46)
Unmarried (62)	13.72±0.25	33.02±4.32	14.51 (9)	51.00 (32)
Pregnant (63)	12.90±0.32	21.63±3.21	26.98 (17)	42.85 (27)

These females were arranged in ascending order for the percentage of anaemia in different marital status/physiological state (e.g., lactating, married, unmarried and pregnant) (Fig. 3.6). As shown in Table 3.16 the lowest anaemic females (7.9%) were from the lactating category and the highest percentages were from pregnant ones (26.98%). Regression analysis of variance showed non significant positive trend in the increase in number of anaemic females in relation to their marital status/physiological states when based on Hb levels ($b=6.11\pm16.2$; $F_{(1,2)}=14.15$; $P>0.06$).

Similarly, when the number of anaemic females in different marital status/physiological states was analysed based on serum ferritin levels <15ug/l, there was a non-significant increase in anaemic females in relation to their marital status/physiological states ($b=4.25\pm3.12$; $F_{(1,2)}=1.85$; $P>0.306$). As with Hb levels, here, too it's the pregnant females where we observe the highest percentage of anaemia (42.85%) and the lowest percentage is in the lactating category (34%).

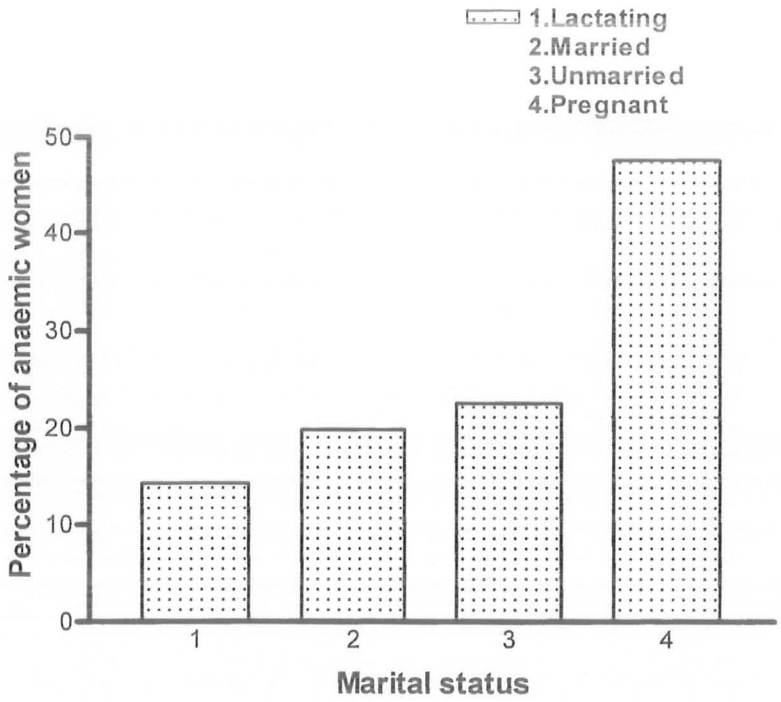


Fig 3.6 Percentage of anaemic women in different marital status/physiological state

General Information About the Subjects

Children:

The children were also included in this study as they were more attached to their mothers not only for maternal care but also for breast-feeding when they were of breast-feeding age. The age groups included in this study thus ranged from 2-12 years of age.

A total of 190 children were scored for IDA study (98 female children and 92 male children). There was difficulty in getting blood of children and that is why blood related data analysis is based on 175 children. Both sexes inclusive of their means of age, altitude, calories intake, height, weight and MUAC are based on 190 children. Mean RBC counts, serum ferritin levels, haemoglobin levels, PCV and MCV were calculated from 175 children. Altogether, 144 (75.8%) children were from rural and 46 (24.2%) from urban areas. Among female children over five years of age (63%) were school going, whereas among male children 52% were attending school.

The mean age of the children (male and females) was 7.92±0.21 years. The mean height and mean weight were 115.26±1.59 cm and 21.20±0.54 kg respectively. Their mean MUAC was 15.45±0.14cm. On average these children were living at a height of 1953.9±36.41m. Analysis of blood samples included the different variables is given in the Table 3.17.

Mean Hb level was 13.16±0.14g/dl and mean serum ferritin level was 36.67±3.21μg/l. Mean RBC count was $3.87\pm0.05\times10^{9}$/l; mean MCV was 108.67±1.62fl and mean PCV was 41.25±0.42l/l. Their mean tea consumption per day was 4.24±0.12 cups. Their mean caloric consumption was 607.9±16.69 k cal.

Seventeen percent of children took milk daily. Overall 54.2% of children had three meals a day on regular basis. Stool samples from 130 children were collected and examined for parasitic ova and cysts. Seventy-two children (38%) gave history of passing worms. The findings of laboratory examination showed

that the 79% of children had ova/cysts in their stool; most common parasite found was *Ascaris lumbricoides (43%),* followed by *Giardia* (16%), *Trichurus trichura* (12%) and *H.nana* (6%).

Table 3.17 **Mean ± SE of different variables in children for IDA study**

S. No	Variable	Mean ± SE
1.	Altitude	1953.9 ± 36.40 m
2.	Age	7.92 ± 0.21 years
3.	Height	115.2 ± 1.59 cm
4.	Weight	21.20 ± 0.54 kg
5.	BMI	15.16 ± 1.80 kg/m^2
6.	MUAC	15.45 ± 0 .14 cm
7.	Serum ferritin	36.66 ± 3.20μg/l
8.	Hb	13.16 ± 0.14g/dl
9.	Iron	2.73±0.12 mg
10.	RBCcount	3.87 ± 0.05x10^9/l
11.	PCV	41.25 ± 0.42 l/l
12.	MCV	108.66 ± 1.61 fl
13.	Tea/day	4.24±0.12 cups
14.	Calories	607.9±16.69 kcal

Nutritional Status of Children

Anthropometrical measurements taken included weight, height and mid upper arm circumference. Of the total children, 29.8% children of 2 to 12 years of age are under weight. There are 5.6% children who are severely malnourished.

Using the anthropometric index Z-score (or standard deviation score), the deviation of the value for an individual from the median value of the reference population divided by the standard deviation of the reference population, 29.8% (95% CI 23.0- 37.6%) of the children of 2-12 years of age are under weight for their age i.e., below –2 Z-score. There are 5.6% (95% CI 2.8-10.7%) children who are severely malnourished (below -3 Z-score).

BMI

The mean BMI for children is 15.16 cm ±1.80kg/m^2 ranging from 6.0 kg/m^2 to 25.7 kg/m^2. There were 47.3% children with BMI less than 15 kg/m^2. More than 18 kg/m^2 BMI include 4.7% children. (Table 3.18).

MUAC

The mean MUAC for all children in the sample is 15.47 (±3.469) cm. The mean MUAC of children of 2 to 4 years of age is 13.86 ±1.12 cm, for 5 to 9 years old children it is 14.75 ±1.413 cm and for children of 10 to 12 years it is 16.97 ±1.82 cm.

Clinical Signs and Symptoms

Eyelid pallor was found in 39% of children. Facial pallor was detectable in 18% of children. Koilonychia (spoon shaped nails) was present in 2% of children. Atrophy of lingual papillae and whitish colouration of tongue was noticed in 7% of children.

Fatigue and exhaustion was reported by 25% of children; 7% of children complained of palpitation; 12% of children reported lack of concentration and forgetfulness. Headache was found in 60% of children.

Table 3.18 **Distribution of children by BMI (kg/m^2)**

BMI Group	Number	Percent
3 to 6	1	0.5%
9 to 11	4	2.1%
12 to 14	85	44.7%
15 to 17	91	47.9%
18 to 20	8	4.2%
24 to 26	1	0.5%

Table 3.19 **Haematological indices of the sample children (male and female)**

Haematological Index	Children	
	2 – 4 (yrs)	5 –12 (yrs)
RBC Count (x 10^9/l)	3.9	3.9
Packed Cell Volume (l/l)	0.411	0.416
Mean Cell Volume (fl)	105	109
Mean Cell Haemoglobin (pg)	31	35
Mean Cell Haemoglobin Concentration (g/l)	31.6	32.7

Haematological Indices

As summary of the findings of other indices including red cells count, MCV, MCH, MCHC and packed cell volume in different children groups is presented in Table 3.19. There was no appreciable difference between red cell count, MCV, MCHC and PCV of the two groups of children (2-4yrs and 5-12 yrs). A slight difference was seen in the mean MCH of the two groups.

Daily Caloric Intakes

Daily caloric intake in children of age group 2-5 years was 529 k cal. It was 636 k cal among children of the age group 6-12 years. The recommended caloric intake for children of age group 2-5 years was 1300 k cal, and for children of age group 6-12 years it was 2100 k cal per day. By calculating their caloric intake it was found that on average the children of age group 2-5 years consumed 40.69% of their recommended allowance, while children of age group 6-12 years consumed 30.3% of their recommended allowance for daily caloric intake. The details are given in Table 3.20.

Iron Intake

Average iron intake per day is given in Table 3.21. Children aged 2-5 years were grouped together while children from 6-12 years of age were separated as male and female children for their daily iron intake consumption. Female children of age group 6-12 years were consuming 2.8 mg of iron per day while their recommended intake of iron was 21 mg per day. It was found that on average these female children consumed 13 % of their recommended allowance for iron (mg). Likewise, male children of age group 6-12 years were consuming 2.9 mg of iron per day while their recommended intake of iron was 16 mg per day. It was found that on average these male children consumed 18 % of their recommended allowance for iron (mg).

Children (both male and female) of age group 2-5 years were having 2.6 mg of iron per day and their recommended iron intake was 10 mg per day. It was calculated that these children of 2-5 years of age were having 26 % of their recommended allowance for iron (mg).

Table 3.20 **Daily caloric intake in children of different age groups**

Population Group	Average Intake (k cal)	Average % of Recommended K calories consumed	Average %of RDA	Percent Under 60% of RDA
Children2 –5 yrs	529	1300	40.7	91.6
Children 6 –12 yrs	636	2100	30.3	97.1

Table 3.21 **Daily iron intake in children of different age groups**

Population Group	Average Intake (mg)	Average % of Recommended K calories consumed	Average% of RDA	Percent Under 60% of RDA
Female 6-12 yrs children	2.8	21	13	100
Male 6-12 yrs children	2.9	16	18	100
Children 2 –5 yrs (Male and Female)	2.6	10	26	100

DATA ANALYSIS

Age Related Changes

Age-related changes in the variables under study are listed in Table 3.22. As the age of the children advanced significant increases in mean calories ($t_{(120)}$=4.93; P<0.001), height ($t_{(120)}$=13.70; P<0.001), weight ($t_{(120)}$=20.62; P<0.001), MUAC ($t_{(120)}$=12.56; P<0.001), Hb ($t_{(111)}$=3.96; P<0.001) and cups of tea/day ($t_{(112)}$=3.10; P<0.01) were observed. In the remaining variables no significant change was seen with increase in age. Significant increase in these variables indicated that there was no nutritional deficiency during their growing period. A significant increase in Hb (13.63±0.25 g/dl) levels at mean age of 11.05±0.09 years was seen compared to the Hb (12.40±0.19g/dl) of children at the youngest mean age of 3.87±0.14 years indicated perhaps resistance increases with age and this helps in defence against IDA. This inference had been drawn from the study of adult females where females with low Hb levels had severe anaemia.

Table 3.22 Age related changes in different variables among children under study

Age Groups (yrs)	Age (yrs)	Altitude (m)	Mean Calories (kcal)	Iron (mg)	Tea/day cups	Ht. (cm)	Wt. (kg)	MUAC (cm)
2-5 yrs.(48)	3.87± 0.14	2041.77±78.19	517.58± .24	2.56± 0.24	3.62±0.22	90.27± 2.21	12.98± 0.34	13.82± 0.15
6-9 yrs, (68)	7.36± 0.13	1970.82±61.04	560.47±24.06	2.40±0.16	4.37±0.19	115.15± 1.35	19.33± 0.43	14.97± 0.16
10-12yrs (74)	11.05± 0.09	1881.34±54.50	710.06±28.90	3.13±2.04	4.52±0.20	131.56± 2.04	28.24± 0.66	16.96±0.21

Age Groups (yrs)	RBCCount ($X10^9$/l)	Hb (g/dl)	Serum ferritin (µg/l)	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (g/l)
2-5 yrs.(48)	3.8±0.10	12.4± 0.19	31.67± 6.46	39.95±0.84	337.107.7±3.6	33.42± 0.91	31.95± 0.64
6-9 yrs, (68)	3.93±0.09	13.10± 0.21	34.01±5.14	41.45±0.74	106.31±2.70	34.35± 0.96	32.23± 0.65
10-12yrs (74)	3.87±0.07	13.63± 0.25	41.63±5.24	41.80±0.63	111.84±2.39	35.76 ± 0.75	32.74± 0.65

Altitude Related Changes

Altitude related changes in different variables are listed in table 3.23. The altitude classes were formed with a class interval of 250 m. In the case of mean calories consumption, the highest mean consumption (715.71±34.09 kcal) was observed at the lowest altitude class (1200 – 1449m). In the higher altitude classes mean caloric consumption decreasing and in the highest altitude class (2950+ m) mean caloric consumption (511.33±26.03 kcal) was very low compared to the lowest altitude class. Regression analysis of variance showed a highly significant decrease in caloric consumption as we go towards higher altitude (b= -34.18 ± 7.42; $F_{(1,6)}$= 21.20; P<0.0037). There was no significant difference in caloric consumption as age advances (b=19.22±13.72; $F_{(1,6)}$= 1.96; P> 0.21) when the mean age was arranged in ascending order.

Mean iron intake showed a non-significant decrease as altitude increased (b= -0.097±0.04; $F=_{(1,6)}$=4.64; P>0.074). The highest iron intake (3.22± 0.36 mg) was at the lowest altitude (1278.57±13.78 m) and lower iron intake (2.27±0.24 mg) at the altitude of 2158.25±6.13 m. Higher mean iron intake with advancing age was also not significant compared to younger age (b= 0.045 ± 0.05; $F=_{(1,6)}$ 0.63; P> 0.45).

Mean MUAC (cm) was highest (16.52±0.69 cm) at the lowest altitude (1278.57±13.78 m) and lowest (13.81±0.38 cm) at the altitude of 2743.90±0.0m. The decrease in mean MAUC from lowest to the highest altitude was not significant (b= -0.069; $F=_{(1,6)}$=0.118; P> 0.74). Likewise, the increase in mean MAUC with increasing age was also not significant (b= 0.23; $F=_{(1,6)}$= 7.76; P>0.23).

Mean number of cups of tea taken per day were lowest (3.35±0.35) at the lowest altitude. The highest mean cups of tea taken/day (6.5±0.34) were observed at the mean altitude of 2743.90±0.0m. Regression analysis of variance showed a highly significant increase in mean number of cups of tea taken/day as we moved towards higher altitude (b= 0.401±0.07; $F_{(1,6)}$= 26.52; P<0.0021).

The highest mean RBC count (4.1± 0.12x 10^9/l) were at the highest altitude and the lowest mean value (3.71±0.17x 10^9/l), at the lowest altitude. The increase in mean RBC count with increase in altitude was not significant (b= 0.04±0.03; $F_{(1,6)}$=2.55; P>0.16).

Mean serum ferritin levels (24.4±4.16 μg/l) at the lowest altitude were very low compared to that at highest altitude (41.28±21.75 μg/l). This increase in mean serum ferritin levels with increase in altitude was not significantly different from zero (b= 0.36±1.62; $F_{(1,6)}$=0.049; P>0.83).

Mean PCV (47.53±1.43 l/l) was highest at the lowest altitude and lowest (40.4±0.67 l/l) at the highest altitude. However, this decrease as we moved towards higher altitude was not significant from zero (b= -0.75±0.33; $F_{(1,6)}$ =5.22; P>0.062). Mean MCV was highest (129.63±4.96 fl) at the lowest altitude but it had its lowest mean value (98.94±3.81 fl) at the highest altitude. Regression analysis of variance showed a highly significant decrease in mean MCV towards higher altitude (b= -3.41 ± 0.96; $F_{(1,6)}$=12.43; P< 0.012).

There was no appreciable difference in mean MCH as we moved towards higher altitude (b= 0.037±0.28; $F_{(1,6)}$=0.016; P>0.90). Mean MCHC (28.41±1.87g/l) was lowest at the lowest altitude and the highest mean was at a (36.40±0.96 g/l) mean altitude of 2743.90±0.0 m. Regression analysis of variance showed a highly significant increase in mean MCHC with increase in altitude (b= 0.988 ± 0.17; $F_{(1, 6)}$ = 30.79; P<0.0014).

Table 3.23 Altitude related changes in different variables in the sample of children under study for IDA

Altitude(m) Mean, (No of subjects)	Mean calories (K cal)	Iron (mg)	Height (cm)	Weight (kg)	MUAC (cm)	Age(yrs)	Tea/day cup
1200 – 1449 1278.57±13.78 (14)	715.71± 34.09	3.22± 0.36	123.05± 5.41	24.69± 2.75	16.52± 0.69	8.92± 0.75	3.35±0.35
14550 – 1699 1511.51±10.29 (72)	648.33± 27.48	2.88± 0.21	118.13± 2.57	22.22± 0.90	15.77± 0.22	8.21± 0.36	3.86± 0.18
1700 – 1949 1829.26± 0.12 (8)	697.75± 63.61	3.14± 0.38	105.56± 5.56	17.17± 2.07	14.18± 0.52	6.37± 0.92	3.62± 0.46
1950 –2199 2158.25±6.13 (43)	569.04± 39.92	2.27± 0.24	112.57± 0.24	21.34± 1.09	15.61± 0.26	8.0± 0.45	4.26± 0.24
2200 – 2449 2341.46±22.83 (20)	631.4± 56.70	3.08± 0.50	114.93± 3.13	20.26± 1.17	14.97± 0.25	7.95± 0.61	4.55± 0.45
2250 – 2699 2596.84±16.16 (17)	489.94± 47.41	2.55± 0.54	116.38± 4.05	20.80± 1.66	15.52± 0.64	7.94± 0.69	4.58± 0.40
2700 – 2949 2743.90 ± 0.0 (10)	472.5± 30.26	2.48± 0.08	108.41± 6.53	17.63± 1.97	13.81± 0.38	6.9± 1.12	6.5± 0.34
2950+ 3109.75±1.58 (6)	511.33± 26.03	2.47± 0.34	104.08± 8.65	15.5± 3.31	13.88± 0.74	5.17± 1.01	5.83± 0.40

Altitude(m) Mean, No of subjects	RBC count x 10^9/1	Serum ferritin (µg/l)	Hb (g/dl)	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (g/l)
1200 – 1449 1278.57±13.78 (14)	3.71±0.17	24.4±4.16	13.34±0.77	47.53±1.43	129.63±4.96	36.95±2.63	28.41±1.87
14550 – 1699 1511.51±10.29 (72)	3.69± 0.08	45.98± 6.86	12.70± 0.27	40.5± 0.62	113.79± 3.53	35.36± 1.01	31.76± 0.69
1700 – 1949 1829.26± 0.12 (8)	3.85± 0.25	30.89±11.72	12.2± 0.52	41.5± 2.65	107.76± 1.75	32.35± 1.94	29.91± 1.62
1950 –2199 2158.25±6.13 (43)	4.14± 0.06	34.45± 3.97	13.55± 0.17	41.62± 0.82	100.58± 1.40	32.94± 0.49	33.03± 0.48
2200 – 2449 2341.46±22.83 (20)	3.92± 0.15	19.76± 4.44	13.21± 0.29	40.42± 1.68	103.49± 2.12	34.42± 1.18	33.38± 1.13
2250 – 2699 2596.84±16.16 (17)	4.14± 0.21	43.16± 11.68	13.76± 0.24	41.43± 1.42	101.59± 2.65	34.39± 1.68	33.61± 1.02
2700 – 2949 2743.90 ± 0.0 (10)	3.74± 0.11	23.49± 6.21	13.87± 0.41	38.11± 0.45	102.5± 3.21	37.35± 1.61	36.40± 0.96
2950+ 3109.75±1.58 (6)(	4.1± 0.12	41.28± 21.75	14.28± 0.76	40.4± 0.67	98.94± 3.81	34.89± 1.91	35.32± 1.66

Changes In Different Variables Based on Different Classes of Mean Caloric Consumption

Caloric intake by all children was grouped into three classes, i.e., caloric intake <500 k cal; caloric intake between 500-1000 k cal and caloric intake > 1000k cal. Changes in variables like mean caloric intake, iron intake, serum ferritin, Hb, tea/day, age, height, weight and MUAC were observed in these classes of caloric consumption (Table 3.24).

The lowest class of caloric intake (<500 k cal) was seen in children of mean age 6.79±0.37 years. The next higher class of caloric intake (500-1000 k cal) was observed in children of mean age 8.28±0.27 years and the highest class of caloric intake (> 1000 k cal) was seen in children of mean age 10.15±0.56 years.

There was no significant difference in Hb level and consumption of tea/day in the three classes of caloric consumption. Significant increase in mean caloric intake was observed in class of calories, 500-1000 k cal, ($t_{(175)}$ =14.91; P<0.001) and >1000 k cal ($t_{(74)}$ =15.18; P<0.001) compared to the lowest class of caloric intake ($t_{(74)}$ = 3.30; P<0.001). Mean iron intake (mg) was significantly higher in class 2 (500 – 1500 kcal) ($t_{(175)}$ = 7.0; P<0.001) and class 3 (> 1000 kcal) ($t_{(74)}$ = 3.30; P<0.001) compared to the lowest class of caloric intake (< 500 kcal).

Difference in height in the class 2 ($t_{(175)=}$ 3.34; P<0.001) and class 3 ($t_{(74)}$=4.84; P<0.001) of caloric consumption is significantly high compared to class 1. Similarly, weight also increases significantly in the 2 higher class of caloric intake ($t_{(175)}$=3.11; P<0.001) and the highest class of caloric intake ($t_{(74)}$=3.81; P<0.001) compared to the lowest class of caloric intake. In the case of mean serum ferritin ($t_{(74)}$=2.26; P<0.05) and mean MUAC ($t_{(74)}$=3.06; P<0.01) significant increase is seen in the highest class of caloric intake compared to the lowest class of caloric intake.

Table 3.24 **Changes in different variables due to different classes of mean calories among children understudy.**

S No.	Classes on the basis of mean caloric intake (No of subjects)	Mean Caloric intake (k cal)	Iron Intake (mg)	Serum ferritin (µg/l)	Hb (g/dl)	Tea/daily cups	Age(yrs)	Height (cm)	Weight (kg)	MUAC (cm)
Class 1	<500kcal (n=63)	393.71± 14.24	1.677± 0.14	35.90± 6.3	13.06± 0.2	4.25± 0.2	6.79± 0.37	106.68± 3.13	18.61± 0.89	14.89± 0.24
Class 2	500 --- 1000kcal (n=114)	662.69± 11.06	3.15± 0.15	39.2± 4.07	13.25±0.20	4.20± 0.15	8.28± 0.27	118.83± 1.84	22.10± 0.69	15.66± 0.18
Class 3	>1000kcal (n=13)	1165.38± 48.79	4.08± 0.72	18.66± 4.3	12.89±0.37	4.53± 0.61	10.15± 0.56	125.47± 3.7	25.81± 1.67	16.36± 0.42

Prevalence of IDA Among Children

WHO Method

By adjusting the cut-off values of Hb according to WHO criteria, the prevalence of IDA was found to be 21% among children.

CDC Method

Using adjustments proposed by CDC, the prevalence of IDA among children was found to be 21%.

-2SD Method

Prevalence of Anemia on the Basis of RBC Morphology

Morphology of RBC was looked at in terms of their normal condition and how severe abnormality affects them. There were only 27 (15.51%) children with normal RBC morphology. In these children mean serum ferritin levels were 42.91±7.47μg/l and Hb levels were 15.13±0.35g/dl.

The low and high cut-off points, for the calculation of prevalence of IDA, were based on the ±2SD area under the curve for mean Hb levels. The low point (-2SD) thus calculated was 11.75 Hb g/dl and the high point (+2SD) was 18.51Hb g/dl.

All the children (male and female) were divided into four groups:

those who have Hb levels below 11.75 g/dl (<-2 SD)

those who have Hb levels between 11.75 g/dl --15.13 g/dl (-2 SD)

those who have Hb levels more than 15.13 g/dl-- 18.51 g/dl (+2 SD)

those who have Hb levels above 18.51 g/dl (>+2SD)

In the first group there were 32(18.38%) children. Their mean Hb level was 10.30±0.24 g/dl and mean serum ferritin level was 18.43±7.53μg/l. These 18.38% children who had Hb levels less than 11.75g/dl were categorized as suffering from severe IDA.

In the second group there were 130 (74.28%) children. Their mean Hb level was 13.56±0.07g/dl and mean serum ferritin level was 40.38±3.63μg/l. These were considered suffering from mild IDA.

In the third group were 10(5.71%) children. Their mean Hb levels were 15.75±0.18g/dl and mean serum ferritin levels were 50.08±15.03μg/l. These children were taken as healthier than the first two groups.

In the fourth group were 2(1.14%) children. Average (as the number of children were 2 we used average instead of mean) Hb level in them was 20.90±0.36g/dl and serum ferritin level was 27.75±2.95μg/l. These were regarded as the healthiest children.

The lowest mean Hb levels (10.30±0.24 g/dl) were in the children who fell below the low cut-off point (11.75g/dl). In the -2SD area under the curve higher mean Hb levels (13.56±0.07g/dl) are seen and a further higher values of mean Hb levels (15.75±0.18gd/dl) were observed in the +2SD area under the curve. The highest Hb levels (average of two Hb values) (20.90±.01g/dl) were in the area outside +2SD area under curve.

In the first group there was no child with normal RBC morphology and the percentage of anaemia was 100.00%. In the second group 16.15% of the children with normal RBC morphology were observed. In the third group 40% of the children had normal RBC morphology. In the fourth group 100% of the children had normal RBC morphology. The Percentage of children with normal cells present in these groups indicates the severity of IDA, the mild form of it and the healthier condition of the subjects.

Regression analysis of variance shows that Hb levels increase significantly with the increase in the number of children with normal blood cells (b=3.39±0.41; $F_{(1,2)}$= 67.76; P=0.014) (Fig. 3.7). However, mean serum ferritin levels do not increase significantly with the increase in normal blood cells (b= 3.76±7.14; $F_{(1,2)}$=0.278; P= 0.65). Regression analysis of variance of children with normal

red blood cells on area under the curve showed a significant increase in the prevalence of IDA ($b = 32.38 \pm 16.14$; $F_{(1,2)} = 28.12$, $P < 0.046$).

The children with normal RBC morphology in the four areas under the curve were analysed separately for their mean haemoglobin and serum ferritin (Table 3.25 a). These children showed higher concentrations of Hb as well as serum ferritin compared to the Hb and serum ferritin concentrations in the overall children sample.

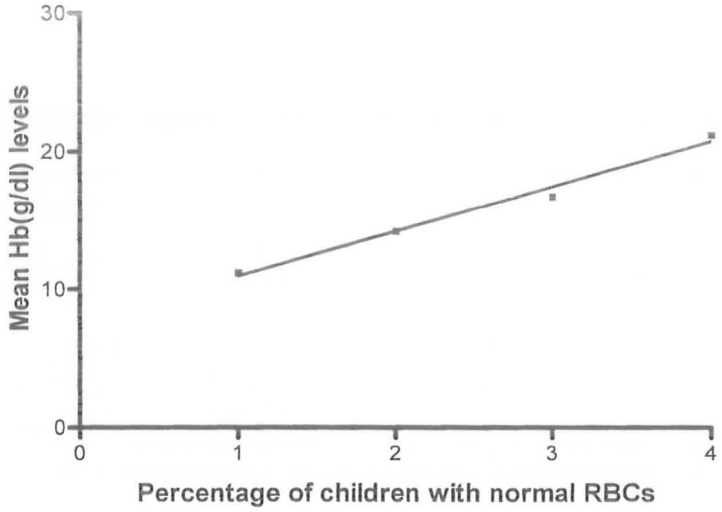


Fig.3.7 Regression analysis of variance shows the increase in mean Hb(g/dl) with the increase in the percentage of children with normal RBCs morphology(P<0.014).

Table 3.25 **IDA based on cut-off values based on morphology of red Blood cells, number and percentage of subjects with normal red blood cells, mean Hb and serum ferritin levels in children (male and female)**

S.no	Hb Classes based on morphology of RBC	Children with*nc (%)	Hb levels g/dl	Serum ferritin µg/l
1	<11.75(n=32)	0 (0.0)	10.30±0.24 (n=32)	18.43±7.53(n=32)
2	11.75-15.13(n=130)	21 (16.15)	13.56±0.07 (n=130)	40.38±3.63(n=130)
3	15.13-18.51(n=10)	4 (40)	15.75±0.18 (n=10)	50.08±15.03(n=10)
4	>18.51(n=2)	2 (100)	20.90±0.36 (n=2)	27.75±2.95(n=2)

*nc= Normal Red Blood Cell Morphology

Table 3.25a **Area under curve, number of children with normal RBC morphology, their mean Hb (g/dl) and mean serum ferritin (µg/l)**

S.No	Area under curve Hb g/dl	Number of Children with *nc	Mean Hb (g/dl)	Mean serum ferritin (µg/l)
1	<11.75	0	0	0
2	11.75-15.13	21	14.37±0.08 (21)	42.45±4.29 (21)
3	15.13-18.51	4	16.22±0.12 (4)	52.92±10.15 (4)
4	>18.51	2	20.90±0.36 (2)	27.75±2.95 (2)

*nc= Normal Red Blood Cell Morphology

Prevalence of IDA In Relation To Different Serum Ferritin Levels

All the children were sorted in ascending order for serum ferritin levels. Serum ferritin level classes were made with an interval of 12μg/l (WHO, 2001) as shown in Table 29.

There were 47(27.01%) children in the first group who had less than 12μg/l serum ferritin. In this group mean serum ferritin levels were 5.73 ± 0.55μg/l and Hb levels were 12.00±0.35 g/dl. The percentage of children with normal RBC was 6.38 (n=3).

In the next higher group, mean serum ferritin levels were 17.43±0.44μg/l and Hb levels were 13.11±0.21g/dl. The number of children in this group was 48(27.98%). The number of children with normal RBC's was 16.67 %(n=8).

In the third group the number of children was 26 (14.94%). Mean serum ferritin levels were 30.08±0.65μg/l and mean Hb levels were 13.81±0.21g/dl. Children with normal RBC's were 23.07 % (n=6).

In the fourth group there were 13(7.47%) children whose serum ferritin levels were 40.86±0.85μg/l and Hb levels were 14.12±0.47g/dl. There were 15.38% (n=2) children with normal RBC's.

In the fifth group there were 5.74 %(n=10) children. Their mean serum ferritin levels were 51.43±1.11μg/l and mean Hb was 14.80±0.69 g/dl. Normal RBC's in them were 30.00 %(n=3).

In the sixth group there were only 4(2.29%) children with no normal RBC's and all of them may be suffering from anemia. Their mean serum ferritin level was 67.95±0.85μg/l and mean Hb level was 14.12±0.47g/dl.

In the seventh group there were 6(3.45%) children. Only one child (16.67%) had normal RBC and 83.33% were considered as anaemic. Their mean serum ferritin level was 75.65±1.20μg/l and Hb level was 13.65±0.32g/dl.

In the eighth group none of the children (n= 04) had normal red blood cells. They were regarded as anaemic children (100.00%). Although their mean serum ferritin level (88.1±2.60μg/l) and mean Hb level (14.05±0.35g/dl) was quite high.

In the ninth group there were 16(9.19%) children. Of these 16 children, only 4 (25.00%) children had normal RBC's and the remaining 75.00% with abnormal RBC's were regarded as anaemic. Their mean serum ferritin level was 145.76±11.20μg/l and mean Hb level was 13.79±0.37g/dl.

Table 3.26 **The prevalence of IDA based on serum ferritin levels (μg/l) in children, mean serum ferritin and Hb levels, children with normal RBC morphology (g/dl) and children with other than normal morphology**

Ferritin Group (< 12 μg/l)	Serum ferritin level (μg/l)	Hb level (g/dl)	Children with *nc (%)	Children with microcytosis and macrocytosis (%)
1 0 – 11.9 (n=47)	5.7±0.55	12.00±0.35	3 (6.38)	44 (93.62)
2 12 – 23. 9 (n=48)	17.43±0.44	13.11±0.20	8 (6.67)	40 (83.33)
3 24 – 35.9 (n=26)	30.08±0.65	13.81±0.21	6 (23.07)	20 (76.92)
4 36 – 47.9 (n=13)	40.86±1.00	13.45±0.25	2 (15.38)	11 (84.61)
5 48 – 59.9 (n=10)	51.43±1.11	14.8±0.69	3 (30.0)	7 (70.00)
6 60 – 71.9 (n=4)	67.95±0.85	14.12±0.47	0 (00.00)	4 (100.00)
7 72 – 83.9 (n=6)	75.65±1.20	13.65±0.32	1 (16.67)	5 (83.33)
8 84 – 95.9 (n=04)	88.1±2.6	14.05±0.35	0 (0.00)	4 (100.0)
9 96+ (n=16)	145.7±11.70	13.79±0.37	4 (25.00)	12 (75.00)

* Normal red cell morphology

Prevalence of IDA in Children at Different Altitude Levels:

The altitude categories were made as mentioned above. In these categories the same cut-off points as were determined with children having normal red cell morphology were used. The low cut-off point (-2SD) was 11.75g/dl.

In the lowest mean altitude level (1278.57 ± 13.78m) there were 14 children in total. Out of these only 3 had Hb below – 2SD (i.e. <11.75g/dl). These (21.42%) children had severe IDA. In this category only 2(14.28%) children had normal red cell morphology.

In the next higher altitude category (1511.51±10.29m), there were 72 children. Out of these 20(27.77%) children had Hb<11.75g/dl (IDA). In this second altitude category 6(8.33%) children had normal red cell morphology.

In the third category (n=8) i.e. mean altitude of 1829.26±0.12m , there were 3(37.5%) children who were considered as IDA children because of Hb levels <11.75g/dl. None of them had normal red cell morphology.

Next comes the forth category of altitude (2158.25±6.13m), which had 43 children. Only 2(4.65%) out of 43 had Hb levels <11.75g/dl and 7 children (16.27%) had normal red cell morphology.

In the fifth altitude category (2341.46±22.83m) there were 20 children. Out of these 2(10%) children had Hb levels <11.75g/dl and hence these were considered as IDA children. Three (15%) out of 20 children had normal red cell morphology.

The next sixth higher altitude class (2596.84±16.16m) had 17 children. Only 1(5.8%) child was diagnosed as severe IDA (i.e. Hb< 11.75g/dl). At this altitude 6(35.29) children were with normal red cell morphology. There were 10 children at mean altitude of 2743.90±0.0 m. Out of them only 1 (10%) child had IDA (Hb<11.75g/dl) and 1 (10%) child had normal red cell morphology.

The last and the highest altitude category eighth at a mean height of 3109.75±1.58m, had no children with severe anaemia and only 2(33.3%) children were with normal red cell morphology

Table 3.27 **Prevalence of IDA in relation to different altitude levels, mean altitude, number of children, percentage of children with normal RBC and prevalence of anaemia (<11.75 g/dl)**

S.no	Altitude classes	Mean Altitude (m)	Number of children	Children with *nc (No.and %)	Prevalence of anaemia <11.75
1	1200 – 1449	127857±13.78	14	02 (14.28%)	03(21.42%)
2	1450 – 1699	1511.5±10.29	72	06 (8.33%)	20(27.77%)
3	1700 – 1949	1829.26±0.12	08	00 (0%)	03(37.5%)
4	1950 - 2199	2158.25±6.13	43	07 (16.27%)	02(4.65%)
5	2200 – 1449	2341.46±22.8	20	03 (15%)	02(10%)
6	2450 – 2699	2596.84±16.1	17	06 (35.29%)	01(5.88%)
7	2700 -- 2949	2743.9±0.0	10	01 (10%)	01(10%)
8	2950+	3109.75±1.58	06	02 (33.3%)	00(0%)

* Normal red cell morphology

General Profile of Male and Female Children:

Table 3.28 gives means of different variables as were studied in adult females. Both male and female children show no appreciable difference in mean age, height, weight, MUAC, iron, RBC counts, serum ferritin levels, Hb levels, PCV, MCH, MCV, MCHC, tea/day consumption and mean caloric consumption.

Table 3.28 **Different mean variables studies in male and female children**

Variables	Male (No)	Female (No)
Age (years)	7.88±0.31 (92)	7.95±0.30 (98)
Height (cm)	113.84±2.41	116.58±21.15
Weight (kg)	21.25±0.80	21.15±0.74
MUAC (cm)	15.27±0.20	15.64±0.20
Mean Calories(kcal)	622.79±26.15	593.91±21.13
Iron (mg)	2.77±0.19	2.68±0.16
Tea/daily (cups)	4.22±0.19	4.26±0.14
RBC counts ($x10^9$/l)	3.82±0.07 (83)	3.92±0.06 (92)
Serum ferritin (μg/l)	37.22±5.01	36.15±4.11
Hb (g/dl)	13.03±0.23	13.28±0.16
PCV (l/l)	41.04±0.65	41.44±0.54
MCH (pg)	34.83±0.79	32.55±0.48
MCV (fl)	108.99±2.45	108.36±2.15
MCHC (g/l)	32.19±0.60	32.55±0.48

Prevalence of IDA in Females:

Prevalence of IDA was also analyzed separately in female and male children. There were 11 (11.22 %) females with normal RBC morphology out of 98 female children (other haematological parameters were found in 92 female children). In these females mean Hb level was 15.08±0.26 g/dl. Area under ±2SD was analyzed in relation to mean Hb levels. Low cut-off point calculated was 13.36g/dl and high cut off point was 16.8g/dl.

There were 44 (47.82 %) female children below low cut-off point of Hb (13.36 g/dl). There was no female child with normochromic normocytic red cell morphology indicated that all 44 female children were suffering from severe anaemia.

In the area between Hb 13.36-15.08 g/dl there were 38(41.33%) female children. These indicated mild anaemic female children. Out of these 38, 7(18.42%) female children had normal RBC morphology.

In the area between Hb 15.08 to 16.8g/dl there were 10 subjects (10.87%) out of them four female children had normal RBC morphology (40.0%) indicating healthier children in this region. No children were in the Hb area >16.8g/dl. Regression analysis of variance of female children with normal RBC morphology on area under curve showed non-significant increase in non-anaemic female children (b= 2.15±10.31; $F_{(1,2)} = 0.043$; P> 0.85) (Fig.3.8).

The number of female children with normal RBC morphology increased significantly towards the +2SD area under the curve (b=20.00±0.91; $F_{(1,1)}$=480.7; P<0.0290). However, mean serum ferritin shows non-significant increase towards +2SD area under curve (b=10.89±1.69; $F_{(1,1)}$=41.26; P>0.09). Similarly, mean Hb increased non-significantly towards +2SD area under curve (b=1.81±0.18; $F_{(1,1)}$ =99.60; P>0.063).

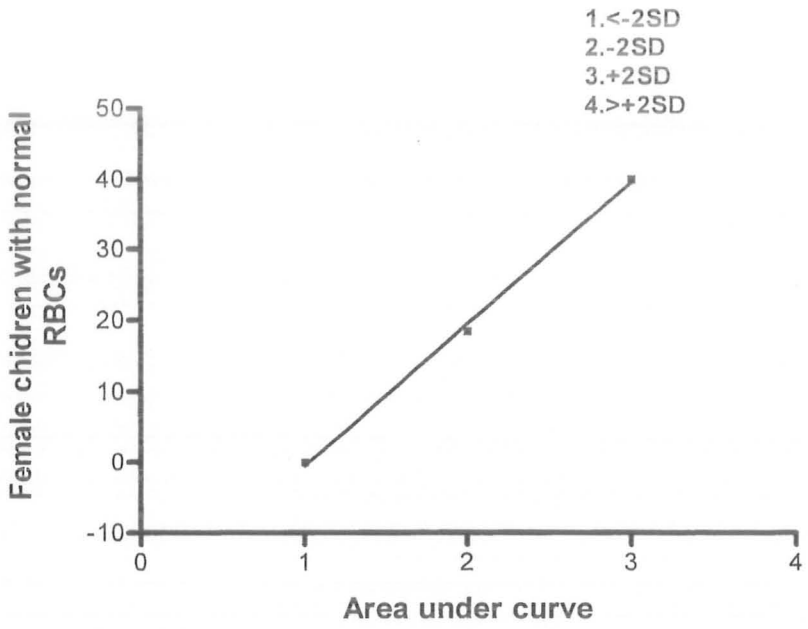


Fig 3.8 Regression analysis of variance shows increase in percentage of female children with normal RBCs in area under curve(P>0.85)

Table 3.29 **IDA based on morphology of red blood cell, number of female children in each class, number. and percentage of female children with normal red blood cell, mean Hb and mean serum ferritin**

S. No	Hb (g/dl)	Number of Female children in each class	Female children with *nc (no & %)	Mean Hb (g/dl) (No)	Mean serum ferritin (μg/l) (No)
1	<13.36	44	0 (0)	12.01+0.19 (44)	30.50+6.64(44)
2	13.36-15.08	38	7(18.42)	14.14+0.07(38)	38.45+4.96(38)
3	>15.08-16.8	10	4(40)	15.64+0.18(10)	52.27+14.84(10)
4	>16.8	0	-	-	-

* Normal red blood cell morphology

Prevalence of IDA in Male Children

In the case of male children there were 16 (17.39%) out of 92 children with normal RBC morphology (other haematological parameters were defined out of 83 male children). In these 16 male children with normal red cell morphology mean Hb level was 15.16±0.58g/dl. The area under the ±2SD calculated showed the low cut-off point 10.56g/dl and the high cut-off point as 19.76g/dl.

Below the low cut-off point of Hb, there were 7(8.43%) male children. Of these, none had normal blood cells, showing severe anaemia in male children in that area.

In the Hb values between 10.56-15.16g/dl, there were 73(87.95%) male children. Out of them 13 (17.8%) had normal red blood cells, the rest had mild anaemia.

Only one male child (1.2%) had Hb level between 15.16 to 19.76g/dl and he had normal red cell morphology (100%).

In the region of >19.76g/dl, there were 2 male children (2.41%) and both of them (100%) had normal RBC morphology, indicating the healthiest children. Regression analysis of variance of children with normal red blood cells on area under curve showed a non-significant increase with the increase in non-anaemic male children ($b = 38.22 \pm 10.74$; $F_{(1,2)} = 12.66$; $P > 0.07$).

Regression analysis of variance showed a highly significant increase in Hb(g/dl) levels as the number of children with normal red blood cells increased ($b=4.02 \pm 0.24$; $F_{(1,2)}=279.6$; $P<0.0036$) (Fig.3.9). There was a non-significant increase in mean levels of serum ferritin (μg/l) with the increase in the number of male children with normal red blood cells ($b=5.07 \pm 6.80$; $F_{(1,2)} =0.55$; $P=0.53$) (Fig.3.10).

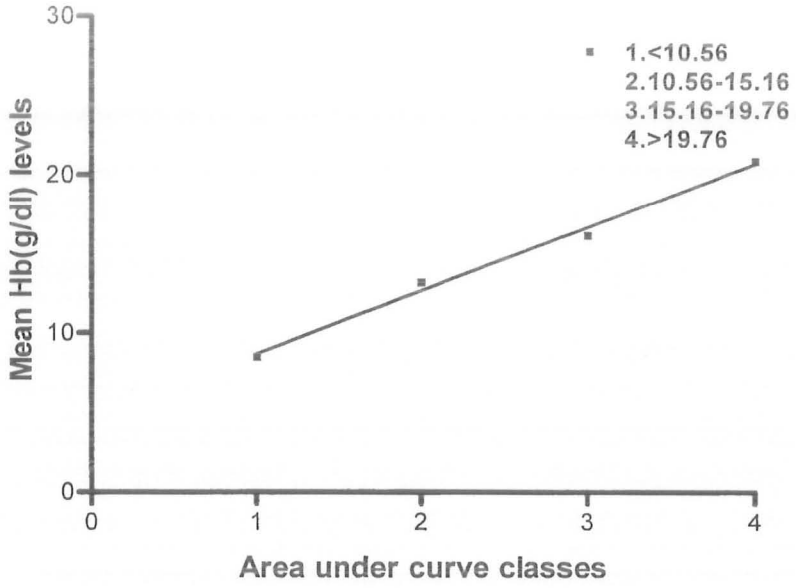


Fig.3.9 Regression analysis of variance of mean Hb(g/dl) levels on different areas under curve classes in male children with normal RBCs (P<0.0036)

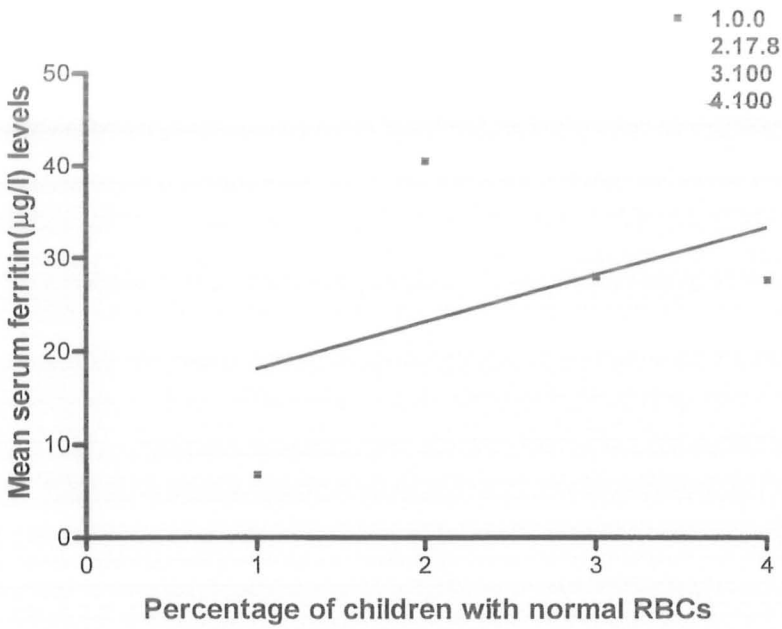


Fig 3.10 Regression analysis of variance shows mean serum ferritin (ug/l) levels increase with the increase in number of male children with normal RBCs (P>0.53)

Table3.30 **IDA based on morphology of red blood cell, number of male children in each class, number. and percentage of male children with normal red blood cell, mean Hb and mean serum ferritin**

S. No	Hb (g/dl)	Number of male children in each class	Male children with *nc (no & %)	Mean Hb (g/dl) (No)	Mean serum ferritin (µg/l) (No)
1	<10.56	7	0 (0)	8.5±0.51 (7)	6.7±2.29 (7)
2	10.56 –15.16	73	13(17.8)	13.2±0.13 (73)	40.53±5.56 (73)
3	>15.16-19.76	1	1(100)	16.2 (1)	28 (1)
4	>19.76	2	2 (100)	20.9±0.1 (2)	27.7±20.9 (2)

* Normal red blood cell morphology

DISCUSSION

The present study is based on 382 women of childbearing age and 190 children (both sexes included). Women of childbearing age ranged from 15-50 years of age with an overall mean age as 28.43± 0.4 years. The female subjects were residing at a mean altitude of 1878 ± 31.73 m. Their mean serum ferritin level was 33.50 ± 2.07 μg/l and mean Hb level was 13.78 ± 0.10g/dl.

The age related changes in these women showed no appreciable change in different characteristics (Table 3.8a, b) particularly in Hb (g/dl) and serum ferritin (μg/l) levels. De Maeyer *et al.* (1985) suggested that reference values of haemoglobin concentration depend on age, but the present study did not find this. This shows that age does not appear to have significant effects on haemoglobin concentration. The higher representation of women was in age groups ranging from 25-34 years.

The study was carried out on a population residing at a higher altitude to investigate prevalence of anaemia. Different authors have put forth different views to predict the prevalence of anaemia in such populations. They have suggested establishing cut-off points for haemoglobin concentration to diagnose anaemia in a population. All those authors have placed the emphasis on concentration of haemoglobin while using analytical methods for the diagnosis of anaemia. Berger *et al.*(1997) were of the opinion that the prevalence of anaemia within a population depends on the criteria for its definition. The measure of haemoglobin concentration in laboratory tests is most commonly used for diagnosis of anaemia in clinical and public health studies. Because the majority of anaemias in children and women of childbearing age are linked to iron deficiency, the principal objective for the diagnosis of anaemia was to detect individuals at high risk of deficiency of this micronutrient.

DeMaeyer *et al.* (1985) observed that the diagnosis of anaemia requires not only an appropriate analytical method but also cut-off values for the concentration of haemoglobin useful for the definition of anaemia. The World Health Organization (WHO) and the International Nutritional Anemia Consultative Group (INACG) have defined the reference values of haemoglobin concentration to define anaemia considering age, sex, and certain physiologic circumstances such as pregnancy. According to Dallman *et al.*(1985) the definition of a cut-off value for haemoglobin that permits the diagnosis of anaemia requires a reference population consisting only of healthy individuals free of all nutritional deficiency that could influence the concentration of haemoglobin.

Similarly, according to Hercberg and Galan (1985); Cook (1982) and Yepez *et al.* (1994) the prevalence of anaemia in a population can be estimated by different methods.

WHO (1975) also recommended that each country may lay down minimum acceptable standards below which an individual is considered unhealthy taking into account the health and other needs of the country. It has also been recognized that there is a homoeostatic mechanism in every individual. In this study the prevalence of anaemia was calculated applying different methods. The results obtained are as follows

Method	**Pregnant Women**	**Non-pregnant Women**	**Children**
WHO (WHO, 2001)	22.2%	15.70%	21.00%
CDC(WHO, 2001)	24.00%	17.00%	21.00%
-2SD(WHO, 2001)	27.00%	13.00%	18.38%

In line with the views presented by Dallman *et al.*(1985); Hercberg and Galan (1985); Cook (1982); Yepez *et al.* (1994) and WHO (1975), I preferred to use the -2SD method for the present study. This method involves both the

normal morphology of RBCs in the subjects and the variation in haemoglobin concentration in relation to the number of subjects with normal RBC morphology in different states of the subjects. Also, in this study I tried to lay down our own minimum acceptable standards for the study of anaemia in Pakistani women following the WHO (1975) recommendation. Kolsteren *et al.* (1994) found that the estimated prevalence of anaemia according to the method of mixed-distribution analysis is approximately four times lower than the estimation based on the adjusted cut-off values for altitude proposed by the CDC (1989). In their study on Tibetans they recorded a different haemoglobin adaptation response. This shows that estimation of prevalence of anaemia is much dependent on the method applied for it.

Hence, prevalence of anaemia was based on RBC morphology and haemoglobin concentration in healthy subjects. In this sample there were 108 (28.27%) women who had normal RBCs. In these women mean haemoglobin level was 15.13±0.16 g/dl and mean ferritin level was 35.95±4.2ug/l. Following the WHO -2SD method (WHO, 2001) cut-off values were set which were 11.77g/dl (low) and 18.49g/dl (high). There were 13.08% women with severe anaemia whose mean haemoglobin level was 10.25±0.15g/dl and mean ferritin level was 16.84±3.1ug/l. The percentage of anaemic women decreased as we moved towards higher haemoglobin cut-off values (Table 3.13) showing an increase in haemoglobin levels. A highly significant positive relationship was observed between percentage of women with normal RBCs and Hb levels (P=0.0091) indicating that with the increase in number of women with normal red blood cells there is proportionate increase in haemoglobin levels

The first of our predictions, at the outset of this study, was that the prevalence of iron deficiency anaemia among women in Gilgit and Ghizer districts would not be less than the national prevalence i.e. around 35%. This was assumed considering the following:

- The remoteness, isolation and backwardness of the area

- The fertility of the land is limited due to harsh and long winters leading to insufficient production of local crops and vegetables.
- Low socioeconomic status of the population, according to Aga Khan Rural support Program Survey the per capital income is 60% of the national per capita income, thereby limiting the purchasing power of the local people to buy food items from market.

Paradoxically, the highest prevalence found among pregnant women (estimated by -2SD method) is 27%, and among non-pregnant women it is 17%, both much lower than the national prevalence. The possible explanations of this inconsistency may include:

1. The haemoglobin cut-off values to diagnose anaemia, even after adjustments for altitude following the known methods, are too low thereby falsely underestimating the prevalence of anaemia. This possibility is further supported by very low serum ferritin levels and daily iron intake.
2. Locally produced foods (vegetables, fruits, grains etc.) may contain more iron compared to food items analysed to determine the iron content. There might be some ingredient in local foods that act as enhancers of iron absorption. The bioavailability of iron may also be different in the population under study. All of these issues need further exploration.

WHO (2001) has established cut-off ranges from 110g/l for pregnant women and for children 6 months-5years of age, to 120g/l for non-pregnant women., to 130g/l for men. Anaemia can be diagnosed by analyzing the haemoglobin concentration in blood or by measuring the proportion of red blood cells in whole blood (haematocrit) at sea level. However, the WHO (2001) cut-off range is for a particular group of women (pregnant and non-pregnant). The mean cut-off range has also been calculated in this study, for pregnant women as 12.90±0.32g/dl and for non-pregnant women i.e., lactating, and married as 13.98±0.12g/dl and 14.03±0.17g/dl respectively. These cut-off ranges are a bit higher than the WHO cut-off ranges, which may have an influence of higher altitude. There were unmarried women (n=62) in

this sample whose cut-off value was 13.72±0.25g/dl. Related to the marital status and haemoglobin levels the percentage of anaemic women, in this study, diagnosed among pregnant were 26.98%; lactating as 7.9%; married 10.68% and in unmarried anaemics were 14.51 percent. We assumed that the prevalence of IDA would be more among pregnant women. Our study showed a higher prevalence, though statistically non-significant, among pregnant women compared to non-pregnant women. It remained a dilemma for the researcher of this study (and might be a point for other researchers) that there was a great different between prevalence of anaemia estimated using heamoglobin concentration and anaemia estimated using other indices e.g. morphology of RBCs, serum ferritin levels and clinical examination of subjects. The anaemia according to Hb concentration was lower than the prevalence according to other indices as well as anaemia in similar subjects elsewhere in the country. Low intake of iron-rich food and lower iron consumption estimated on the basis of food history also complicates the interpretation of these results. Therefore, further studies are needed to explore this disparity. According to Fleming (1989), pregnant women are more likely than non-pregnant women to be anaemic for a variety of reasons including haemodilution and increased demand on iron and folate stores. Severe anaemia in pregnancy has been reported as the main cause of up to 20% of maternal deaths in some hospital series in sub-Saharan Africa (Armon, 1979; Mtimavalye *et al.*, 1980) and 11-13% in community-based studies (Marchant, 2002). A study in Malawi found anaemia to be significantly associated with maternal death (McDermot *et al.*, 1996). Marchant (2002) investigated that of 507 pregnant women in southern Tanzania 11% were severely anaemic (Hb<8g/dl).

In this study the prevalence of anaemia in relation to different serum ferritin levels showed that the percentage of normal women (according to Hb concentration) increased significantly as the serum ferritin level increased (P=0.0071). However, there was no relationship between increase in ferritin level and the number of subjects with normal RBCs (P=0.97).

Altitude also has a role in the prevalence of anaemia as we saw an increase in haemoglobin level and subjects with normal RBC counts as we move towards higher altitude. There was a non-significant decrease in anaemic women towards higher altitude (P=0.0522). Regression analysis of variance also shows a non-significant increase in haemoglobin concentration with higher altitude.

Hurtado *et al.*(1945) and Dallman *et al.* (1985) observed that on the international level, reference values have been established on the basis of studies done on populations living at sea level. However, adaptation to living at high altitude carries an increased blood capacity for the transportation of oxygen. Persons living at high altitudes have higher concentrations of haemoglobin and haemotocrit than do those living at sea level. An increase in haemoglobin concentration was also observed in the present study towards higher altitudes.

Adaptation to high altitude can have an effect on haemoglobin concentration. According to Pawan and Jest (1978); Kolsteren and Van der Stuyft (1994); Baker *et al.*, (1979) and a report from Instituto Nacional de Estadistica (1989-1990) around 20-30 million people worldwide live at high altitudes higher than 3000 m, defined as high altitude. Especially in the high plains of Ethiopia and the Tibetan plateau of Himalayas, where the adaptation of life to high altitudes could occur without an increase in the concentration of haemoglobin, and above all in the Andean high plateau. Approximately 17 million people live at high altitudes in the Andean region of Latin America, and 38% of them are Bolivians.

In Bolivia, data on the prevalence of anaemia are poor. One report by UNICEF (1994) states that the prevalence is approximately 48-50% in children less than 15 years of age residing in Cochabama (2400m). The same report indicates that anaemia is very uncommon in the Bolivian altitplano,

which seems unlikely in view of the two studies that found a prevalence of anaemia oscillating between 67.2% and 14.6% in children between 6 months and 9 years of age (Berger *et al.* 1994) and around 56.5% in pregnant women (Fernandez, 1996). This discrepancy might be due to the utilization of different cut-off values for the definition of anaemia.

One of our predictions was that as found elsewhere, there would be a strong association between haemoglobin levels and altitude above the sea-level. The findings of our study too show that there is an association between altitude and mean Hb levels i.e. the mean Hb level at 1000-1499 meters above sea level is 13.3 gm/dl compared to 15.2 gm/dl at more than 3000 meters.

There were 190 children included in this study, of which 98 were females and 92 males. The age of the children ranged from 2-12 years. Their mean age was 7.92±0.21 years. Among female children 63% were school going and in males 52% were school going. In the sexes combined the mean haemoglobin level was 13.16±0.14g/dl and mean serum ferritin was 36.67±3.21ug/l. The cut-off point was 11.75g/dl (low) and 18.51 g/dl (high) for sexes combined. The percentage prevalence of anaemia in children was 18.38% in this study.

Studies in Cote d'Ivoire (Staubli *et al.*, 2001) and Benin (Hercberg *et al.,* 1988) estimated that iron deficiency anaemia accounted for about 50% of the anaemia observed. In the Cote d'ivoire study, the proportion of anaemic individuals with iron deficiency varied by age and sex. About 80% of the anaemic pre-school age children had iron deficiency anaemia, compared with 50% of the school-age children and women and 20% of the men. In these areas malaria and other infections or inflammatory disorders contributed significantly to the high prevalence of anaemia, particularly in young children, but these infections and/or disorders and iron deficiency could not explain all of anaemia cases. A study carried out on the Kenyan coast (Scoltzfus *et al.*

1997), 76% of the pre-school age children were anaemic (Hb<110g/l) and 3% severely so (Hb<50 g/l).

Our study showed that in children mean Hb levels increased significantly as the number of children with normal RBCs increased (P=0.014) but mean ferritin levels did not increase significantly with the increase in number of children with normal RBC morphology.

Iron deficiency anaemia is the most severe degree of iron-deficiency and ensues if the haemoglobin falls below a statistically defined threshold lying two standard deviation below the medium of a healthy population of the same age, sex and stage of pregnancy (WHO/UNICEF/UNU, 1996). Haemoglobin distribution vary with age, sex, and different stages of pregnancy, and with altitude and smoking (CDC, 1989) and possibly is genetically determined (Perry *et al.*, 1992). Our study showed that mean hemoglobin levels increased significantly towards the higher altitude (P=0.038). We saw variation in the distribution of haemoglobin concentration at different mean altitude levels (Table 3.15).

Over 2 billion people worldwide are iron deficient with a total prevalence estimated at about 40% of the world's population (WHO, 1991). This estimation compares prevalence of anaemia in developed and under developed countries as well. Prevalence among various sub-groups was estimated ; 51% for pregnant women, 48% for infants and 1 to 2 year old children, 35% for preschool children. These are global mean estimates-prevalence in these sub-groups tends to be up to three to four times higher in developing than developed countries. Regional data from WHO (1996), derived from a total of 32 existing national-level surveys available globally, showed the prevalence of anaemia (Hb <11g/dl) for pregnant women: south-east Asia (79%), eastern Mediterranean (61%), Africa (44%), western Pacific (39%), Americas (29%), and Europe (20%). In central Asia, very high prevalence among women (about 80%) and children (about 60%) has been

reported in demographic and health surveys in Kazakhstan and Uzbekistan. Nearly half of the global total number of anaemic women live in the Indian Subcontinent (WHO, 1991) and, in India alone, prevalence of anaemia among pregnant women may be as high as 88% (ICMR, 1989).

Different explanations have been given for variations in the prevalence of anaemia and haemoglobin concentrations. According to Dirren *et al.* (1994) adolescents and males living in the Himalayas have mean Hb values lower than Andean residents living at the same altitudes. Reasons for this are not clear but they suggested that there might be different adaptations among ethnic groups that atmospheric pressure changes with latitude as well as altitude, that the methodologies for the studies were dissimilar and/ or that different environmental and disease patterns exist.

According to Hurtado *et al.* (1945) and Dallman *et al.* (1985) reference values have been established on the basis of studies done on populations living at sea level. However, adaptation to living at high altitudes carries an increased blood capacity for the transportation of oxygen. Persons living at high altitude have higher concentrations of haemoglobin and haematocrit than do those living at sea level. This variation is due to the decrease in the partial pressure of oxygen at high altitudes, which induces a decrease in the absolute rate of oxygen available per unit of pulmonary surface (Tufts, 1982) and induction in the saturation of oxygen in the blood (Hurtado *et al.*, 1945).

In this study non-pregnant non-lactating women, pregnant and lactating women were consuming 49.6%, 46.9%, and 32.3% of their recommended calories. Similarly, iron intake by these women was 15%, 14%, and 17% of their recommended allowances. This study also shows non significant decrease in mean calories consumption towards higher altitude ($b=-136.8\pm53.79$; $F_{(1,3)}=6.46$; $P>0.084$), Similarly, there was a non-significant decrease($b=-0.912\pm0.58$; $F_{(1,3)}=2.45$; $P>0.21$) in iron intake towards higher altitude. Nutritional anaemia is related to low caloric consumption and iron

intake. This is what we observed in this study as well. The present study showed tea intake increased significantly towards higher altitude ($P<0.004$). This study also showed decrease in anaemic subjects towards higher altitude. The two results indicate that increase in tea intake may not have an effect on the increase/ decrease of number of anaemic subjects.

The effects of nutritional deficiencies have been described by different authors. They are of the view that ill health caused by deficiencies of calories, proteins, vitamins and minerals interacting with infections and other poor health and social conditions, saps the strength and well-being of millions of women and adolescent girls around the world. It weakens women's ability to survive childbirth, makes them more susceptible to infections, and leaves them with fewer reserves to recover from illness (Galloway *et al.*, 2002).

According to the MI/INF (1999) report, iron deficiency and anaemia are the most prevalent nutritional deficiencies in the world. The body uses iron to produce haemoglobin, protein that transports oxygen from the lungs to other tissues in the body via the blood stream, and anaemia is defined as having a haemoglobin level below a specific level (less than 12 gms of haemoglobin per deciliter. According to Allen (2000) women are specially susceptible to iron deficiency and anaemia during pregnancy, and about half of all pregnant women in less developed countries are anaemic, although rates vary significantly among regions.

According to Shubhada and Rashmi (2000), in communities where many adolescent girls are underweight, supplements may improve the girl's overall health and their pregnancy outcomes, including reducing their risk of bearing low birth-weight babies. In India and Egypt where iron supplements have been given out in schools, the prevalence of anaemia has fallen significantly.

This study did not include supplementations to anaemic women as this is a vertical study of a population to investigate prevalence of anaemia at higher altitudes. I predict that if supplements were given to anaemic subjects of the present population reduction in rates of anaemia would be expected as has been observed the successful outcomes in India and Egypt. Not only this but improvement in health care, medical aids, health education and awareness about anaemia are very essential factors to overcome this problem. The symptoms of anaemia and knowledge of infections becomes important for self-education and awareness. Localities where anaemia is prevalent should be provided easy access to clinics and sufficient number of health visitors should be available to help alleviation of disease. In the study area the education level is poor. Not many even turn up to schools and there are few who go for school level education. Incentives need to be given to younger generation to get at least school education. Policy makers should take care of these things at community level.

This study as well as other studies has shown that haemoglobin concentration increases with higher altitude. Exceptions to this also exist where no change in haemoglobin concentration have been observed in populations residing at high altitude. This is because these populations are adapted to the conditions at higher altitude. This is possible that over the period of time these populations have gone through different environmental conditions. Natural selection must have led to the survival of those who had adjusted themselves in high altitude conditions. It is just possible that the populations, like the one under study, which have not attained the adaptability to higher altitude conditions, may still be in the transitory stage of evolution in this regard.

On the basis of the findings of this study and comparison with available data it is recommended that further exploratory and preferably intervention studies are needed to ascertain the prevalence of anaemia at mountainous populations of Pakistan. The dynamics of life in isolated rugged mountainous

terrain have a number of differences from the life on plains where most of the studies related to prevalence of anaemia have been carried out. The enormous capacity of the human body to adopt to its environment might be a key factor in differences of prevalence of anaemia in our study area. Particularly the disparity between the scarcity of iron rich food and consumption of large quantity of tea, morphology of RBCs, serum ferritin levels, clinical signs of anaemia and the estimated prevalence of anaemia on the basis of haemoglobin level justifies more studies especially experimental studies to be able to estimate the prevalence of anaemia and/or appropriate cut-off values for diagnosis of anaemia.

Further research on native foods, their nutritional value/iron content and any special foods at higher altitudes (may be more than 2500 meters) can open new vistas to our understanding of IDA in the Northern Areas.

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