

**ACUTE RESPIRATORY TRACT INFECTION (ARI)  
ASSOCIATED WITH LOW ZINC LEVEL IN  
SEVERELY MALNOURISHED CHILDREN.**

**A THESIS SUBMITTED TO THE UNIVERSITY OF DHAKA  
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE  
INSTITUTE OF NUTRITION & FOOD SCIENCE**

**BY**

**GIFT**

382493

**Dr. Md. Salim Shakur**

Dhaka University Library



382493

ঢাকা  
বিশ্ববিদ্যালয়  
গ্রন্থাগার

**DIGITIZED**

**NUTRITION UNIT  
DHAKA SHISHU (CHILDREN) HOSPITAL  
DHAKA  
MAY 2000**

## ACKNOWLEDGEMENT

I am indebted to Prof. Dr. M.A. Malek for his kind support in accepting me as Ph D student and for having spared a large share of his valuable time in guiding and supervising me in my research work.

I would like to thank Atomic energy center, Dhaka for granting me permission to work in their Chemistry division, to have the laboratory facilities for determining the zinc concentrations in serum and hair. I would like to thank in this regard to Dr. S.A.Tarafder, Chief Scientific Officer, Chemistry division, Atomic Energy Centre, Dhaka for his support and cooperation. I would like to express my gratitude to Mrs. Shohela Akhter, Senior Scientific Officer, Chemistry division, Atomic Energy Centre for her advice and support.

I would like to thank Prof. Dr. Atahar Ali, Ph.D of department of statistics, Dhaka University for his advice and cooperation for statistical part of the study.

I would like to thank Management Board and Director of Dhaka Shishu Hospital for giving me permission to carry out the research and for allowing me to study on the relevant patients of Dhaka Shishu Hospital. In this regard I am thankful to Miss Nasreen Bano, Nutritionist, Nutrition Unit of Dhaka Shishu Hospital and doctors and nurses of Nutrition Unit of Dhaka Shishu Hospital for their help and co-operation during the research.

I thank the member of the Institute of Nutrition and Food Science for their help and friendship.

382493



I would like to thank my beloved parents, my wife Dr. Parveen Akhter, who is a Consultant radiologist in Bangladesh Private Medical College, and my two daughters Parisa and Salonee for their patience and feelings for my work.



## CONTENTS

|                                                                                                     | Page      |
|-----------------------------------------------------------------------------------------------------|-----------|
| <b>ABSTRACT</b>                                                                                     | <b>1</b>  |
| <b>ABBREVIATION</b>                                                                                 | <b>3</b>  |
| <b>SPECIFIC INTRODUCTION</b>                                                                        | <b>5</b>  |
| State of ARI and malnutrition in world's children and their contribution in child mortality         | 5         |
| Effect of zinc deficiency in increased incidence and case fatality from pneumonia and malnutrition. | 8         |
| Protean and nonspecific clinical and biochemical features of zinc deficiency.                       | 9         |
| Significance of assessment of zinc status by simultaneous estimation of serum and hair zinc         | 11        |
| <b>GENERAL INTRODUCTION</b>                                                                         | <b>14</b> |
| Historical background of zinc deficiency                                                            | 14        |
| Protein energy malnutrition and zinc deficiency                                                     | 16        |
| Effect of Zinc on Malnutrition                                                                      | 19        |
| Zinc Nutriture During Recovery                                                                      | 22        |
| Clinical features of zinc deficiency                                                                | 25        |
| Human zinc deficiency and toxicity                                                                  | 32        |
| Zinc and T- and B – lymphocytes                                                                     | 32        |
| Metabolism of zinc in adulthood                                                                     | 36        |
| Dietary intake and absorption of zinc                                                               | 37        |

382493



|                                                                                  |           |
|----------------------------------------------------------------------------------|-----------|
| Systemic distribution and peripheral uptake of zinc by tissues                   | 40        |
| Zinc Homeostasis                                                                 | 41        |
| Causes of zinc deficiency                                                        | 42        |
| Detection and diagnosis of zinc deficiency and excess: Zinc 'status'             | 43        |
| Plasma and serum zinc concentrations                                             | 45        |
| Leucocyte and erythrocyte zinc content                                           | 47        |
| Hair and Urinary zinc content                                                    | 48        |
| Functional indices of zinc                                                       | 49        |
| How to interpret data and detect zinc deficiency or excess                       | 50        |
| General conclusions concerning the suitability of hair Zn as an indicator in man | 54        |
| <b>RATIONALE</b>                                                                 | <b>60</b> |
| Justification for undertaking this protocol                                      | 60        |
| <b>OBJECTIVES OF THE STUDY</b>                                                   | <b>66</b> |
| <b>SUBJECT AND METHOD</b>                                                        | <b>67</b> |
| Clinical Procedure                                                               | 67        |
| Anthropometric measurement                                                       | 68        |
| Subjects and sample size                                                         | 68        |
| Body composition                                                                 | 71        |
| Sample collection and preparation                                                | 72        |
| Statistical analysis                                                             | 74        |

382493





|                                                                                                                      |            |
|----------------------------------------------------------------------------------------------------------------------|------------|
| <b>RESULTS</b>                                                                                                       | <b>76</b>  |
| Baseline and general characteristics                                                                                 | 76         |
| Result of Body composition                                                                                           | 77         |
| Outcome of zinc analysis                                                                                             | 78         |
| Correlation of serum and hair zinc with age and with each other                                                      | 81         |
| Zinc concentrations in different types of malnutrition                                                               | 81         |
| Serum Proteins and Haemoglobin in Malnourished (PEM) children and in malnourished children (PEM) associated with ARI | 82         |
| Correlations of serum and hair zinc with serum proteins and haemoglobin                                              | 82         |
| <b>DISCUSSION</b>                                                                                                    | <b>83</b>  |
| <b>CONCLUSION</b>                                                                                                    | <b>106</b> |
| <b>LIST OF FIGURES</b>                                                                                               | <b>108</b> |
| <b>LIST OF TABLES</b>                                                                                                | <b>122</b> |
| <b>REFERENCES</b>                                                                                                    | <b>137</b> |

## **ACUTE RESPIRATORY TRACT INFECTION (ARI) ASSOCIATED WITH LOW ZINC LEVEL IN SEVERELY MALNOURISHED CHILDREN.**

### **ABSTRACT:**

#### **Objective:**

To determine the zinc status in severely malnourished children suffering from ARI, by simultaneous estimation of serum and hair zinc.

#### **Subject and Method:**

A case control 4 cell prospective study was conducted on 152 children aged between 6 months to 60 months. Of them 35 children had ARI (Pneumonia) without malnutrition, 47 children had severe protein energy malnutrition (PEM) without Pneumonia or other significant clinical problem and 32 children had severe PEM complicated by ARI. Thirty eight well nourished children without significant clinical problem of the same age group were taken as control. The study was carried out in Dhaka Shishu Hospital and the duration of the study was from July 1995 to February 1998. Zinc status was assessed by determining Zinc concentration in serum and hair simultaneously. Zinc concentrations were estimated by Flame Atomic absorption spectrophotometry (AASP) in chemistry division of Atomic Energy Centre, Dhaka after proper sample collection and after properly processing the samples for determination of zinc by AASP. Results were expressed in parts per million (ppm).

#### **Results:**

The results of the study showed that serum zinc was significantly low ( $p < 0.05$ ) in ARI ( $1.26 \pm 0.60$  ppm) PEM ( $1.31 \pm 0.55$  ppm) and PEM associated with ARI ( $0.90 \pm 0.51$  ppm)



than control ( $1.76 \pm 0.98$  ppm). Serum zinc in ARI with PEM was also found significantly low ( $P < 0.05$ ) than ARI without PEM. Corresponding hair zinc was also found significantly low ( $P < 0.05$ ) in ARI ( $158 \pm 48$  ppm), PEM ( $174 \pm 75$  ppm) and in ARI associated with PEM ( $177 \pm 70$  ppm) than control ( $249 \pm 154$  ppm). However unlike serum zinc, there was no significant difference between hair zinc in ARI with that of ARI associated with severe PEM. In addition to bivariate analysis, multivariate linear logistic regression model used, showed significantly negative association of serum zinc with ARI (PE = -3.664,  $p = 0.0139$ ), PEM (PE = -2.933,  $p = 0.0016$ ) and with ARI associated with PEM (PE = -3.694,  $p = 0.0034$ ). Hair zinc although statistically not significant, also showed negative association with ARI (PE = -0.003,  $p = 0.884$ ) PEM (PE = -0.006,  $p = 0.128$ ), and with ARI associated with PEM (PE = -0.003,  $p = 0.447$ ).

### **Conclusion:**

The results of the study suggest that low zinc status is associated with ARI and PEM and zinc status is further decreased when ARI occurs in PEM children. Assessment of zinc status which is unreliable by estimating zinc in single body fluid or tissue can be removed by simultaneous estimation of zinc in serum and hair which will reflect more reliable index of zinc status of body. Interventions to improve zinc intake in children of developing countries like Bangladesh particularly to malnourished children will improve zinc status and thereby expected reduce ARI and PEM related morbidity and mortality.

## ABBREVIATION

|        |   |                                                        |
|--------|---|--------------------------------------------------------|
| AASP   | : | Atomic absorption spectrophotometry                    |
| AE     | : | Acrodermatitis enteropathica                           |
| ALRI   | : | Acute lower respiratory tract infection                |
| ARI    | : | Acute respiratory tract infection                      |
| BIA    | : | Bioelectric impedance analysis                         |
| BINP   | : | Bangladesh integrated nutrition intervention programme |
| BMI    | : | Body mass index: weight in kg/height in m <sup>2</sup> |
| Ca     | : | Calcium                                                |
| CI     | : | Confidence interval                                    |
| CMI    | : | Cell mediated immunity                                 |
| CNS    | : | Child Nutrition Survey                                 |
| CRIP   | : | Cysteine rich intestinal protein                       |
| CRP    | : | C reactive protein                                     |
| Cu     | : | Copper                                                 |
| DTH    | : | Delayed type cutaneous hypersensitivity                |
| FFBM   | : | Fat free body mass                                     |
| FM     | : | Fat mass                                               |
| H/A    | : | Height for age                                         |
| IAEA   | : | International atomic energy agency                     |
| ICU    | : | Intensive care unit                                    |
| IRR    | : | Increased respiratory rate                             |
| LCI    | : | Lower chest indrawing                                  |
| NAHRES | : | Nutrition and health related environmental studies     |
| NCHS   | : | National centre for health statistics                  |

|        |   |                              |
|--------|---|------------------------------|
| OR     | : | Odds ratio                   |
| PEM    | : | Protein energy malnutrition  |
| RBP    | : | Retinol binding protein      |
| RDA    | : | Recommended daily allowance  |
| SD     | : | Standard deviation           |
| TBW    | : | Total body water             |
| UNICEF | : | United nations children fund |
| W/A    | : | Weight for age               |
| W/H    | : | Weight for height            |
| WHO    | : | World health organization    |
| Zn     | : | Zinc                         |



# **SPECIFIC INTRODUCTION**

## **ACUTE RESPIRATORY TRACT INFECTION (ARI) ASSOCIATED WITH LOW ZINC LEVEL IN SEVERELY MALNOURISHED CHILDREN.**

### **SPECIFIC INTRODUCTION:**

#### **State of ARI and Malnutrition in World's Children and their Contribution in Child Mortality**

Malnutrition is a major child health problem of developing countries like Bangladesh (Kabir and Mosleuddin 1996)<sup>1</sup> and continues to be a serious problem. According to UNICEF, the state of world children 1998 report some 56% of Bangladeshi children under five suffer from moderate to severe malnutrition, 21% of whom are severely under weight (UNICEF)<sup>2</sup>. About 25% - 50% of children in developing countries die before their fifth birthday and over 50% of these deaths are related directly or indirectly to malnutrition (Puffer RR and Serrand CU 1973)<sup>3</sup>. Of the nearly 12 million children under five who die each year in developing countries mainly from preventable causes the death of over 6 million or 55% are either directly or indirectly attributable to malnutrition (WHO,1996)<sup>4</sup>. Malnourished children are more vulnerable to infection. In majority of deaths in developing countries, infections are common association (Tomkins A, Whatson F 1989)<sup>5</sup>. Malnourished patients are more vulnerable to infection including pneumonia (Frank S,1985)<sup>6</sup>. Most of the severely malnourished children admitted in hospitals of developing countries like Bangladesh have various infections for which admission are sought (Kenneth, Robert and others 1981)<sup>7</sup>. Study carried out in Dhaka Shishu Hospital found acute respiratory tract infection (ARI) was the commonest infection in severely malnourished children (Shakur, Bano and Ehsan 1991)<sup>8</sup>. Acute respiratory tract infection (ARI) is now one of the most important causes of mortality in children of developing countries like Bangladesh. According to WHO 1991 report about 13 million children die

every year in the world, 95% of them in developing countries where pneumonia is one of the leading causes, accounting for about four million death (WHO,1991)<sup>9</sup>.

Various other studies done in various parts of the world including world health statistics also showed pneumonia as leading cause of death in developing countries accounting for one third of all childhood death in developing countries (Leoski J, 1986; Denny FW,1986; Garenne M, 1992)<sup>10,11,12</sup>.

Of the nearly twelve million children who die every year in developing countries 19% are attributable to ARI, 19% are attributable to diarrhoea & 55% are either directly or indirectly attributable to malnutrition (WHO, 1996)<sup>4</sup>. However in Bangladesh in particular the figure is even worse. Out of 380,000 death under 5 year, 120000 that is 35% are attributable to acute respiratory tract infection, 95,000 i.e. 25% attributable to diarrhoea and 65% are directly or indirectly attributable to malnutrition (Baqui AH,1998)<sup>13</sup>.

Malnutrition is one of the risk factors for ARI including pneumonia causing increasing case fatalities from Pneumonia (James JW,1972; Zaman K,1996; de Francisco A,1993)<sup>14,15,16</sup>. Severely malnourished children die in majority cases due to infection and among infections ARI is the leading cause of death (WHO,1981)<sup>17</sup>. At Balbela hospital in Djibouti mortality was 12% in children with acute respiratory tract infections or gastroenteritis but when children admitted with these infections had the added complication of PEM (Protein Energy Malnutrition) death rates rose to nearly 25% emphasising for need for improved skills in the treatment of PEM, when it is associated with these conditions (Mohammed 1993)<sup>18</sup>.



Literature review revealed that over the past five decades median case fatalities has remain unchanged among severe PEM which range from 20%-30% (UNICEF 1993)<sup>19</sup>. Even in 1990s, mortality rates of 49% have been reported for malnourished children in hospital, although low rate also occurs (Schofield C et al,1995)<sup>20</sup>. But according to WHO in the management of severely malnourished children a mortality rate of more than 20% is unacceptable, 11% - 20% is poor 5% to 10% is moderate, 1% to 4% is good and <1% is excellent (WHO 1999)<sup>21</sup>. High case fatalities in hospitals have been attributed to faulty case management, which arises because staffs are unaware of how to treat severely malnourished children and lack prescriptive guideline (WHO,1999)<sup>21</sup> Scherbaum concluded that therapeutic feeding is predominantly influenced by protein dogma of past decade with no consideration of recent scientific knowledge (Scherbaum et al. 1992)<sup>22</sup>. A survey of treatment centres world wide to identify the cause of unchanged high mortality showed that for acutely ill PEM children inappropriate diets, high in protein, energy and sodium and low in micronutrient including zinc are common place (Schofield C et al. 1995)<sup>20</sup>. Even in 1990s mortality rates of 49% have been reported for malnourished children in hospital, although very low rate also occur<sup>20</sup>. The contemporary view is that early dietary management should be cautious in terms of protein and energy (Waterlow JC 1992)<sup>23</sup>. Appropriate feeding with micronutrient supplementation together with aggressive antibiotics therapy and less use of intravenous fluid for rehydration are factors that are believed to reduce mortality and morbidity of these children (Suskind D, 1990)<sup>24</sup>. Dietary intake of protein of 1-2 gm/kg body weight and 80-120 kcl/kg body weight are recommended. Higher intakes are inappropriate and may be dangerous in the first few days of treatment because the malnourished child is in brittle metabolic state and unable to deal with extra metabolic stress. Zinc which plays an important role as antioxidant engaged in scavanging free radicals, thus preventing lipid peroxidation and cell

membrane damage and which is present in more than 70 important metalloenzymes carrying various metabolic activities are practiced as supplement in only 15 nutrition centres (25%) out of 65 nutrition centres observed under multicenter studies all over the world. (Schofield C, Ashworth N, 1995)<sup>20</sup>.

### **Effect of Zinc Deficiency in Increased Incidence and Case Fatality from Pneumonia and Malnutrition.**

The increased unchanged high mortality in severe PEM and increased incidence and severity and case fatality of pneumonia in malnourished children as mentioned above may be at least partly if not wholly attributable to zinc deficiency. Schofield and Ashworth in their study also found among other factors, death rates in malnourished children are significantly higher in those nutrition centres where zinc is not routinely given to malnourished children<sup>20</sup>. The increased susceptibility to ARI and other infections in malnourished children may be attributable to zinc deficiency in malnourished children (Chandra, 1983)<sup>25</sup>. In experimental models, isolated protein energy malnutrition does not result in impaired cellular immunity (Good RA, 1979)<sup>26</sup>, which is usually observed in severely malnourished children, which make malnourished children vulnerable to infectious including pneumonia. From Good's study we can therefore assume that it is the low zinc status in malnourished children, which causes impaired immunity and subsequently more susceptibility to infections in malnourished children. Malnourished children are often reported to have associated micronutrient deficiencies (Khanam S, 1988)<sup>27</sup>, including low zinc nutrition (Smith ZM, 1964)<sup>28</sup>. There are numerous reports from different parts of the world that zinc deficiency can occur with protein energy malnutrition (Hansen JDL 1969 & Kumar S, 1977)<sup>29,30</sup>. Low zinc and other trace elements were detected in hair, serum, liver and muscles in PEM patients



in many studies all over the world including Bangladesh (Cheek DB, 1970; Golden BE, 1979; Warren PJ, 1969, Malek MA, 1987)<sup>31,32,33,34</sup>. On the other hand, it has been indicated that deficiency of zinc may be a cause of failure to thrive and malnutrition in infancy as also a cause of geophagia and poor appetite in infants and children (Hambridge KM, 1973)<sup>35</sup>. Zinc deficiency may cause anorexia, nausea, hypogonadism, anosmia, parosmia and weight loss (Hambridge KM, 1972)<sup>36</sup>. On the absence of oedema, there were significant relation between plasma zinc concentrations and stunting, skin ulceration and wasting (Golden BE, Golden MHN, 1979)<sup>32</sup>. Most of the enzymes involved in DNA and protein synthesis are zinc metallo-enzyme.

#### **Protean and Nonspecific Clinical and Biochemical Features of Zinc Deficiency.**

The clinical and biochemical findings in zinc deficiency are similar to that found when protein synthesis is inhibited by other mechanism. Indeed there are close similarities between zinc deficiency and essential amino acid deficiency or antimetabolic drug administration. Like the essential amino acids, zinc is a component of many key proteins and is released only during general protein catabolism. Features common to zinc and essential amino acid deficiency are:

- (1) Rapid development of anorexia and growth failure
- (2) No change in the bound pool
- (3) Rapid and profound fall in the free pool
- (4) Relative insensitivity to dietary carbohydrate or fat intake
- (5) Increased blood ammonia concentration
- (6) Increased blood urea
- (7) High risk of oxidation of administered amino acid
- (8) A decreased incorporation of amino acid into protein



- (9) Reduction of polysomes with relative increased in monosome
- (10) Reduced RNA to DNA ratio and
- (11) Rise in free pool with starvation (Golden MHN, 1982)<sup>37</sup>.

The similarities between essential amino acid deficiency and zinc deficiency itself suggests that zinc deficiency caused a block in protein and nucleic acid synthesis. Moreover, a relative block in protein synthesis and large requirement for zinc as a constituent of cells (about 110 mg/kg muscle) may result in a limitation of lean tissue synthesis when zinc is deficient. As described before many of the enzymes involved in nucleic acid and protein metabolism are zinc metallo-proteins and are dependent on zinc (Kratzer FH, 1959)<sup>38</sup>. Thus the most vulnerable zinc pool is probably represented by enzymes of protein synthesis. There are striking clinical similarities between malnutrition and acrodermatitis enteropathica (AE). They both show anorexia, diarrhoea, skin excoriation and break down immune incompetence with fungul infections, cachexia and an altered affect. Furthermore, the circulating zinc levels of PEM frequently approach those seen in AE. It is therefore tempting to speculate that actual zinc deficiency has some effect on the expression of malnutrition apart from the alteration in zinc metabolism occasioned by the other components of the condition. However low zinc status often found in PEM is not universal and there is independent relationship between hypozinaemia and clinical feature of zinc deficiency (Golden and Banett 1988)<sup>39</sup>. For example zinc deficiency associated with severely malnourished children in Indian subcontinent may not be found in severely malnourished children of Africa or South America. In some parts of the world most children with malnutrition have xerophthalmia, whereas it is very uncommon in other geographical location. Now it is said that in some parts of the world, malnutrition is complicated by superimposed vitamin A deficiency, whereas it is not a complicating factor in other location (Golden MHN, Golden BE,

Benett FI,1985)<sup>40</sup>. Trace element deficiencies particularly zinc deficiency have been considered analogous in this regard to vitamin A deficiency and zinc or other trace element deficiencies may or may not be associated with severe PEM depending on geographical locations. Very low zinc found in Acrodermatitis enteropathica was not found in that extent in hypogonadal dwarfs associated with mild zinc deficiency in children of Middle East and signs of zinc deficiency such as frequent infections, skin lesions, diarrhoea are not common features of stunted children associated with mild zinc deficiency of children in Colorado, USA, which are common in zinc deficiency associated with malnutrition in the Third World (Goldem MHN,1982)<sup>37</sup>.

#### **Significance of Assessment of Zinc Status by Simultaneous Estimation of Serum and Hair Zinc.**

There is no single specific, sensitive and non invasive assay of zinc that can confidently and comprehensively reflect zinc status of the body (Golden and Golden,1981; Jackson,1991)<sup>41,42</sup>. Since until now, there is no single specific non invasive assay of zinc that can confidently and comprehensively reflect the zinc status of body clinical understanding of diagnosing zinc deficiency will only come through simultaneous comparison of the predictive value of different biochemical and physiological parameters in different compartments of body (Solomon 1979)<sup>43</sup>. Assessment of zinc status by determining zinc by simultaneous zinc determination in multiple body fluids or tissue have also been found very useful in other studies as well (Prasad AS, 1973; Prasad AS, 1976, Mcbean LD, 1991)<sup>44,45,46</sup>. We also therefore took the tedious job of assessing zinc status by simultaneous estimation of zinc in two compartments, in serum and in hair. Intelligent interpretation of serum zinc is still considered at present the best way to assess zinc status as far as zinc estimation in only



single body tissue or fluid is concerned (Aggett PJ, 1994)<sup>47</sup>. On the other hand the popularity of using hair as a biopsy tissue for trace elements including zinc resides in parts, in the ease with which samples may be collected in a non invasive and non traumatic way which is best suited for children, in the ease with which samples may be stored and in the stability of the samples after collection. In addition, the concentration of most trace elements including zinc are frequently one or two orders of magnitude greater in hair than they are in more conventional biopsy tissues such as urine or blood (NAHRES 22, IAEA, Vienna,1994)<sup>48</sup>. The above facts were the rationale of selecting serum and hair for determination of zinc concentrations simultaneously in those two compartments. However, serum zinc which reflect the zinc status of the moment varies with various physiological and pathological conditions and with food spacing. Strong zinc homeostasis of the body tends to keep serum zinc to almost normal level inspite of severe clinical zinc deficiency. On the other hand, hair zinc, which is more stable and indicate zinc status of the past do not fluctuate readily with various patho-physiological conditions of the body. However it holds good to estimate zinc in suboptiomal zinc deficiency only (Pallauf J,1973)<sup>49</sup>. In severe zinc deficiency when zinc supply becomes limiting to hair follicles, zinc accumulates in higher concentration in hair follicles thereby increasing the hair zinc concentration leading to paradoxically high hair zinc concentration (Agette PJ, 1994)<sup>47</sup>. On the other hand with marginal zinc supply, that is when it is less limiting on hair growth, hair zinc concentrations do reflect marginal zinc deprivation, features of which such as growth retardation, responds to zinc supplementation (Cavan KR,1993 and Gibson RS, 1989)<sup>50,51</sup>. However if we can assess zinc status by simultaneous estimation of serum and hair zinc and by careful interpretations of their concentration in these two compartments, keeping in mind, the various physiological and pathological conditions which may influence there



concentration in various ways, we can more reliably and less erroneously assess zinc status of malnourished children who are more vulnerable to various stressful conditions. Although until now zinc deficiency is best demonstrated only by observing the effects of zinc supplementation under specific conditions with appropriate controls, a beneficial effect of zinc supplement is observed only in the presence of preexisting biochemical zinc deficiency (Sanstead HH,1970)<sup>52</sup>. It has been found by Hallbook et al that chronic leg ulcer healed faster with zinc therapy, only if the pretreatment serum zinc level was found low, the cut off point in their study was  $<110\mu\text{gm}/100\text{ ml}$  (Hallbook T, 1972)<sup>53</sup>. We therefore also hypothesized that malnourished children will be more benefited and will suffer less from pneumonia and severity of pneumonia if occurred at all on malnourished children will be significantly reduced, if zinc supplement are given to those malnourished children who are biochemically zinc insufficient as assessed by simultaneous estimation of serum and hair zinc.

On the basis of the background mentioned and considering the high and unchanged mortality of severe malnutrition over the last five decades the study was undertaken to determine the zinc status in severely malnourished children (severe PEM) and in severe malnutrition associated with pneumonia, with the hypothesis that if zinc status found low, intervention policy may be undertaken to improve the zinc status in malnourished children, which in turn may decrease mortality and morbidity from severe malnutrition and severe malnutrition associated complications like severe malnutrition associated with pneumonia.

# **GENERAL INTRODUCTION**

## GENERAL INTRODUCTION

### Historical Background of Zinc Deficiency

Zinc is an essential trace mineral in the nutrition of microorganisms (Raulin J, 1869)<sup>54</sup>, plants (Sommer AI, 1926)<sup>55</sup>, and animals (Todd WR, 1934)<sup>56</sup>. This element has been found to be an integral part of several metalloenzymes, including carbonic anhydrase, alkaline phosphatase, lactic dehydrogenase, and carboxypeptidase (Parisi AF, 1969)<sup>57</sup>; zinc is also required for metabolism of nucleic acids and synthesis of protein (Holt AB, 1970; Mills CF, 1969; Prasad AS, 1967; Prasad AS, 1969; Underwood EJ, 1971)<sup>58,59,60,61,62</sup>. Since 1934 when zinc deficiency was first described in animals (Todd WR, 1934)<sup>56</sup>, and particularly since 1955 when zinc deficiency was reported to be responsible for parakeratosis in pigs (Tucker HF, 1955)<sup>63</sup>, the effects of zinc deficiency have been described in a number of different animal species (Barney GH, 1967; Dynna O, 1963; Hurley LS, 1966; Mills CF, 1967; O'Dell BL, 1959)<sup>64,65,66,67,68</sup>. Anorexia and poor growth are prominent features of zinc deficiency in young animals (Macapinlac MP, 1966; Mills CF, 1969; Prasad AS, 1969)<sup>69,59,61</sup>.

Human zinc deficiency occurs in Egypt (Prasad AS, 1961; Prasad AS, 1963)<sup>70,71</sup> and other Mid East countries (Halsted JA, 1970)<sup>72</sup>, where it is associated with retardation of growth and failure of sexual maturation in adolescent males. In other countries, including the United States, there have been isolated reports of zinc deficiency complicating chronic illness (Caggiano V, 1969)<sup>73</sup> or malabsorption (Mac Mahon RA, 1968)<sup>74</sup>. Pories et al. (Pories WJ, 1967)<sup>75</sup> have shown that oral supplementation with zinc accelerates healing of wounds in surgical patients, and Sandstead et al. (Sandstead HH, 1970)<sup>52</sup> have demonstrated that, in the rat, a beneficial effect of such supplementation is observed only in the presence of a pre-existing zinc deficiency. Henkin and associates have reported



recently that hypogeusia and dysgeusia are relatively common disorders in adults and that these abnormalities of taste can frequently be corrected with oral therapy with zinc (Henkin RI, 1971; Henkin RI, 1970)<sup>76,77</sup>.

The possibility of nutritional zinc deficiency in man was first postulated by Prasad et al. (Prasad AS, 1961)<sup>70</sup> in 1961. They collected a group of boys from Iran who had severe iron deficiency anemia, hepatosplenomegaly, short stature, hookworm infestation and marked hypogonadism. The diets of these patients consisted mostly of bread and beans and very little animal product. Prasad et al in 1963 described a second group of patients from Egypt with hypogonadal dwarfism, anemia and hepatosplenomegaly (Prasad AS, 1963)<sup>71</sup>. These patients had low zinc content in plasma, sweat, hair and red blood cells. The plasma turnover rate of zinc was enhanced; there was a smaller 24 hour exchangeable pool and reduced excretion of zinc in stool and urine. It thus appears from these studies that zinc has some role in normal growth and development and that some cases of hypogonadal dwarfism may be due to zinc deficiency. Similar clinical pictures have been described from other places e.g. from Turkey, Portugal, Morocco (Halsted JA, 1974)<sup>78</sup> and aboriginal settlement in Australia (Cheek DB, 1981)<sup>79</sup>. Some doubts have been cast on Prasad's observation by studies made by Coble et al. (Coble YD, 1966)<sup>80</sup> in 1966. They showed that inspite of low zinc level, the cases described by Prasad later developed normalcy and that a group of randomly selected normal people had also low zinc levels. It is probable that other nutritional factors contributed to the development of total picture of hypogonadal dwarfism. There is now little doubt that zinc is an essential nutrient for man and clinical zinc deficiency can naturally occur. This has been further confirmed by experience with total parenteral nutrition (Solomons NW, 1979)<sup>81</sup>. What has recently become apparent is that hypozincaemia is associated with hyperuicaemia

(Cheek DB, 1981; Prasad AS, 1979)<sup>79,82</sup>. An increased relationship between plasma zinc and plasma copper concentrations was noted in rat (Smith SE, 1946; VanReen R, 1953)<sup>83,84</sup>.

### **Protein Energy Malnutrition and Zinc Deficiency.**

Protein energy malnutrition in the developing parts of the world is probably the most common cause of zinc deficiency. Many reports have described a low plasma zinc concentration in malnourished children (Golden MHN, 1978)<sup>85</sup>. Recovery of children from malnutrition appears to have difficulty with lean tissue formation. They become relatively obese with normal skinfold measurements while they are still underweight for their height (Brooke OG, 1976)<sup>86</sup>. Muscle biopsy shows that recovered children have small muscles that are still actively growing (Hansen Smith FM, 1979; Belton H, 1980)<sup>87,88</sup> and physiological measurement suggests that white muscles fibres (low in zinc) are laid down in preference to red muscles fibres with high in zinc (Belton H, 1980)<sup>88</sup>. Zinc supplementation in diet solves the problem (Golden MHN, 1978)<sup>85</sup>.

There are numerous reports from different parts of the world that zinc deficiency can occur with protein calories malnutrition (Hansen JDL, 1969; Kumar S, 1977)<sup>29,30</sup>.

It has been indicated that deficiency of zinc may be a cause of failure to thrive in infancy as also a cause of geophagia and poor appetite in infants and children (Hambidge KM, 1973; Hambidge, 1977)<sup>35,89</sup>. Children with severe PCM during recovery on a milk based or a soya protein based diet become zinc deficient and show a low rate of weight gain and high energy cost of tissue deposition. The deficiency is more marked in soya protein based diet due to high phytate content. Zinc supplementation increases the rate of weight

gain and reduces the energy cost of tissue deposition. It favours lean tissue formation rather than causation of adiposity (Golden MHN, 1981)<sup>90</sup>.

Zinc was used topically by ancient Egyptians in the form of calamine for promotion of healing and is mentioned in the Ebers papyrus from 1550 B.C. (Failla MI, 1977; Pories WJ, 1967)<sup>91,92</sup>. Over 3,500 years later it is still considered reportable that zinc therapy may promote healing of infectious and noninfectious skin lesions (Pories WJ, 1967; Cross RF, 1981; Verma KC, 1980)<sup>92,93,94</sup>.

Perusal of most medical textbooks shows that childhood malnutrition in the tropics has been consistently ascribed to feeding a diet deficient in either protein or some combination of the two. Kwashiorkor is said to result from "pure" protein deficiency, whereas marasmus arises from protein and energy deficiency. These concepts are enriched in the term protein-energy malnutrition.

We now know that the malnutrition of these children is much more complex and is unlikely ever to be the result of "pure" protein deficiency (Landman J, 1980)<sup>95</sup>. Lack of other essential dietary components, toxins in fresh and spoiled food, and the grossly disturbed relationship between the child and his flora are all now thought to play central roles in the genesis and maintenance of malnutrition. To this must be added the role of infection with recognised pathogenic organisms, which nearly always precipitates and usually accompanies malnutrition (Jackson AA, 1983)<sup>96</sup>.

Of the dietary components, the individual essential amino acids, the vitamins, and the major electrolytes have received most attention, whereas fats, nonelectrolyte minerals,



and the trace elements have been relatively neglected. Past reports of abnormalities in metabolism of these nutrients have been viewed as being superimposed on the primary defects of protein and energy deficiency.

The view that the other deficiencies are epiphenomena is supported by consideration of xerophthalmia. In some parts of the world most children with malnutrition have xerophthalmia, whereas it is very uncommon in other geographic locations. This difference in clinical signs posed a problem as to whether clinicians were dealing with two pathological entities. The recognition that xerophthalmia was specially caused by vitamin A deficiency resolved the conundrum: Now it is said that in some parts of the world malnutrition is complicated by superimposed vitamin A deficiency, whereas it is not a complicating factor in other locations; therefore it is not an integral part of the condition. Trace element deficiencies have been considered, analogous in this regard to vitamin A deficiency, as nonessential epiphenomena.

Recently, the central dogma of protein deficiency as the dominant cause of kwashiorkor has been challenged. The basis of this challenge is that the sine qua non of kwashiorkor - nutritional edema, can resolve without any change in plasma albumin concentration (Golden MHN, 1980)<sup>97</sup> and on a diet which by conventional criteria is itself deficient in protein (Golden MHN, 1982)<sup>98</sup>. Furthermore, there is no relationship between the rate of loss of edema and the dietary protein intake. At present the cause of nutritional edema is unknown. Several of the other cardinal features of malnutrition, such as dermatosis, hair discoloration, and fatty liver, are unexplained: They are not seen in experimental animals malnourished with formula diets containing a sufficient vitamin and trace element supplement; they are seen in animals given the foods ingested by malnourished

populations. The other features of malnutrition, such as anorexia, diarrhea, wasting, stunting, and hypoalbuminemia, are common to many noxa apart from protein and energy deficiencies. It is quite possible that trace element deficiencies singly or in combination play a primary role in the genesis of some of the features of malnutrition.

### **Effect of Zinc on Malnutrition**

There are striking clinical similarities between malnutrition and acrodermatitis enteropathica (AE): They both show anorexia, diarrhea, skin excoriation and breakdown, immunoincompetence with fungal infections, and as altered affect. Furthermore, the circulating zinc levels frequently approach those seen in AE. A priori, it is tempting to speculate that actual zinc deficiency has some effect on the expression of malnutrition apart from the alterations in zinc metabolism occasioned by the other components of the condition.

Malnourished children present with spectra of clinical signs. In a study done by Golden BE, the plasma zinc concentration was examined in relation to various clinical features of malnutrition (Golden BE, 1979)<sup>32</sup>. There was no relationship with a history of acute or chronic diarrhea, observed stool frequency after admission to the ward, or anorexia. There were statistically independent relationships between plasma zinc and nutritional edema, skin ulceration, and stunting in height. These relationship do not, of course, demonstrate cause and effect. Although plasma zinc concentration is probably the best biochemical indicator of zinc status currently available, zinc deficiency can be demonstrated only by observing the effects of supplementation under specific conditions and with appropriate controls.

One of the major problems in studying the malnourished child is finding the appropriate control. The complexity and heterogeneity necessitate either very large series of carefully matched individuals or the use of the children as their own controls. Golden et al. (Golden BE 1979)<sup>32</sup> found in their study that during the first few days after admission the clinical changes in the children were rapid. Their infections were treated, diarrhea controlled, and major electrolyte imbalances corrected. They had a remarkable improvement in affect, and they lost their edema. Large supplements of potassium, magnesium, and vitamins were given therapeutically.

Over this time of rapid metabolic changes it was very difficult to ascribe any measured changes to provision of a particular nutrient despite the correlation which often exists, unless the response was dramatic and consistently failed to occur when that specific nutrient alone was withheld from the therapeutic regimen.

When examining the relationship between zinc and the skin lesions of malnutrition, the problem of control was circumvented by specifically selecting children with symmetrical skin lesions on their limbs. In this way one side of the child could act as control, and the other was treated with local zinc. Any change in the general condition of the child thus affected each side identically. A double-blind sequential trial showed much more rapid healing of the sores with zinc supplementation (Golden MHN, 1980)<sup>99</sup>. Since that time, zinc has been routinely prescribed for children with open skin lesions: They heal rapidly and completely, and we no longer encounter lesions that become indolent, as in the past. We can probably ascribe these lesions, which are very similar to those found in AE, to zinc deficiency.



The interesting association between stunting and low plasma levels may well be due to chronic mild zinc deficiency, similar to that described by Hambidge's group in Denver (Hambidge KM, 1972)<sup>36</sup>. Because the children do not receive the same diets after returning home as they received while developing malnutrition, this hypothesis is not as easily amenable to experimental varification as it was in Denver.

Another cardinal feature common to both AE and malnutrition is increased susceptibility to infection. Again, when assessing the immune status of these children the results are likely to be confounded by the clinical changes that occur after admission. Topical application of zinc and placebo ointments were applied by Golden et al. in their study to increase the local cutaneous zinc concentration at the site of testing for delayed cutaneous hypersensitivity (Golden MHN, 1978)<sup>100</sup>. Golden et al used Candida antigen to which all the children had been naturally exposed. The results of this study showed that all the tests in the zinc-supplemented arm gave positive reactions, whereas on the unsupplemented side some children gave normal reactions and some hardly reacted at all. The children with the most depressed responses on the unsupplemented arm had the lowest plasma zinc concentrations. This result indicates that much of the susceptibility to infection in malnutrition may be attributable to inadequate supplies of zinc.

As with vitamin A-associated xerophthalmia, all the children do not have skin lesions or such infections as candidiasis. Severe zinc deficiency does not seem to be central to the genesis of malnutrition (either marasmus or kwashiorkor), but it often complicates malnutrition. It can be recognized clinically by the skin lesions and mucosal candidiasis, and appropriate supplements can be given.

## Zinc Nutriture During Recovery

We cannot conclude from above studies that zinc deficiency always occurs in all malnourished children or zinc status is similar in malnourished children in different part of the world. However, it is obvious that to return the muscle and liver to normal will require additional zinc, as well as all the other protoplasmic constituents. On an individual cell basis (DNA) the content of zinc is less than half that of a normal cell in both liver and muscle. Tissues, particularly muscle, form the major components of body weight. Mixed muscle tissue contains about  $1.7 \mu\text{mol Zn/g}$  wet weight (Golden BE, 1981)<sup>90</sup>. If the body is about one-third muscle tissue and the concentration of zinc in muscle has to be doubled to allow for repletion of its full normal activity, there will need to be a retention of  $1.7 \times 300 \times 0.5 = 255 \mu\text{mol zinc/kg body weight}$ . Thus just to return existing muscle tissue to its normal state (per DNA which infers hypertrophy) will require a retention of  $255 \mu\text{mol}$  (16.6 mg) zinc/kg. For a 5-kg child this represents the total recommended dietary allowance for more than 2 weeks. When we consider that no account has been taken of the repletion requirements for other tissues or of the facts that zinc is not 100% utilized and there are continuing losses, it is obvious that large quantities of zinc will be required during rehabilitation if the metabolic adaptations are to be successfully reversed. To this must be added the requirement of zinc for new tissue synthesis.

Typically, a recovering malnourished child may gain weight at a rate of  $15 \text{ g/kg/day}$ . If this were lean tissue with a zinc content similar to normal mixed muscle, a 5-kg child would need to retain an additional  $127 \mu\text{mol}$  (8 mg) zinc/day. Of course, if half the weight gained was fatty tissue, only half this amount would be required; nevertheless, it

then represents nearly the total recommended daily allowance, again with no allowance for continuing losses or incomplete absorption.

It is obvious that during rehabilitation very large amounts of zinc are required to replace depleted tissues and to permit growth. None of the currently used diets supply anywhere approaching the amounts of zinc required to return the child to normal. It is perhaps for these reasons that anthropometrically recovered children still have thin atrophic muscle fibres (Hansen-Smith FM, 1979)<sup>87</sup> with a much reduced zinc concentration (Cheek DB, 1970)<sup>31</sup>.

Malnourished children who are given maintenance diets, which do not permit growth and supply only 1.5  $\mu\text{mol}$  zinc/kg/day (less than the content of 1 g of muscle), maintain their plasma zinc concentration (Golden BE, 1981)<sup>90</sup>. When they are given an increased diet so that they can gain weight rapidly, even provision of the recommended dietary allowance does not prevent a profound fall in the plasma zinc concentration. This remains low throughout recovery and returns to normal only when the children cease gaining weight rapidly. There is a direct relationship between the fall in plasma zinc and the rate of weight gain, emphasizing the dominant role growth has on zinc requirements (Golden BE, 1981)<sup>41</sup>.

Although zinc deficiency may not be a feature of malnutrition on presentation, failure to provide sufficient zinc may well limit the rate of growth and give rise to a conditioned zinc deficiency. Walravens et al found an existence of growth limiting syndrome of mild zinc deficiency in children (Walravens PA, 1983)<sup>101</sup>. A similar growth limiting zinc deficiency syndrome has been reported to affect older children in Yugoslavia (Buzinia R,



1980)<sup>102</sup>. Poor growth is a notable problem in acrodermatitis enteropathica and “catch up” growth follows the correction of the severe zinc deficiency state that characterizes this disorder (Walravens PA, 1978)<sup>103</sup>.

To test whether zinc deficiency does in fact supervene during recovery from malnutrition, in one study children were supplemented with zinc in the later stages of rehabilitation. There was a dramatic and immediate increase in the rate of weight gain (Golden MHN 1981)<sup>90</sup>. Accompanying this is a decreased energy cost of growth from over 60 kJ/g weight gained to about 30 kJ/g weight gained. One can interpret this to mean that more lean tissue is being synthesized with the provision of zinc, in accordance with the isotopic measurements of changes in muscle mass that have been made in similar children (Jackson AA, 1977)<sup>104</sup>.

It is important to emphasize that zinc is only one of the constituents of protoplasm. If any one other constituent is not supplied in adequate amounts, there is a similar limitation on growth and conditioned deficiency. Zinc is very unlikely to be unique in this regard.

That these children are indeed zinc-deficient after recovery is demonstrated by the growth of their thymus gland with zinc supplementation (Golden MHN, 1977)<sup>105</sup> and stimulation of their WBC sodium pump activity (Patrick J, 1980)<sup>106</sup>, as well as their growth responses. These children did not display the overt clinical signs of dermatosis, diarrhea, or anorexia classically associated with severe experimental zinc deficiency.

That these signs are displayed only by children with severe zinc deficiency is illustrated by the case of one subject who grew very rapidly on a phytate-containing soya formula.

The child's plasma zinc concentration fell to 3.5  $\mu\text{mol/liter}$  (23  $\mu\text{g/dl}$ ) and remained at that level for several weeks. The child then developed mild diarrhea and a minimal skin rash on his upper lip and anus. He was given a single dose of 92  $\mu\text{mol}$  (6 mg) zinc, equivalent to 15  $\mu\text{mol}$  (1 mg)/kg body weight. Within 1 day the lesions had healed and the diarrhea stopped. There was no change in his plasma zinc level, which remained at 3.5  $\mu\text{mol/liter}$ . Following this single dose the child completed a metabolic balance study without any recurrence of symptoms or signs.

When a child presents with zinc-responsive skin lesions and diarrhea he is analogous to the iron-deficient child presenting with koilonychia and a cricoid web. Mild zinc deficiency can cause abnormalities of immune function, electrolyte homeostasis, body composition, and growth without dramatic or even overt clinical signs. The responses to supplementation in humans (Golden MHN, 1981)<sup>107</sup> and experimental animals (Chesters JK, 1970)<sup>108</sup> are extremely rapid, being measured in hours or at most days; this means that a therapeutic trial of zinc supplementation is a practicable test of zinc deficiency. However, we are as yet unsure of the most sensitive physiological or biochemical variable to monitor for a response: It is likely to be some aspect of T-cell function, as these cells seem to be particularly sensitive to zinc deficiency.

### **Clinical Features of Zinc Deficiency.**

The recommended dietary allowance (RDA) for zinc is 15 mg/d for a healthy adult (US Food and Nutrition Board, 1974)<sup>109</sup>. It is not generally appreciated that as much zinc as iron is required in the diet. Indeed the zinc content of muscle, liver, pancreas and gut is higher than that of iron. Two-thirds of body iron occurs in red cell haemoglobin. No corresponding protein has been identified for zinc. The problem is not one of finding a

relevant zinc metallo protein - there is a plethora of them. Over 80 zinc metalloenzymes have been described. They occur in all the major classes of enzyme and major metabolic pathways. Enzymes familiar to the clinician such as lactate dehydrogenase, alkaline phosphatase and carboxypeptidase are all zinc metalloenzymes. Unlike selenium, iodine and iron the limiting and vulnerable zinc pool has not been identified. Indeed animals may die from zinc deficiency with very little change in the gross zinc concentration of their tissues. Virtually all the zinc in the body is locked away in bone and protein: to release it the whole tissue has to be catabolized. There does not seem to be any functional store of zinc. Animals given a zinc-deficient diet develop symptoms in a few days and even preimplantation embryos are morphologically abnormal if a zinc deficient diet is given from the time of copulation (Hurley LS & Sharda, 1975)<sup>110</sup>.

Most of the enzymes involved in DNA and protein synthesis are zinc metalloenzymes. The clinical and biochemical findings in zinc deficiency are similar to those found when protein synthesis is inhibited by other mechanisms. Indeed, there are close similarities between zinc deficiency and essential amino acid deficiency or antimitotic drug administration. Thus, in these conditions, the rapidly turning over tissues such as the white cells, skin and gut mucosa are those that are most affected by the insult. Immunoincompetence, diarrhoea and skin lesions together with an inability to repair wounds are the hallmarks of all these conditions. The striking correspondence between the effects of zinc deficiency and of essential amino acid deficiency is shown by the following developments (Golden MHN & Golden BE, 1981)<sup>90</sup>:

- (1) anorexia and growth failure;
- (2) profound teratogenesis;
- (3) little change in the bound pool with dietary deficiency;



- (4) a rapid and dramatic fall in the small free pool with dietary deficiency;
- (5) particular sensitivity to dietary nitrogen intake;
- (6) relative insensitivity to dietary carbohydrate or fat intake;
- (7) increased blood ammonia concentration;
- (8) increased blood urea concentration;
- (9) excessive oxidation of administered labelled amino acids;
- (10) decreased incorporation of amino acids into body proteins;
- (11) reduction in polysomes with an increase in monosomes;
- (12) reduced RNA to DNA ratio;
- (13) a rise in the concentration of the free pool with starvation.

It is likely that the most vulnerable zinc pool will be one or more of the enzymes involved in protein or nucleic acid synthesis. Unfortunately, we do not know which of the many zinc enzymes involved will be the limiting one and it is unlikely that it will be as amenable to biopsy and quantitation as the proteins containing the other trace elements.

The most clear-cut human zinc deficiency disorder is acrodermitis enteropathica (AE), a rare congenital defect of zinc absorption. The report of Moynahan EJ (1974)<sup>111</sup> that AE patients invariably respond clinically and biochemically to physiological doses of zinc has been confirmed by at least 50 subsequent reports. The clinical picture seen in AE, which we can take as the index of severe human deficiency, has been produced by parenteral feeding with low zinc solutions (Wolman SL et al., 1979)<sup>112</sup> and closely resembles experimental zinc deficiency in animals.

Characteristically skin lesions appears first, starting around the nostrils and corners of the mouth as erythematous crusted lesions which do not itch or respond to steroids. They spread to the fingers, toes, perineum and any other area which is traumatised. The skin lesions may be associated with discrete papulopustular lesions resembling acne vulgaris (Schlappner OLA et al., 1972)<sup>113</sup> and vesico-bullous lesions (Van Vloten WA & Bos LP, 1978)<sup>114</sup>. The patients develop alopecia, intractable diarrhoea and mental depression (Kay RG et al., 1976)<sup>115</sup>. Most patients develop chronic infections, particularly mucocutaneous candidiasis (Singh KP, 1992)<sup>116</sup>. Definitive human zinc deficiency thus has clinical features in common with malnutrition and other trace element deficiencies (Golden MHN, 1985)<sup>40</sup>. In brain and its constituents parts, whose composition is largely preserved in the malnourished children, no difference have been found in copper zinc or manganese concentrations between malnourished and control children (Lehman BH, 1971)<sup>117</sup>. Anorexia, diarrhoea, stunting, wasting, skin desquamation with ulceration, a reduction in lymphoid tissue and an increased susceptibility to infections are common to both zinc deficiency and malnutrition. We know that the malnutrition of children is much more complex and is unlikely ever to be the result of "pure protein deficiency (Lendman J, 1980)<sup>118</sup>. The features of malnutrition are also common to any other insult which interferes with protein and nucleic acid synthesis (Fujioka M, 1964)<sup>119</sup>. It is only when zinc is the particular limiting nutrient for protein synthesis that we should ascribe these features of malnutrition to zinc deficiency. With our current knowledge the best way to diagnose zinc deficiency is to supplement with zinc and observe the response. O'Rian S et al found incised wound heals better in rats by zinc sulphate supplement (O'Rian S, 1968)<sup>120</sup>. The cell mediated immune (CMI) response was evaluated by Sazawal S et al before and after 120 days zinc supplementation in preschool children (Sazawal S, 1997)<sup>121</sup>. The study found an improved cellular immune status, which may

have been one of the mechanisms for observed improved impact of zinc supplementation on diarrhoeal morbidity<sup>121</sup>.

During recovery from malnutrition an enormous amount of zinc is required as a constituent of the cells that are newly synthesized (up to 110 mg zinc/kg muscle). It is in recovering children that Golden MHN et al. have shown a growth response to zinc supplementation of standard diets (Golden MHN et al, 1977)<sup>109</sup>. They have also demonstrated regrowth of their atrophic thymus glands and stimulation of the sodium pump in their white cells (Golden MHN et al, 1977)<sup>109</sup>. It seems, therefore, that zinc deficiency can be one of the many deficiencies seen in children with malnutrition. It is likely that the specific pattern of deficiencies determines the different clinical features of individuals with malnutrition. This is the probable reason for the heterogeneity of presentation of so-called 'protein-energy malnutrition'. Thus although the skin lesions and defective CMI may usually be due to zinc deficiency, supplementation does not seem to affect other features such as the oedema, hepatomegaly or red hair seen in many of these individuals. The diarrhoea of PEM seems to be multifactorial: supplementation with zinc gives equivocal results.

A syndrome comprising hypogonadism, dwarfing, hepatosplenomegaly, anaemia and achlorhydria occurs in the Middle East, and was the subject of the original claims that zinc deficiency occurred in man. The dwarfing and hypogonadism in particular were said to be due to zinc deficiency (Prasad AS, 1963)<sup>71</sup>. This conclusion was based on the low zinc concentrations in plasma, red cells, urine and sweat, and on turnover of intravenous <sup>65</sup>Zn. Over a hundred patients with this syndrome have been described in great detail, with photographs published of at least a quarter of them. None of the patients had skin



lesions resembling those in AE, alopecia, chronic diarrhoea, recurrent infections or other features seen in definite zinc deficiency. Although hypogonadism is a cardinal feature of this syndrome it is not a usual feature of AE. The two conditions are thus quite distinct clinically.

At the biochemical level it should be noted that very low plasma zinc values found in AE, in zinc deficiency associated with parental nutrition and even in malnutrition were not seen in the hypogonadal dwarfs.

Marginal zinc deficiency in young animals does cause growth retardation and anorexia. That these are also the clinical signs of zinc deficiency in young children is suggested by workers from Colorado, USA. School children who were shorter than their peers and had anorexia were distinguished by having a much lower hair zinc concentration (Hambidge KM et al., 1972)<sup>36</sup>. These workers went on to supplement a commercial infant feeding formula with zinc and found that the male, but not the female, infants grew more rapidly on the supplemented formula (Walravens PA & Hambidge KM, 1976)<sup>122</sup>.

The signs of zinc deficiency such as frequent infections, skin lesions, diarrhoea and stunting are not common features of infants in Colorado, but are common in much of the Third World where malnutrition is rife. There have been very few studies of zinc status in these countries, partly because of the difficulties in defining and assessing zinc status unequivocally.

In contrast to iron and iodine, zinc has not yet established itself in clinical medicine, except as a specific treatment of AE, despite enthusiastic reports by interested clinicians

and circumstantial evidence to suggest that zinc deficiency, often in combination with other deficiencies, is a common clinical and public health problem. This is because of the considerable confusion that still surrounds the clinical features of mild zinc deficiency; they are non-specific and we lack insight into which of the many zinc pools is the most vulnerable to deficiency and the mechanism by which this pool exerts its effects. As a consequence there is uncertainty and difficulty in defining and assessing zinc status in the individual patient.

The most consistently reported effect of zinc on mammalian cells is that of membrane stabilization (Failla MI, 1977; Chvapil M, 1973; Chvapil M, 1972)<sup>91,123,124</sup>. Zinc is present in many mammalian cell membranes (Chvapil M, 1973)<sup>123</sup>, and the stabilizing action of certain steroid hormones on lysosomal membranes may be secondary to the effects of zinc (Failla MI, 1977)<sup>91</sup>. The exact mechanism by which zinc stabilizes cell membranes is not clear and could be different for lysosomal, ribosomal, and other membranes. Four general theories, not mutually exclusive, have been proposed. First, zinc may inhibit peroxidation of membrane lipids by forming mercaptides with thiol groups of membrane proteins (Chvapil M, 1976; Chvapil M, 1973; Chvapil M, 1972)<sup>125,123,124</sup>. Second, zinc may bind to and inactivate membrane-regulating enzymes attached to plasma membranes (Chvapil M, 1976)<sup>125</sup>. Third, zinc could displace calcium for proper functioning of microtubules or microfilaments (Failla MI, 1977; Chvapil M, 1976)<sup>91,125</sup>. Fourth, changes in membrane fluidity associated with zinc binding could also alter membrane function (Failla MI, 1977)<sup>91</sup>.

## Human zinc deficiency and toxicity

Chronic zinc deficiency has been associated with dwarfism, hypogonadism, dermatitis, and alopecia, decreased wound-healing, taste and smell, and, a disease called acrodermatitis enteropathica (AE) or Danbolt's disease (Hsu JM, 1980; Burch RE, 1976; Prasad AS, 1978; Hambidge KM, 1978; Williams RB, 1979)<sup>126,127,128,129,130</sup>. AE is a rare autosomal recessive disease of children associated with decreased levels of zinc, diarrhea, bullous-pustular dermatitis of the extremities, and alopecia (Hambidge KM, 1978)<sup>129</sup>. Children with this disease also commonly have dermatitis due to *Candida* species or *Staphylococcus aureus* (Prasad AS, 1978; Hambidge KM, 1978; Rodin AE, 1969)<sup>128,129,131</sup>. Untreated children die from cachexia, usually associated with infection (Hambidge KM, 1978; Rodin AE, 1969; Julius R, 1973)<sup>129,131,132</sup>.

Specific studies of immune function in laboratory animals with zinc deficiency have noted hypoplasia of the thymus, lymph nodes, spleen, and Peyer's patches (Anderson E, 1973)<sup>133</sup>. In children with AE, decreased thymus size has also been reported (Rodin AE, 1969)<sup>131</sup>, and one child with decreased immunoglobulins had almost no lymphoid tissue at autopsy (Julius R, 1973)<sup>132</sup>. Another child had normal B-and T-cell functions but decreased neutrophil chemotaxis; after treatment with 40 mg of ZnSO<sub>4</sub> daily, neutrophil chemotaxis was normal (Businco L, 1980)<sup>134</sup>. It is difficult to determine whether reported susceptibility to infection in patients with untreated AE is due to zinc deficiency itself or to zinc-deficiency-induced protein calorie malnutrition, damage to the skin, or immune function defects.

**Zinc and T- and B - lymphocytes.** In many experimental systems, zinc deficiency decreases T-cell functions but has little or no effect on B-cell functions. Because animals



fed zinc-deficient diets eat much less food (Fernandes G, 1979; Luecke RW, 1978)<sup>135,136</sup>, caution must be taken to separate the effects of zinc deficiency from the effects of protein calorie malnutrition.

Lymphocytes are one of the few, possibly the only, types of cells activated by zinc (Chvapil M, 1976)<sup>125</sup>, and zinc transferrin receptor sites are present on human lymphocytes (Phillips JL, 1976)<sup>137</sup>. Zinc is necessary for DNA replication by phytohemagglutinin (PHA)-stimulated lymphocytes (Williams RO, 1973)<sup>138</sup>; this requirement probably is related to zinc-containing DNA polymerase and reverse transcriptase enzymes (Chvapil M, 1976)<sup>125</sup>. Zinc chelators can inhibit these enzymes, and supplemental zinc or nickel, but not other cations, can reverse the inhibition (Williams RO, 1973)<sup>138</sup>. Healthy adult humans with normal serum levels of zinc who were fed 660 mg of ZnSO<sub>4</sub> daily for one month were noted as having small but significant increases in levels of zinc in serum; there were also similar responses of lymphocytes in vitro to the T-cell mitogens, PHA, and concanavalin A (Duchateau J, 1981)<sup>139</sup>. In another study, 15 people over 70 years of age were fed 440 mg of ZnSO<sub>4</sub> daily for one month, and 15 controls were not. The people given zinc had increases in numbers of circulating T-lymphocytes, delayed-type hypersensitivity responses to various skin-test reagents, and IgG antibody response to tetanus toxoid; however, in vitro lymphocyte response to three mitogens were unchanged. Unfortunately, zinc status prior to therapy was not evaluated (Duchateau J, 1981)<sup>140</sup>. After several days of incubation of human peripheral lymphocytes with 10<sup>-4</sup>M zinc salt preparations, increased uptake of tritiated thymidine and increased DNA synthesis occurred (Kirchner H, 1970; Ruhl H, 1971)<sup>141,142</sup>. Two patients receiving hyperalimentation who did not have protein calorie malnutrition had serum levels of zinc of <30 µg/100 ml. They both had <30% of the

control response to PHA by their circulating T-lymphocytes; this increased to >130% of the control response after at least two weeks of zinc replacement. Also, one of the patients had no delayed-type hypersensitivity responses prior to zinc therapy but did afterward (Allen JI, 1981)<sup>143</sup>. Several reports of malnourished and zinc-deficient patients (mostly children) have noted absent or decreased skin-test responses, reduced thymus size, and reduced PHA-induced lymphoblastic transformation, all of which increased after (1) the application of topical ZnSO<sub>4</sub> at the skin-test site (Golden MHN, 1978)<sup>85</sup>, (2) weeks of orally administered zinc (Pekarek RS, 1979)<sup>144</sup>, or (3) orally administered zinc and increased caloric intake (Golden MHN, 1977)<sup>105</sup>.

The immune response in zinc-deficient animals has been evaluated by a variety of techniques, including reports that the appearance and progression of autoimmunity in New Zealand Black mice is delayed by dietary-zinc deprivation (Beach RS, 1981)<sup>145</sup> and the demonstration that T-cell mitogenesis is decreased (while no change in B-cell mitogenesis is observed) in zinc-deficient culture media of spleen cells from mice fed a normal diet (Zanzonico P, 1981)<sup>146</sup>. However, most investigators have studied mice or rats fed a zinc-deficient diet for at least several weeks. General decreases in thymus size (Fernandes G, 1979; Luecke RW, 1978; Chandra RK, 1980; Fraker PJ, 1978; Fraker PJ, 1977; Good RA, 1980)<sup>135,136,147,148,149,150</sup>, in the production of thymic hormone (Good RA, 1980)<sup>150</sup>, and in the amount of lymphoid tissue (Fernandes G, 1979)<sup>135</sup> occur after weeks, but in most of these studies protein calorie malnutrition may also have played a role. Spleen size may (Rabinovitch M, 1973)<sup>151</sup> or may not (Chandra RK, 1980)<sup>147</sup> be decreased under these conditions.

The antibody-mediated responses to a T-cell-dependent antigen assayed as direct and indirect plaque-forming cells produced by mouse spleens in response to sheep erythrocytes (Jerne plaque assay) have been used to evaluate T-cell helper function. In a number of studies, animals fed zinc-deficient diets for weeks had decreased plaque assays (Fernandes G, 1979; Luecke RW, 1978; Fraker PJ, 1978; Fraker PJ, 1977)<sup>135,136,148,149</sup>, but much of this may have been due to decreased dietary intake (Luecke RW, 1978)<sup>136</sup>. After weeks on a zinc-adequate diet, plaque assays and thymus size returned to normal (Fraker PJ, 1978)<sup>148</sup>. Other studies of immune function in mice and rats fed a zinc-deficient diet for weeks have reported decreased killer T-cell formation as measured by cytotoxicity response of spleen cells (Fernandes G, 1979)<sup>135</sup> and increased (Chandra RK, 1980)<sup>147</sup> or decreased (Fernandes G, 1979)<sup>135</sup> natural killer-cell activity as measured by release of <sup>51</sup>Cr (Chandra RK, 1980; Fraker PJ, 1977)<sup>147,149</sup>. Little or no effect on B-cells was measured by antibody-dependent cell-mediated cytotoxicity of spleen cells against chicken erythrocytes (Fernandes G, 1979; Chandra RK, 1980)<sup>135,147</sup>; similar results were noted by assays of antibody production or anti-sheep-erythrocyte plasmacyte production after reconstruction of the zinc-deficient mice with thymocytes (Fraker PJ, 1977)<sup>149</sup>.

Miscellaneous factors related to mammalian immunity. ZnCl<sub>2</sub> protects mice against the lethality of endotoxin, perhaps by stabilizing lysosomal membranes that may be involved with the development of endotoxin shock (Snyder SL, 1976)<sup>152</sup>.

Mice given 0.4 mg of ZnCl<sub>2</sub> and inoculated 1 hr later with a lethal dose of *Salmonella typhosa* lipopolysaccharide had a 97% survival rate (Snyder SL, 1976)<sup>152</sup>. Mortality was 100% if 0.08 mg of ZnCl<sub>2</sub> was injected instead of 0.4 mg. Zinc at a concentration of 0.3



mM in the incubation medium inhibits the lysis of rabbit erythrocytes by a variety of toxins, perhaps by stabilizing the membrane (Avigad, 1976)<sup>153</sup>. Zinc also promotes wound healing (Pories WJ, 1967)<sup>92</sup>; however, one study, which assayed serum levels of zinc prior to the institution of therapy, reported that chronic leg ulcers healed faster with zinc therapy only if the pretreatment level was  $<110 \mu\text{g}/100 \text{ ml}$  (Hallbook T, 1972)<sup>53</sup>.

### Metabolism of Zinc in Adulthood

The systemic distribution of the metal is summarised in table I. The total zinc content in adult men is thought to be in the region of 1.0-2.0 g (Jackson MJ, 1989)<sup>154</sup>. This represents about 30 mg of zinc/g fat free mass. There are large variations in concentrations of zinc both between and within tissues and cells. The highest concentrations are in the retina and male reproductive organs (prostate gland), and in the integuments where, as well as being a biologically active pool, much of the metal might reflect the homeostatic sequestration of metabolically unnecessary zinc.

**Table I: Relative sizes of tissue zinc pools in adults.**

| Tissue                              | Content (g) | Distribution (%) |
|-------------------------------------|-------------|------------------|
| Muscle                              | 1.5         | 60               |
| Bone                                | 0.5 - 0.8   | 20 - 30          |
| Skin and hair                       | 0.21        | 8.0              |
| Liver                               | 0.1 - 0.15  | 4-6              |
| Gastrointestinal tract and pancreas | 0.03        | 2.0              |
| Kidneys                             | 0.02        | 0.8              |
| Spleen                              | 0.003       | 0.1              |
| Central nervous system              | 0.04        | 1.6              |
| Blood                               | 0.02        | 0.8              |
| Plasma                              | 0.003       | $<0.1$           |

The most liable zinc pools are those in the plasma and in the liver (Jackson MJ, 1989)<sup>154</sup>, and a small pool in the liver may act as a specific reserve at times of imminent deficiency (Lowe NN, 1991)<sup>155</sup>. Otherwise most (85-95%) of the body zinc is locked away in the much larger pools. Of these the zinc in skin and hair probably never re-joins systemic metabolism whereas that in muscle and bone can do so if it is released adventitiously when the turnover or catabolism of these tissues is increased by physiological or pathological processes other than zinc deprivation.

### **Dietary intake and absorption**

The intake of zinc by adults is about 10 mg daily. Meats are a good source of zinc (20-40 mg/g wet weight in dark meat but less than this in paler meats such as poultry). Many plant sources provide a high zinc concentration (e.g. lentils and grains such as wholemeal wheat: 30 mg/g, maize and polished rice: 10-15 mg/g) but the efficiency with which their intrinsic zinc can be released and absorbed is limited by the presence in plants, particularly seeds, of the abundant phosphorus compound, phytate, which binds zinc (Torre M, 1991)<sup>156</sup>.

Zinc is absorbed throughout the small intestine, and possibly, by the colon. The highest rate of absorption is in the jejunum compared with the duodenum which in turn is more efficient than the ileum (Lee HH, 1989)<sup>157</sup>. However because there is a large entero-pancreatic circulation of zinc in which the intestinal secretion of endogenous zinc can be at least twice the amount consumed daily it is probable that distal intestinal absorption of zinc is functionally very important (Mateshe JW, 1980)<sup>158</sup>. This point is important to remember in managing patients with intestinal resections.

The mechanisms and regulation of the intestinal mucosal uptake and transfer of zinc are not known precisely (Cousins RJ, 1985)<sup>159</sup>. Intestinal mucin is probably an important initial binding agent that concentrates zinc, and other cations, at the mucosal surface (Cornad, 1991)<sup>160</sup>. Enterocytic uptake of zinc probably involves both specific and non-specific carrier and binding mediated processes. Absence of the specific enterocytic mechanism of zinc uptake and/or transfer probably underlies the inherited zinc deficiency disease Acrodermatitis enteropathica (Aggett PJ, 1983)<sup>161</sup>. Within the enterocyte there is a cysteine-rich intestinal protein (CRIP) that has specific binding sites for zinc and which may be involved in the mucosal transfer of zinc (Hempe JM, 1991)<sup>162</sup>. This possibility awaits confirmation.

The factors which influence the intestinal uptake of dietary zinc are summarised in table II. All of these collectively influence the efficiency with which zinc is absorbed and used by the body (Cousins RJ, 1985)<sup>159</sup>. This process is called "bioavailability" and it is often expressed as a percentage of the dietary intake of zinc. Unfortunately the term is often used incorrectly to describe the efficiency with which zinc would be available from a particular foodstuff, as if the food itself were the only item limiting the bioavailability. Table II clearly illustrates that many other factors influence the bioavailability of zinc and it is therefore misleading to ascribe to any foodstuff a particular percentage bioavailability for its constituent zinc and to assume that this value applies to all dietary and physiological circumstances.

Iron can interfere with the intestinal absorption of zinc (Causins, 1985; Conrad ME, 1991; Crofton RW, 1989)<sup>159,160,163</sup> and this can assume ractical significant in clinical care. Additionally, and intriguingly, folic acid supplements, 1 mg(2.27  $\mu$ moles), given



daily to preterm low birth weight infants up to 16 weeks of age have been reported to reduce serum zinc concentrations (Fuller NJ, 1992)<sup>164</sup>, but the pathophysiological basis of this and its implications for clinical care are unclear.

**Table II: Factors which will influence the intestinal uptake and transfer of zinc**

---

|                                                                                                                |
|----------------------------------------------------------------------------------------------------------------|
| Dietary presence of                                                                                            |
| Chemical form and oxidation state of element in diet                                                           |
| (a) antagonistic ligands (phosphate, carbonate, tannates, polyphenols, oxalate).                               |
| (b) Facilitatory ligands (carboxylic acids, some sugars particularly glucose, amino acids, fatty acids)        |
| (c) Competing metals (iron and copper)                                                                         |
| Luminal (intestine)                                                                                            |
| pH and redox state                                                                                             |
| Efficiency of dietary hydrolysis                                                                               |
| Bacterial fermentation (non-starch polysaccharides)                                                            |
| Mucosal (intestine)                                                                                            |
| Genetic influences, inborn errors of absorption                                                                |
| (Acrodermatitis enteropathica)                                                                                 |
| Entrainment of uptake and transfer mechanisms                                                                  |
| (influenced by systemic factors listed below)                                                                  |
| Changes in mucosal structure and function                                                                      |
| Alcohol, drug, smoking                                                                                         |
| Systemic factors                                                                                               |
| Anabolic requirements: growth, pregnancy and lactation                                                         |
| Homeostatic setting for nutrient determined by previous dietary supply and specific systemic reserves of metal |
| Supply and metabolism of other nutrients                                                                       |
| Post-catabolic states                                                                                          |
| Endocrine influences                                                                                           |
| Hepatic and renal function                                                                                     |
| Infection and stress                                                                                           |

---

## **Systemic distribution and peripheral uptake of zinc by tissues**

In the circulation, zinc is bound to albumin (80%), alpha-2 macroglobulin (15%), low molecular weight protein, and possibly to transferrin and a histidine rich protein, less than 1% is associated with amino acids or with metalloenzymes. Virtually no zinc would be present in an ionic form.

The uptake of zinc by peripheral cells such as hepatocytes, fibroblasts, and pancreatic acinar cells show characteristics of carrier mediation as well as possible passive diffusional non-saturable mechanisms. Hepatic extraction of recently absorbed zinc is highly efficient and is impaired with liver disease. Although the liver plays an important role in controlling the metabolism of zinc we do not know how this is achieved or regulated. One important entity in this process is metallothionein. This is the generic name for a group of isometallothioneins which are intracellular monomeric polypeptides (relative molecular mass 6500): they comprise about 60 amino acids of which 30% are cysteine residues (Coleman JE, 1992)<sup>165</sup>. Each molecule can bind 6-10 atoms of several metals but of these, only copper, zinc and cadmium can induce its synthesis.

The primary function of metallothionein is unknown. It might be: (i) an intracellular zinc depot, (ii) a sequestor to prevent the potential damage of excessive intracellular accumulation of metals, (iii) a regulator which because it binds copper more avidly than it does zinc prevents copper from interfering with zinc binding sites; (iv) a homeostatic modulator of zinc; or (v) an intracellular source of cysteine in neonates and a source of reducing sulphhydryl antioxidant groups. It might well have all of the above roles but because metallothionein is not inducible in severe zinc deficiency, it would seem that the primary purpose of the protein involves the metabolism of zinc. Other factors, such as

endotoxemia, starvation, infection, glucocorticoids, hypothermia, exercise, and oestrogens induce metallothionein, as a component of the acute phase response. A mediator of this phenomenon, interleukin 1, increases the expression of metallothionein-1 and -2 genes in liver, bone marrow, and thymus and this is accompanied by an increased uptake and retention of zinc by these organs (Cousins RJ, 1988)<sup>166</sup>. Simultaneously, less zinc is deposited in bone, skin, and intestine. This redistribution of zinc may also explain the fall in the plasma concentrations of the metal which occur when people experience such stress.

### **Zinc Homeostasis**

At adequate and marginally adequate dietary intakes, homeostasis of zinc metabolism is effected primarily by the liver and intestine (Taylor CM, 1991)<sup>167</sup>. When the body is at risk of zinc deprivation the intestinal absorption of exogenous zinc increases and there is a reduction in intestinal losses of endogenous zinc arising from intestinal conservation with an upregulation of carrier sites, and from a reduction in the pancreatico-biliary secretion of zinc (Lee HH, 1989)<sup>157</sup>, and to a lesser extent, increased renal conservation of the element. These mechanisms are important because with the exception of the possible hepatic labile pool (Lowe NN, 1991)<sup>155</sup>, there are no specific systemic stores of zinc and we are dependent on acquiring zinc from external sources.

Intestinal metallothionein mRNA and metallothionein protein are induced by parenteral zinc. With inappropriately high zinc intakes and increasing body burden, the systemic load is regulated by increased intestinal secretion of endogenous zinc and by reduced mucosal uptake. At high intakes of zinc the mucosal transfer of the metal is related inversely to intestinal metallothionein which is thought to sequester zinc within the



enterocyte (Hempe JM, 1991)<sup>168</sup>. Enterocytic CRIP concentrations have not been seen to alter with high intakes of zinc. At high intakes of zinc the element also accumulates in the skin and hair, and possibly bone.

### **Causes of zinc deficiency**

The conditions which are associated with an increased risk of deficiency of trace elements are summarised in table III (Hambidge KM, 1989; Aggett PJ, 1989)<sup>169,170</sup>. Symptomatic zinc deficiency has been seen in all these conditions and this discussion will focus on some particular points. The cited pathogenetic categories are arbitrary, because in most clinical situations more than one of the listed mechanisms would be operative. For example, in coeliac disease as well as there being a failure of the intestinal uptake and transfer of the element, there is also an increased loss of endogenous zinc possibly arising from the coexistent malnutrition, catabolism of lean tissue, increased bone turnover and protein losing enteropathy (Crofton RW, 1990)<sup>171</sup>.

**Table III: Mechanisms and conditions which predispose to dietary and systemic deprivation of zinc.**

|                                                                                                                                  |
|----------------------------------------------------------------------------------------------------------------------------------|
| Inadequate intake and absorbability                                                                                              |
| Malnutrition states                                                                                                              |
| Vegetarianism                                                                                                                    |
| Therapeutic and synthetic diets (e.g. enteral and parenteral nutrition, and diets for management of inborn errors of metabolism) |
| Intestinal infestation (bacteria, protozoa, helminths)                                                                           |
| Nutrient interactions with dietary components and drugs                                                                          |
| Maldigestion and malabsorption                                                                                                   |
| Immunity of absorptive mechanisms                                                                                                |

---

Acrodermatitis enteropathica (inborn error of absorption)

Gastric surgery and intestinal resection

Enteropathies, inflammatory bowel disease

Exocrine pancreatic insufficiency

Biliary obstruction

Hepatic disease

Increased loss

Catabolic states

Protein losing enteropathies

Renal failure, renal dialysis and diuretic therapy

Chronic blood loss and haemolysis (thalassaemia, sickle cell disease)

Chelating agents (specific and non-specific)

Exfoliative dermatoses

Increased utilisation

Rapid tissue synthesis

Post-catabolic convalescence

Neoplastic disease

Resolving anaemias

---

### **Detection and Diagnosis of Zinc Deficiency and Excess: Zinc 'Status'**

Evidently this depends on a high degree of suspicion and an awareness of the features of zinc deficiency or excess and of the circumstances in which they might occur. Frequently the term zinc 'status' is used, but it is not clear what this really means, no single measurement will inform one about the total amount of zinc in the body and in any case such information would be of limited status relevance to the real risk or existence of zinc deficiency, or toxicity. When considering the issue of zinc status it is better to ask the question "Does this individual have, or is he or she at risk of deficiency?" or less frequently similar questions relating to zinc toxicity. No single assay of zinc will reflect the entire spectrum from zinc deficiency through adequacy to zinc excess and toxicity, in

fact none is sufficiently specific on its own to assess the possibility of zinc deficiency. Table IV is an attempt to illustrate the metabolic spectrum of zinc status. It can be seen from this that by the time any standard biochemical or physiological parameter is abnormal then deficiency or excess of zinc is well established. Sufficiently sensitive unambiguous indices of homeostatic adaptation to marginal changes, as would be ideal to detect incipient deficiency or excess, are not yet available (Aggett PJ, 1993)<sup>172</sup>. It should be appreciated also that the zinc content of pools and tissues which are frequently used to measure zinc "status" is susceptible to other physiological and pathological disturbances and to deficiencies and excesses of other essential nutrients apart from changes in zinc supply (Golden MHN, 1989)<sup>173</sup>.

**Table IV: A postulated scheme of the spectrum of zinc "status" and its associated pathophysiological phenomena.**

| Death                       | Zinc Excess                                                                                                                                                                                                                             |
|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Toxicity                    | Clinical, biochemical and pathological features: homeostatics overwhelmed; metabolism of other nutrients (e.g. Cu, Fe) disturbed; depressed RBC Cu-Zn superoxide dismutase activity.                                                    |
| Compensatory homeostasis    | Excretion and tissue sequestration adequate; no chemical disease; hepatic, hair, skin, bone zinc increased                                                                                                                              |
| Initial excess              | Reduced absorption and increased net intestinal loss from endogenous Zn pools                                                                                                                                                           |
| Adequacy                    |                                                                                                                                                                                                                                         |
| Initial deprivation         | Increased absorption and renal conservation; reduced loss of endogenous Zn                                                                                                                                                              |
| Compensated metabolic phase | Vital functional pools protected; no alterations in tissue and fluid concentrations; mobilization of possible stores; no clinical disease but homeostasis becoming inadequate; growth retardation, increased Zn plasma clearance, white |



|                         |                                                                                                                                                                                                                |
|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                         | cell Zn falls, plasma/serum Zn may fall                                                                                                                                                                        |
| Decompensated metabolic | "early" functional defects so that the metabolism of other nutrients is becoming disrupted and health effects may occur; depressed Zn metalloenzyme activities, specific functional defects.                   |
| Gross clinical phase    | Metabolism of other nutrients (e.g. aminoacids, fatty acids, iron) grossly disturbed; extensive specific and non specific functional defects, release of zinc from tissue breakdown may maintain plasma levels |
| Death                   | Zinc Deficiency                                                                                                                                                                                                |

### Plasma and serum zinc concentrations

Plasma and serum zinc concentrations are similar. Previously reported discrepancies between the two have now been attributed to delays in separating the fluids from the blood cells and the use of citrate, rather than deionised heparin anticoagulant for plasma collection. Plasma and serum contain between 10% and 20% of circulating zinc and their zinc concentrations (reference range about 9-22  $\mu\text{mol/l}$ ) are often used to screen for deficiency. These values are depressed by infections and other stresses such as those which induce the synthesis of metallothionein, even so these factors do not depress these concentrations to those seen in severe hypozinaemic zinc deficiency; values below 8  $\mu\text{mol/l}$  are strongly suggestive of zinc deficiency.

The release of zinc from tissue breakdown can maintain plasma/serum zinc levels within the reference range although the patient has clinical and biochemical features of zinc deficiency (Conrad ME, 1991)<sup>160</sup>. For example the catabolism of only 10g of muscle with an assumed zinc content of 40  $\mu\text{g}$  (0.6  $\mu\text{mol}$ )/g wet weight would release sufficient zinc to contribute 2  $\mu\text{moles}$  of zinc/l plasma volume. At times of rapid tissue synthesis an

opposite phenomenon can happen; plasma zinc concentration might fall, presumably because zinc is being taken up from the plasma pool quicker than it is replaced from the intestine or from other compartments. This has been seen in children during the anabolic phases of recovery from malnutrition, rapid growth in ex-preterm infants (Altigani M, 1989)<sup>174</sup>, and in large cross-sectional studies of plasma zinc concentrations in healthy children in which declines in plasma zinc concentrations have coincided with the ages of growth spurts (Butrimovitz GP, 1979)<sup>175</sup>, and even when zinc supplements have been started to treat zinc deficiency.

Plasma and serum zinc concentrations are increased (by up to 15%) if they are collected with venous stasis and care must always be taken to avoid haemolysis and contamination of the samples by rubber (zinc free rubber is available). Postprandial collections give higher values than preprandial samples but levels fall within 2 hours of the meal. With short term fasting and starvation zinc concentrations increase. These phenomena contribute to a diurnal variation in plasma/serum zinc concentrations.

Plasma zinc concentrations in healthy infants match those of adults. A higher concentration has been noted in breast-fed infants at 6 months than in those fed formula; this could reflect the relative efficiencies of absorption of the metal from the respective feedings. In preterm neonates there is a progressive decline in serum or plasma zinc concentration with a nadir ( $9.1 \pm 2.6 \mu\text{mol}$ ) at about 6-12 weeks of age; it increases subsequently (Altigani M, 1989; Gibson RS, 1981)<sup>174,176</sup>. This depression correlates with the rate of weight gain, being greater in those infants who grow fastest and is more marked in males.

## Leucocyte and erythrocyte zinc content

Assays on leukocytes in attempts to detect deficiency are difficult to perform reliably and to interpret. The zinc content of the small amount of white cell pellet which would be available from children might be altered considerably by contamination with plates or environmental zinc during the separation procedures. Polymorphonuclear leucocytes can be collected relatively free from contamination with platelets but monocytes which are in themselves a very mixed population are difficult to collect free of contamination with platelets.

Leucocytes subsets have different zinc contents e.g. mean (SD): neutrophils 1.26 (0.27); monocytes 2.58 (0.65); lymphocytes 1.85 (0.32)  $\mu\text{mol Zn/mg protein}$ . Furthermore each has a different biological half-life (e.g. neutrophils 6h; monocytes 1-3d; T lymphocytes 30h-200d). Thus pathophysiological changes in the relative proportions of the leucocytes subsets would lead to significant changes in the zinc content of the entire population. These phenomena could seriously limit the interpretation of mixed white cell analyses and the use of selected white cell subsets may seem to be more valuable. Analyses on selected separated subsets may prove to be more useful but are for the moment too impractical for routine or emergency use. In a systematic study of zinc supplemented children leucocyte zinc concentrations were found to be a useful indicator of zinc deprivation (Simmer K, 1988)<sup>177</sup>.

Since erythrocytes have a long half life (approximately 40 days) their zinc contents cannot be regarded as reliable indicators of recent or acute changes in zinc supply, unless zinc toxicity is suspected, but even so there are no adequate reference ranges to enable data to be evaluated.



## **Hair analysis**

The limitations of determining hair zinc concentrations to detect severe zinc deprivation has been shown in animal models in which there is a paradoxical increase in zinc content of hair with severe zinc deprivation. This has been noted in malnourished children. A possible explanation for this is that as zinc supply becomes limiting hair synthesis in the follicle is reduced such that despite the reduced supply of zinc it accumulates in the hair root at a normal or increased rate. On the other hand with marginal zinc supply, that is when it is less limiting on hair growth, hair zinc concentrations do reflect marginal zinc deprivation, features of which, such as growth retardation, respond to zinc supplementation (Cavan KR, 1993; Gibson RS, 1989)<sup>50,51</sup>. In hair analyses care must be taken to avoid possible spurious results arising from contamination such as dirt, hair dyes and shampoos and from the leaching of zinc from hair by cleaning procedures. There is no evidence that analyses of zinc in nails is at all reliable.

## **Urinary zinc content**

Urinary zinc values are difficult to interpret. Usually, in adults, about 0.5 mg zinc is excreted in the urine in 24 hours, there are no comparable estimates in children. With increasing zinc deprivation renal conservation of zinc can reduce urinary zinc concentrations before any reliably discernible changes in plasma zinc concentration. Urinary zinc excretion is increased in catabolic states poorly controlled diabetes mellitus, treatment with drugs with chelating abilities, and hepatic dysfunction. When the plasma zinc pool is reduced during anabolism the urinary excretion of zinc could also be depressed. Hence urinary zinc losses could be increased and reduced in situations predisposing to zinc deprivation.

### **Tissue zinc concentrations**

The zinc contents of muscle, bone and liver have not been well characterised in terms of human zinc metabolism and even if they were it is unlikely that performing the necessary biopsies would be particularly practical on clinical to help in the routine care of children.

### **Functional indices of zinc**

Functional indices of zinc "status" include the determination of the activities of zinc metalloenzymes such alkaline phosphatase, carboxypeptidase, lactase dehydrogenase, 5-nucleotidase, thymidine kinase,  $\delta$ -amino-laevulinic acid dehydratase in erythrocytes, and angiotensin 1-converting enzyme and  $\alpha$  D-mannosidase in serum or plasma. Assays have been developed to determine alkaline phosphatase activity in leucocytes, neutrophils and red cell membranes but, with the possible exception of ecto 5' nucleotidase in lymphocyte plasma membranes (Meftah S, 1991)<sup>178</sup> there is as yet no good evidence that such demanding enzymatic assays are of much practical value.

Serum thymulin activity falls within induced zinc deficiency in man and animals and reduced activities of thymulin in plasma obtained from individuals with nephrotic syndrome and sickle cell anaemia has been restored after incubation in vitro with zinc (Prasad A, 1988)<sup>179</sup>. Other functional tests of zinc deprivation include measurement of cutaneous hypersensitivity, white cell (macrophage) chemotaxis, taste acuity and electroretinography. None of these methods lend themselves to routine clinical or population studies but they may be useful parameters to measure in monitoring responses to zinc supplementation (Cavan KR, 1993; Gibson RS, 1989)<sup>50,51</sup>.

Plasma metallothionein concentrations reflect hepatic metallothionein concentrations and might therefore be useful for differentiating the hypozinaemia of stress, when metallothionein levels would be increased, from that of true zinc deprivation when the levels of metallothionein would be low. Metallothionein can be measured in the plasma and urine but levels in red cells may be less susceptible to various interferences (Grider A, 1990)<sup>180</sup>. Unfortunately results to date have shown that other factors such as iron deficiency may influence circulating and erythrocyte metallothionein concentrations, thus this assay may not be as useful as was hoped initially.

### **How to interpret data and detect zinc deficiency or excess**

According to Aggett PJ (Aggett PJ, 1994)<sup>47</sup> currently the best ways to assess possible zinc deficiency are the intelligent interpretation of plasma zinc concentrations in the context of the subject's metabolic health and the monitoring of the clinical and/or functional and biochemical response to zinc supplements. In this context clinical (Aggett PJ, 1980)<sup>181</sup>, in particular growth responses in children do appear to be useful (Hambidge KM, 1989; Cavan KR, 1993; Schlesinger L, 1992; Walravens PA, 1992; Walravens PA, 1989; Golden BE, 1992)<sup>169,50,182,183,184,185</sup>. If appropriate resources and expertise are available leucocyte or neutrophil zinc levels may be of value, but, perhaps, again, only if they are measured serially in parallel with supplementation (Simmer K, 1988)<sup>177</sup>.

It is important to realise that studies in unselected populations (Hambidge KM, 1989)<sup>169</sup> will produce less clear cut results than those in groups of children or in individuals in whom there are other clues of possible zinc deprivation such as growth faltering with prolonged breast feeding alone (Walravens PA, 1992)<sup>183</sup>, or failure to thrive (Walravens PA, 1989)<sup>184</sup>. Additionally such population studies with zinc supplements may produce



tantalisingly brief responses as was seen in Gambian children in whom small but significant increases in mid arm circumferences and in in vitro zinc stimulation of plasma thymulin occurred (Bates CJ, 1993)<sup>186</sup>. Such results could imply the existence of a responsive sub-cohort, the absence of zinc deprivation, or that the adequate supply of zinc had made another essential nutrient rate limiting.

In the case of zinc the levels in blood is kept within rather narrow limits whereas the level in bone may vary to a large extent. The levels in the liver where Zn is mainly bound to metallothionein and in muscle also seem to exhibit only small fluctuations. Growing animals have a higher demand for Zn than adults. Biological effects of deficiency are the inhibition of growth as well as lesions of the skin (parakeratosis) and the atrophy of hair follicles. Hsu and Anthony (Cagnetti P, 1975)<sup>187</sup> have described the essential role of Zn in the synthesis of keratin. In severe deficiency, loss of hair is a most prominent feature (Wiadrowskia, B., 1983)<sup>188</sup>.

After a subcutaneous injection of <sup>65</sup>Zn the "steady state level" of the tracer in the pelt of the mouse was higher than that in the liver and the kidneys but lower than that in bone (Greenwood MR, 1977)<sup>189</sup>. In severe dietary Zn deficiency an even higher transfer of the trace has been observed indicating that the increased efficiency of the intestinal resorption and the decreased turnover of Zn in a period of deficiency "preserve" Zn in the central pool and leads to an increase of the tracer in this pool which was reflected in hair (Kalman S.M, 1977; Campbell B C, 1977; Kopito L, 1969)<sup>190,191,192</sup>. In this case the tracer in hair reflects the homeostatic counteraction against the dietary Zn deficiency.

In long term feeding of <sup>65</sup>Zn a build up of the tracer was observed in hair and in bone of rats demonstrating the continuous supply of the tracer to hair and its very slow turnover

in bone (Kopito, L., 1969)<sup>192</sup>. The level of stable Zn was about the same in bone and hair of the rat. Both were higher than the levels in the kidney and the liver. A response to dietary deficiency of Zn could not be observed in induced hair growth between day 21 and 31 of the deficiency but it has been seen in naturally grown hair harvested at day 77 (Kopito, L., 1967)<sup>193</sup>. Obviously the homeostasis could not be maintained over the prolonged period of deficiency. In accordance with the relatively large supply of Zn with milk in suckling infants, the content in their hair was found to be high compared to that in adults. After weaning the level in hair decreased sharply and subsequently rose slowly to the adult level which was reached about the age of sexual maturity. This development is the same in mammals and man and may indicate a transient scarcity of the element in the central pool at the time of weaning. Only a minor influence of pregnancy and lactation has been reported on Zn levels in the hair of mother animals (Versieck J, 1980)<sup>194</sup>.

Pallauf and Kirchgessner (Marzulli, F.N., 1978)<sup>195</sup> found that the reflection in hair of the levels on Zn in the diet was relatively good for a slightly suboptimal supply. Based on this finding and on the results of previous investigators in various domestic animal species (Niculescu T, 1983; Clayton E., 1983; Fergusson JE 1981; Benguera, J.L., 1987; Weber C.W., 1984; Paciga J.J., 1974; Jarvis R.E., 1977; Chattopadhyay A., 1977; Wibowo A.A.E., 1980; Schroeder H.A., 1970; Iyengar G.V., 1988)<sup>196,197,198,199,200,201,202,203,204,205,206</sup> and in laboratory rats, they arrived at the conclusion that homeostatic regulation severely interfered with the reflection of dietary supply in hair. Similar findings have also been reported by Deeming and Weber (Thimaya S, 1982)<sup>207</sup>. Supplements in the diet did not lead to a linear increase in hair. Instead a plateau was reached, above which no further increase occurred. The

conclusions are compatible with the concept of a strong homeostatic regulation of the level of Zn in the pool supplying the hair matrix (central pool). In Zn deficiency this mechanism affects a gradual increase of the fractional intestinal resorption and simultaneously a gradual decrease of the excretion thus, prohibiting the fast depletion of the stores and maintaining an "above critical" level at the sites where Zn is essential (Salbe A.D., 1990)<sup>208</sup>. Accordingly, a gradual decrease of the fractional resorption combined with a gradual increase of the excretion may be observed during excessive Zn supply.

Deeming and Weber (Thimaya S., 1982)<sup>207</sup> reported that in infant rats the dietary supply of the element was reflected in hair over a large range of concentration. This was also found with bone zinc while kidney zinc was decreased in animals on diets with levels below requirement but did not increase with an intake above requirements. Zinc in the liver revealed no relation to dietary supply.

The Zn contents of hair samples collected at the end of the experiment done by Jacob et al (Limic N., 1986)<sup>209</sup> who supplied rats with optimal or slightly higher than optimal level of zinc, found Zn content of hair were neither correlated to dietary Zn nor to liver Zn. These findings also support the conception of the influence of homeostatic control on the level of Zn in hair. Furthermore, an influence of thyroid function on Zn has also been found in hogs bristles. The levels were low in the initial phase of a treatment with methylthiouracil which produces hypofunction of the thyroid (Limic N., 1987)<sup>210</sup>. This demonstrates also that Zn in hair may be influenced by factors other than the dietary supply on Zn.



Influence of Zn deficiency on other elements in hair which may be due to interactions at various metabolic levels, have also been reported. Iyengar et al. (Iyengar G.V., 1988)<sup>206</sup> have described an increase of Ca concomitant with the decrease of Zn in the hair of the rat. The increased Zn in hair of weaning rats fed a diet low in vitamins and its decrease when the diet contained a large amount of sucrose (Salem H., 1989)<sup>211</sup> or the changing levels of Zn in hogs bristle due to dietary supplements of Ca, Cd or Cu (Versieck, 1980)<sup>194</sup>, are additional evidence of the many factors which exert an influence on the level of Zn in hair. Often the variation of Zn in hair was rather large between individual animals even if these were from an inbred strain and of the same age and sex and kept on a Zn supplemented diet and under strictly controlled experimental conditions. It may be suspected that this may be even more so in humans due to the larger individual differences in man.

#### **General conclusions concerning the suitability of hair Zn as an indicator in man**

It may be concluded that Zn is transferred to hair in proportion to its availability in the central compartment. In dietary Zn deficiency the level in this compartment may be kept normal in circumstances where homeostatic mechanisms counterbalance the low supply in the diet. Furthermore, a low intestinal uptake of Zn may not always be due to low levels in the diet but also due to excessive amounts of Ca, Cd or Cu or other constituents of the diet or hormonal regulations interacting with the kinetics of Zn. Thus the level in hair may indicate sufficient or insufficient internal availability rather than the level in the diet. In adult animals the response of Zn in hair to dietary deficiency or supplementation was weak or even absent. This may also be the case in adult man. Therefore, hair analysis does not appear to be suitable for indicating dietary adequacy or marginal

deficiency of zinc in adults. Due to the relatively high demand for Zn in infancy combined with relatively low body stores the levels in hair or infants or of growing individuals may be more susceptible to low dietary supply than those of adults. Low levels in hair of infant animals are obviously a relatively reliable indicator of a state of Zn deficiency while normal levels may not always indicate adequate supply. This is also in accordance with the experience in man where low levels in hair of subjects of 16 to 20 years of age are obviously a relative reliable indicator of a state of Zn deficiency (Strain WH, 1966)<sup>212</sup>. On the other hand, findings in animals suggest, however, that normal levels in hair may not always indicate an adequate supply.

As mentioned before, it has been found in various studies that malnutrition often associated with zinc deficiency. On the other hand various studies as mentioned above zinc deficiency may cause anorexia, hypogausia, growth retardation cachexia and overt malnutrition. If not solely responsible for malnutrition zinc deficiency at least may help in clinical expression of severe malnutrition. Moynahan has drawn a provocation comparison between the hallmark manifestations of acrodermatitis enteropathica, namely apathy, alopecia scaly skin lesions diarrhoea and immunodeficiency and those of kwashiorkor suggesting a role for zinc deficiency in the expression of kwashiorkor syndrome (Moynahan EJ, 1976)<sup>213</sup>. Again apart from growth promoting factors in children another important property of zinc is its antiinfective property, which it usually performs by improving cell mediated immunity particularly by lymphocytic stimulation. Therefore zinc deficient children are hypothesized irrespective of nutritional status to be more susceptible to infectious diseases particularly pneumonia, which is probably most frequent and important infectious causes of mortality in children of least developed country like Bangladesh. Therefore zinc deficient children are more vulnerable to



pneumonia. Although zinc deficiency can occur isolatedly without malnutrition, it is more frequent in malnourished group as have been found in many studies. Therefore infectious diseases particularly pneumonia is theoretically more frequent and severely in malnourished group who are usually more vulnerable to zinc deficiency. In fact ARI including pneumonia has been shown to increase both in incidence and severity in various studies (Tupasi TE, 1990; James JW, 1972, Zaman R, 1996, de Francisco A, 1997)<sup>214,14,15,16</sup> in malnourished children. Therefore it is essential to know whether malnourished children who usually die of infectious diseases of which pneumonia is most frequent are really zinc deficient or not, and how zinc status of malnourished children with or without pneumonia differs with the children who are better nourished and having no pneumonia.

Unfortunately very little is known especially of children about the zinc status of our children particularly those severely malnourished children, who are suffering and dieing of pneumonia. No clinical study has so far been done in Bangladesh. However, unfortunately there is no single specific sensitive and non invasive assay of zinc that can confidently and comprehensively reflect zinc status of the body. Serum zinc which reflects zinc status of the moment varies with various physiological and pathological condition and with food spacing. Strong zinc homeostasis of the body tends to keep serum zinc to almost normal level inspite of severe clinical zinc deficiency. On the other hand hair zinc which is more stable and indicate zinc status of the past do not fluctuate with various patho-physiological condition of the body. However it holds good to estimate zinc in suboptimal zinc deficiency only. Similarly estimation of zinc in WBC, urine and in various zinc metallonzymes have fallacies. Therefore many authors now believe the best way to demonstrate zinc deficiency is to observe clinical response with



zinc supplement in specific conditions with appropriate control. Growth response for example in children is very useful in this regard (Graham GG, 1981; Halsted JA, 1972; Sandstead HHKP, 1976)<sup>215,216,217</sup>.

However having said that it has been found in various studies that effect of zinc supplement are observed best where preexisting biochemical zinc status were found low. It has been found by Hallbook et al., that chronic leg ulcer healed faster with zinc therapy only if the pretreatment level was found low the cutoff point in their study was  $<110\mu\text{gm}/100\text{ ml}$  (Hallbook T, 1972)<sup>53</sup>. They have found that in patients with venous leg ulcers where the patients had initially low serum zinc level the healing frequency was higher in those treated with zinc than those given placebo. In patients with normal serum zinc level, no significant difference in healing frequency was observed between patients treated with zinc sulphate and those given placebos. We therefore also hypothesized that if zinc status (serum and hair zinc) found low (pretreatment low zinc status) in malnourished children associated with ARI, then routine zinc supplement to malnourished children may be more useful in reducing the incidence, mortality and morbidity from ARI (pneumonia) than children who are not zinc deficient. In other words routine zinc supplemented children, whether malnourished or not are expected to have lesser frequency and severity of pneumonia if their presupplemented biochemical zinc status are found low. The world Health Organization Technical Report "Trace Elements in Human Nutrition" called upon nutritional scientists to intensify their research into the nutritional status of population with regards to the trace elements. Recent technological advances in atomic absorption spectrophotometry has improved the ability to measure trace elements concentrations in body fluids and tissues. The mandate of the World Health Organization Task force however require that a less tedious

diagnostic parameters for zinc and other trace elements nutriture be devised. However as mentioned earlier there is not yet single specific and sensitive assay of zinc of body fluids or tissue that can confidently and comprehensively assess the zinc status of body. Major advances in our clinical understanding of diagnosing zinc deficiency will come only through simultaneous comparison of the predictive values of different biochemical and physiological parameters of nutrition in transverse or longitudinal studies (Solomon NW, 1979)<sup>81</sup>. A model of this approach is seen in the studies of Prasad et al. in sickle cell disease in which determination of plasma, hair RBC and urinary zinc were detected simultaneously for the assessment of zinc nutrition (Prasad AS, 1973; Prasad AS, 1976)<sup>44,45</sup>. Walravens and Hambidge used a similarly multiple indices to measure the effect of zinc supplementations to infant formula (Walravens PA, 1976)<sup>122</sup>. On the basis of the background mentioned so far particularly considering diagnostic dilemma to assess zinc status biochemically and considering high unacceptable unchanged mortality from severely malnutrition during last fifty years despite improved understanding of pathophysiology and treatment of severely malnourished child, the study was undertaken to assess zinc status of severely malnourished children by simultaneous estimation of serum and hair zinc, with the hypothesis that if zinc status is found low from the study results, than intervention policy may be undertaken by routine zinc supplements to malnourished children, which by improvement of zinc status and thereby improvement of immunity will help to reduce the incidence, severity and mortality from infectious disease particularly pneumonia, which is still the major cause of high unchanged mortality of severely malnourished children. So far no such study has been done in Bangladesh. The study results will therefore will be interesting and useful not only for researcher working in this field, but also for clinicians dealing with malnutrition and infectious disease. The study results may be helpful for health policymakers nationally

and internationally particularly organizations like WHO, UNICEF to undertake strategy to prevent or reduce malnutrition and to reduce malnutrition associated high mortality. However further studies are required in Bangladesh in particular in this field. Interventional study that is finding the clinical response of zinc supplement to malnourished children together with determination of zinc status both in presupplement and postsupplement stage will provide more useful and conclusive message about the beneficial effect of zinc supplement to malnourished children in reducing incidence, severity and mortality from penumonia.



# **RATIONALE**

## RATIONALE

### Justification for Undertaking this Protocol

Malnutrition and ARI are both major child health problems of Bangladesh and continuing to be great threats to child health and child health survival of Bangladeshi children. Malnutrition is a silent emergency. But the crises is real and its persistence has profound and frightening implications for children, society and of future humankind. More attention is lavished on the gyrations of world stock markets than malnutrition's vast destructive potentials or on equally powerful benefits of sound nutrition. Therapeutic feeding to malnourished children in the past was predominantly influenced by protein dogma of past decade with no considerations of recent scientific knowledge particularly no considerations to micronutrient supplement including zinc. Micronutrient deficiency which include vitamin A and zinc deficiencies are estimated to cost some countries the equivalent of more than 5 percent of their gross national products in lost lives, disability and productivity. By this calculation, Bangladesh and India forfeited a total of \$ 18 billion in 1995 (World Bank Report, Washington DC, 1997)<sup>218</sup>. Malnutrition has long been recognized as a consequence of poverty. It is increasingly clear that it is also a cause. In some parts of the world, notably Latin America and East Asia, there have been dramatic gains economically in reducing child malnutrition. But overall, the absolute number of malnourished children world wide has grown. Severely malnourished children have high mortality rates. Despite improved understanding of pathophysiology and treatment of severely malnourished child, the median case fatality rate has remain relatively unchanged at 20% - 26% for past 50 years (Schofield et al, 1995)<sup>20</sup>. Of the nearly twelve million children, who die every year in developing countries, 19% are attributable to ARI, 19% are attributable to diarrhoea and 55% are either directly or

indirectly attributable to malnutrition (WHO 1996)<sup>4</sup>. In Bangladesh, the figure is even worse as far as malnutrition and ARI are concerned. Out of 380,000 death under 5 in Bangladesh, 1200000 that is 32% are attributable to ARI, 95,000 that is 25% attributable to diarrhoea and 66% are directly or indirectly attributable to malnutrition (Baqui AH, 1998)<sup>13</sup>. Illness particularly infectious diseases like pneumonia, diarrhoea etc are important cause of malnutrition and malnourished children on the other hand are more vulnerable to infectious disease particularly ARI and diarrhoeal diseases, which help to maintain high case fatality of malnourished children. In Africa, of the total malnourished children, approximately 33% develop malnutrition after being infected with malaria. The malnutrition, infection, malnutrition vicious and notorious cycle is a well known fact. The nutritional status of children in Bangladesh like many developing countries have shown significantly positive relationship with infectious disease like diarrhoea, cough, fever along with other sociodemographic condition (Child Nutrition Survey of Bangladesh, Bangladesh Bureau of Statistics, 1997)<sup>219</sup>. It was found by child nutrition survey (CNS) of Bangladesh, that nutrition status by height for age Z score decreased by 0.14 and weight for age Z score decreased by 0.23 points, for each episode of prolonged fever and caught. Severely malnourished children die in majority cases due to infections and among infections ARI is the leading cause of death (WHO,1981)<sup>17</sup>. Increased susceptibility to infection in malnourished children is due to reduction in immunological capacity, especially in cellular immune status. Impaired immunity in malnourished children is postulated to be due to zinc deficiency as zinc takes part in improving cell mediated immunity by lymphocytic stimulation. Ashworth and Schofield of London school of Hygiene and Tropical Medicine, in their multicenter nutritional study to find the cause of unchanged high mortality of severely malnourished children for last fifty years have found that among other factors, the mortality rates in nutrition centres were



significantly higher, where malnourished children were not routinely supplemented with zinc. Zinc is an important micronutrient that has long been known to be essential for the growth and development of cells and for the functioning of the immune system. However, because zinc deficiency is extremely difficult to measure, little attention was paid until recently to the possibility that it might impair child health and development and may cause increased susceptibility to infections including ARI. Although until now zinc deficiency is best demonstrated by observing the effects of zinc supplementation under specific conditions with appropriate controls, a beneficial effect of zinc supplement is observed only in the presence of preexisting biochemical zinc deficiency, as mentioned before (Sanstead HH, 1970)<sup>52</sup>. The world Health Organization Technical Report "Trace Elements in Human Nutrition" called upon the nutritional scientists to intensify their research into nutritional status of population with regards to trace elements. Recent technological advances in atomic absorption spectrophotometry has improved the ability to measure trace elements concentration in body fluids and tissues. The mandate of the World Health Organization Task force however require that a less tedious diagnostic parameters for zinc and other trace elements nutriture to be devised. However as mentioned before there is no single, specific noninvasive assay of zinc in tissue or body fluid that can confidently and comprehensively assess the zinc status of the body. Major advances in assessing zinc status have been achieved by assessing zinc by simultaneous estimation of zinc in more than one compartments of body fluids or tissues (Solomon 1979; Prasad AS, 1973; Prasad AS, 1976)<sup>81,44,45</sup>. Assessment of zinc by estimating zinc in multiple body fluid or tissue, therefore is expected to provide more accurate and less erroneous status of zinc. Assessment of zinc by simultaneous estimation in multiple body tissue or fluid has not been performed so far in Bangladesh, on children suffering from severe malnutrition associated with pneumonia or suffering

from pneumonia without significant malnutrition. Therefore (1) this study will be first of its kind in Bangladesh, which will reveal zinc status in Bangladeshi children suffering from ARI and from malnutrition associated with ARI. In the study zinc status has been assessed by determining zinc concentration in two body compartment, in serum and in hair. The (2) rationale of choosing this two compartment were due to the fact that until now it is believed that intelligent interpretation of serum zinc is still considered the best way to assess zinc status as far as zinc estimation in single body tissue or fluid is concerned (Aggett PJ,1994)<sup>47</sup> and serum zinc predominantly indicates zinc status of the moment. On the other hand the popularity of using hair as biopsy tissue for trace elements including zinc resides in parts in the ease with which samples may be collected in a non invasive and non traumatic way which is best suited for children, in the ease with which samples can be stored and in the stability of the samples after collection. In addition the concentrations of most trace elements including zinc are frequently one or two folds of magnitude greater in hair than they are in more conventional biopsy tissue such as urine or blood (NAHRES 22 IAEA Vienna 1994)<sup>48</sup>. Hair zinc is more stable, do not fluctuate easily and can indicate zinc status of the past. Therefore combination of estimation of serum zinc which indicates current zinc status and hair zinc indicating long term index of zinc status and by careful interpretations of their concentration in this two compartment, keeping in mind the various physiological and pathological conditions which may influence their concentrations in various ways will reflect more comprehensive and reliable zinc status of children, particularly of malnourished children who are more vulnerable to various stressful conditions including infections. The above facts were the rationale of selecting serum and hair for determination of zinc concentrations simultaneously in those two compartments of body. On the basis of the study results (3) further broad based population study may be undertaken by nutrition



researchers of Bangladesh or by Bangladesh Bureau of Statistics, Ministry of Planning by conducting child nutrition survey (CNS) of Bangladesh, which will be more realistic approach to assess zinc status in the community because in hospital based study where children are referred and admitted selectively, many clinical and biochemical conditions are often over represented. If zinc is found low interventional research (4) may be done in future by giving zinc supplement to Bangladeshi children particularly to malnourished children and by observing clinical response for example how the zinc supplemented group are attacked by ARI in terms of frequency or severity than non supplemented groups, a conclusion can be made regarding reduction of incidence, severity and mortality from ARI in zinc supplemented group.

The study was also undertaken with the hypothesis (5) that if zinc status is found low from study results than the children suffering from malnutrition will be greatly benefited by routine zinc supplement and will be less susceptible to ARI, thereby helping to reduce mortality from severe malnutrition. We also hypothesised that if zinc status is found low in children suffering from pneumonia (ARI) even without significant malnutrition, than routine zinc supplement in recommended daily allowance (RDA) to wellnourished Bangladeshi children may prevent even wellnourished children to suffer from increased incidences and severity of ARI. Recognizing that malnutrition is hindering national development the Government of Bangladesh has formulated a National Nutrition Policy and launched a nation wide integrated nutrition intervention programme (BINP). The initiation aims to address malnutrition directly and help promote faster socioeconomic development. If zinc is found low from study results, than the study result may be used by policy makers and programme managers for addressing child malnutrition and ARI, the two major health problems prevailing in the country and intervention policy by



giving zinc supplement to undernourished children may be taken by the Government along with other nutritional intervention programme. As mentioned before zinc deficiency may cause growth retardation and failure to thrive in a previously well nourished children just as malnutrition may be associated with zinc deficiency. Therefore if zinc status is found low from study results (6) then routine zinc supplement to Bangladeshi children may help to maintain satisfactory nutritional status and may decrease the incidence of high malnutrition in Bangladeshi children and thereby decreasing high infant mortality in Bangladesh where malnutrition shares major role contributing directly or indirectly to 66% mortality under 5 in Bangladesh.

Attempts have been made to present a multivariate analysis to identify the important determinants of child malnutrition and ARI. The relationship (7) of malnutrition, ARI and malnutrition with ARI have not only made with zinc (both serum and hair) but also with various paediatric age group and sex, which will identify age group and sex at risk of severe malnutrition, ARI and ARI associated with malnutrition so that special attention for interventional programme can be properly taken to target vulnerable paediatric age group and sex.

## **OBJECTIVES OF THE STUDY**

## **OBJECTIVES OF THE STUDY:**

- (1) To assess zinc status in severely malnourished children, in children suffering from pneumonia and in severely malnourished children associated with pneumonia and to compare their zinc status with zinc status of wellnourished healthy children without significant clinical problems.
- (2) To assess zinc status by estimating zinc concentration in multiple body compartments (serum and hair) simultaneously.
- (3) To find the relationship of malnutrition, pneumonia and malnutrition associated with pneumonia with zinc status (serum and hair zinc) with multivariate factors (age, sex) by using logistic relation method, which will identify the specific paediatric age group and gender at risk of developing malnutrition, ARI or combined malnutrition associated with ARI.
- (4) To find the relationship of serum and hair zinc concentration with various anthropometric (weight for age, weight for height and height for age) state of body.



## **SUBJECT AND METHOD**

## **SUBJECT AND METHOD**

It was a case controlled clinical non randomized cross sectional 4 cell study. Children were assigned in 4 groups.

1. Severe protein energy malnourished (PEM) group.
2. Severe PEM associated with Acute Respiratory Infection (ARI).
3. ARI without significant malnutrition and
4. Wellnourished children without significant clinical problem, which was regarded as comparison or control group. It was a hospital based study, done in Dhaka Shishu (Children) Hospital, which is the largest children hospital in Bangladesh, having total bed of about 345 of which 20 bed is exclusively reserved for severely malnourished children in Nutrition ward. Children aged between six months to sixty months both male and female were included in the study.

### **Clinical Procedure**

ARI: We considered pneumonia as ARI. Pneumonia was diagnosed clinically, according to WHO guideline having lower chest indrawing (LCI) or increased respiratory rate (IRR) more than age specific value or both (WHO Geneva, 1991)<sup>220</sup>.

Severe Malnutrition: Children having weight for age less than 60% of the US National Center for Health Statistics (NCHS) median (Gomez, 1952)<sup>221</sup> or whose weight for height was less than 70% of NCHS WHO median reference value (termed severely wasted) or who had symmetrical nutritional oedema ( termed oedematous malnutrition) at least in lower limbs were considered as severe malnutrition (WHO Geneva 1999)<sup>222</sup>. We considered severe malnutrition and severe PEM as synonymous, and used the term synonymously. Height for age less than 85% of NCHS median was considered as severe stunting.

**Anthropometric measurement:** Body weight was measured with a digital scale (Seca) after admission having precision of 5 gm. Supine length was measured on a locally constructed wooden length board for the children less than two years of age or if the children cannot stand properly due to sickness or weakness. For other children erect posture length was measured and recorded with Leister plastic height scale of child health foundation, Leistershire UK.

**Subjects and sample size:** Total one hundred and fifty two children having age group of six to sixty months of both sex were enrolled in the study. They were included in 4 groups (4 cell study) (a) severe malnutrition (b) severe malnutrition with ARI (pneumonia) (c) Pneumonia without malnutrition (d) wel-nourished children without pneumonia or significant clinical problems (control). The estimation of sample size was performed taking probability at the 5% level and assuming a power of 70%. The sample size was determined according to formula

$$\frac{Z^2 pq}{d^2} \text{ when}$$

Z = standard normal variate at 5% level of significant which is 1.96

p = probability of success (sample)

q = 1 - p = probability of failure

P = probability of true value (incidence)

d = P - p

Assuming probability of success (p) of severe PEM in sample (hospitalized PEM) to be 30% (0.3) and probability of true value (P) of severe PEM as 17% (0.17), that of ARI, p as 35% (0.35) and P 10% (0.1) and that of ARI + PEM p and P being 20% (0.2) & 5% (0.05) respectively.



For example, sample size of severe malnutrition (Severe PEM)

$$\begin{aligned} n &= \frac{Z^2 pq}{d^2} \\ &= \frac{(1.96)^2 (0.3 \times 0.7)}{(0.3 - 0.17)^2} \\ &= \frac{3.84 \times 0.21}{(0.13)^2} = 47.71 \end{aligned}$$

Similarly sample size of ARI was  $n = 22$  and that of ARI + PEM was  $= 27$

In group A 38 well-nourished children without ARI or significant clinical problem were taken as control. The control were taken from medical out patient department of Dhaka Shishu Hospital and from surgery of private practice from healthy siblings who accompanied their parents with the sick child. Only those children who were co-operative and whose parents gave valid consent for study on their children were enrolled in the study.

In group B, 35 children suffering from pneumonia but having no significant malnutrition (weight for age  $>90\%$  of NCHS median) were enrolled in the study. We considered pneumonia as ARI. Pneumonia was diagnosed clinically by finding lower chest indrawing (LCI) or by increased respiratory rate (IRR) more than age specific value according to WHO guideline (WHO Geneva 1991)<sup>220</sup>. Accordingly, children aged between 2 months to 1 year having respiratory rate more than 50/minute and children above 1 year having respiratory rate more than 40/minutes were considered to have IRR. IRR other than pneumonia like heart failure, bronchial Asthma, metabolic acidosis etc were excluded. Children suffering from pneumonia were taken from general paediatric ward of Dhaka Shishu Hospital. They were enrolled in the study after taking valid consent from their parents.

In group C, 47 severely malnourished children (severe PEM) having weight for age or weight for height less than 60% or 70% of the NCHS median respectively or who had nutritional oedema and having no clinical evidence of pneumonia or other obvious significant clinical problems were enrolled in the study. Severe malnutrition due to chronic medical or surgical conditions were also excluded and thereby only primary severe malnutrition were enrolled in the study. We considered severe malnutrition and severe PEM to be synonymous. The malnourished children were taken from nutrition ward of Dhaka Shishu Hospital. Parent's consent were taken before enrolling the malnourished children in the study.

In group D 32 children with severe malnutrition associated with pneumonia (according to WHO guideline) but no other obvious acute clinical condition (Acute Gastroenteritis, meningitis etc) were included in the study. They were also taken from Nutrition ward of Dhaka Shishu Hospital and they were also enrolled in the study after taking valid consent from their parents.

Randomization was not possible as we found very difficult to get and had to wait unduly prolonged time to get well-nourished children (control), whose parents will agree to allow to take blood due to needle phobia and allow scalp hair sample collection for cosmetic blemish. Similarly, we found great deal of difficulty to get children with severe malnutrition and pneumonia in higher age group (> 2 years) in randomized fashion to match wellnourished children of higher age group, as combined PEM and ARI are more frequently found in lower paediatric age groups.

Informed consent from parents or legal guardian were taken in all cases before they were enrolled in the study. The study was approved by ethical review committee and management board of Dhaka Shishu Hospital. Guardians were given the right to withdraw their consent to continue the study with their children. Even if they do not agree to enroll their children in the study or withdraw their consent during their study, they received the usual schedule treatment.

### **Body composition**

Body composition were measured in all four groups by using Bioelectric impedance (BIA) analysis using a tetrapolar electrode, placing two electrode in right lower limbs and two in right upper limbs. In the upper limb, the red electrode was placed at the level cutting the mid points of the radial and ulner tubrosities and the black one just behind the middle finger adjacent to metatarso phalangeal joint. Similarly two electrode were placed on the right foot, the red electrode cutting the mid points of medial and lateral malleolae at the distal end of tibia and fibula and the black one being placed just behind the middle toe, adjacent to metatarsophalangeal joints. An electric impulse of 800 MA at 50 KHz (Lukasaki HC 1985)<sup>223</sup> was then passed through the body of the child and reading of the resistance (electric impedance) was recorded. A linear regression equation (Fjeld CR 1990)<sup>224</sup> was used to calculate the Total Body Water (TBW), Fat free body mass (FFBM) or lean mass using age specific formulas and age specific constants of body density (Famon J 1982)<sup>225</sup>. Fat mass and percentage of fat mass and percentage of body solid were then calculated simply from percentage of FFBS and percentage of TBW respectively. Oedmatous malnutrition (Kwashiorkor and marasmus kwashiorkor) were excluded from the study.



### Sample collection and preparation

Zinc status was assessed by determining zinc concentration in hair and serum. Each hair sample (minimum 1 gm) was collected from different areas of head with a clear stainless steel scissor. They were kept in small polythene envelop with proper labeling of the patient's name, age and sex until sent for analysis. According to recognized procedure (Report IAEA Vienna 1978)<sup>226</sup>, the samples were washed twice with acetone, three times with double distilled water and finally once with acetone. The washed samples were air dried for 24 hour in a clear room. Dry weight was minimum 0.5 gm. Samples were then prepared by wet ashing method according to Parkin elmer method of analysis (Perkin Elmer, Norwalk, 1982)<sup>227</sup> in the following manner.

6 ml of  $\text{HNO}_3$  was added and was allowed to react at room temperature. The digested hair was then warmed and 1 ml of  $\text{HClO}_4$  was added and heated at  $200^\circ\text{C}$  until dense white fume were evolved. The solution was accepted for analysis when it became water clear. It was then transferred to 5 ml volumetric flask of deionized water. The solution was used for determination of zinc after further 1:10 dilution. The solution was preserved in dessicater for analysis.

For serum zinc 5 ml of venous blood was collected in de ionized vials using deionized syringe, centrifuged and serum was stored at  $-20^\circ\text{C}$  until ready for analysis. The serum was diluted to one fold with deionized water and the standard solution were made in 10% glycerol to match the viscosity of serum solution. Standards were made from spectroscopic graded salts of elements and suprapure acid (2 ml of 65% nitric acid). All the solution were made with double distilled deionized water. The predigested samples

were then heated at 110°C for 6 hour and subsequently at 150°C until the ashing procedure was completed.

The zinc concentrations in hair and serum after proper processing and preparation were measured by flame atomic absorption spectrophotometry (AASP), using Perkin Elmer model no 3110, using wave length of 213.9 nm with 0.7 nm slitwidth. It was also performed in chemistry division of Bangladesh Atomic Energy Centre, Dhaka. Result were expressed in parts per million (ppm). Serum zinc was considered low if the value was <1ppm which corresponds to 1µgm/ml or 100µgm/dl or 15.3 µmol/L. We also considered hair zinc to be low if zinc concentration was <150 ppm corresponding to 150 µgm/gm of hair. There is still no single reference normal range for serum or hair zinc. The wide divergence for the normal values of zinc in hair make cut off point for low hair zinc extremely difficult. The literature contains broad range of results for the normal value of zinc in human hair. Some (Hilderbrand DC, 1974; Hambidge DM 1982, Rivlin RS 1983, Barette SJ, 1985)<sup>228,229,230,231</sup> interpret these broad range of normal value as a serious impediments to the utilization of hair analysis for assessing nutritional status. Some authors (Katz 1980, Chatt A 1983, Katz & Chatt 1988)<sup>232,233,234</sup> have considered variable such as length of hair, age, gender, race and habitat of donor as well as the procedure used for the collection and preparation of hair sample to influence hair zinc concentration. Moreover mean values for the same age varies between one study and another. This could be at least partially explained by lack of internal standard or a common reference standard material in the various laboratories. In addition, quite often the extremely high standard deviation, in some instances even larger than mean zinc value indicate great individual variation (Dorea JO 1985)<sup>235</sup>.

Similarly there is no single reference normal ranges for serum zinc. Therefore the term “frequent normal values” is used for serum zinc also. Serum zinc concentrations ranging about 9-22  $\mu\text{mol/l}$  (0.59 – 1.44 ppm) are often used to screen zinc deficiency (Agette PJ 1994)<sup>47</sup>. ‘The frequent normal values’ for zinc used range from 0.8 – 1.1  $\mu\text{gm/ml}$  equivalent to 0.8 – 1.1 ppm (IYENGAR V 1988)<sup>236</sup>. Golden BE et al (Golden BE 1979)<sup>32</sup> considered serum zinc concentration to be low, if found below 14  $\text{mmol/l}$  (0.93 ppm) which almost equivalent to 1 ppm (1 ppm is exactly equivalent to 15.3  $\mu\text{mol/l}$ ). The same cut off point was used by Roy (Roy SK 1997)<sup>237</sup> for their study in malnourished Bangladeshi children with acute diarrhoea. Serum zinc in my study was considered low if it was below 1 ppm, equivalent to 15.3  $\mu\text{mol/l}$ , the cut off point almost similar to that used by Roy SK et al in malnourished Bangladeshi Children. In case of malnourished and ARI patients, hair and blood samples were collected immediately after admission to hospital before any treatment was initiated and serum proteins were estimated from collected blood in case of malnourished children with or without ARI.

## STATISTICAL ANALYSIS

Data cleaning, validation and analysis was performed using SPSS/PC plus an SAS software.

### Bivariate analysis

The zinc concentration in hair and serum in the three clinical conditions of 4 cell study was compared with the control and also with each other using Duncan Multiple Range test (DMRT) and Tukey’s test using SAS software and was considered significant if  $p$  was  $<0.05$ .



Odds ratio (OR), 95% confidence interval (CI) of OR, standard error of OR was also used in bivariate analysis to know the degree of difference of zinc concentration in between two groups. An odds ratio was considered significant if the upper and lower limit of confidence interval do not includes unity. Z statistics were also used to compare the two values at various significant level to reject null hypothesis ( $h_0$ ) and the minimum cut of point was 0.05 i.e.,  $p < 0.05$ .

#### **Multivariate logistic regression analysis:**

Multivariate logistic regression analysis was also used using serum zinc, hair zinc, age and sex in PEM, ARI and ARI+PEM response profile and it was accepted for statistical analysis if the model were found statistically significant (  $p < 0.05$ ). The results were considered statistically significant if p was found  $< 0.05$ .

To find the direct relationship between two variables we also used correlation coefficient (r) and it was considered only of statistical importance if there was a linear relationship between two variables. The linear relationship was considered significant if p was  $< 0.05$ .

## **RESULTS**

## RESULTS

### Baseline and general characteristics

Thirty eight children were allotted to welnourished control group (Group A). Thirty five children were allotted to ARI(Pneumonia) in group B and forty seven and thirty two children were allotted to severe malnutrition (PEM) in group C and PEM with ARI in group D respectively (Table V).

The mean age (in months) in 4 groups were  $32 \pm 6.52$  months,  $28 \pm 6.54$  months,  $25 \pm 5.62$  months and  $22 \pm 4.25$  months in group A,B,C and D respectively (Fig.1, table-V) showing mildly lower trend of age group in malnourished children with or without pneumonia, as it is more frequent in lower age group and it was very difficult to find higher age group (more than 24 months) in malnourished group with or without ARI to match exactly the healthy control group. Similarly to get more lower age group in healthy control to match exactly the two malnourished groups (group C & D) were also found difficult as most parents refused to allow their small children to take blood samples and hair samples from their smaller children.

Sex distribution in 4 groups were reasonably welmatched with male female distribution in group A(Control) being 22 and 16, and in group B,C & D, 20 and 15, 27 and 20 and 18 and 14 respectively. Male female ratio having approximately 1.3:1 in all groups (Fig.2, Table-V).

The result of the anthropometric in 4 groups are given in table VI and in Figure 3.

As expected according to inclusion criteria, anthropometric measurement are within normal range in welnourished control (group A) and in welnourished ARI group (Group



B), having no underweight according to weight for age, no wasting according to weight for height and no stunting according to height for age. Severely malnourished group with or without ARI (group C & D) on the other as expected from inclusion criteria were severely (<50%) under weight (mean 48.12 & 47.63 in group C & D respectively), severely wasted (weight for height <70% of NCHS WHO median) and severely stunted (height for age below 85% of NCHS WHO median). Out of total 79 severely malnourished children (47 in group C and 32 in group D) 50 was marasmic (30 in group C and 20 in group B) and 29 were oedematous malnutrition (combined kwashiorkor and marasmic kwashiorkor) Distribution of kwashiorkor and marasmic kwashiorkor in group C and D were 6 & 3 kwashiorkor (total 9 kwashiorkor) respectively and 11 and 9 were marasmic kwashiorkor (Total 20) respectively (Fig.4, Table VII).

### **Result of Body composition**

The result of body composition in 4 groups are shown in tabular form (Fig. 5, Table VIII-XI). Percentage of TBW was obtained from impedance value obtained by BIA method and by linear regression equation used for obtaining TBW for malnourished children (Fjeld CR 1990)<sup>224</sup>. From TBW, percentage of fat free body mass (% FFBM), percentage of fat mass (% fat) and percentage of body solid were obtained, using age specific formula and age specific constant of the body density (Famon J 1982)<sup>225</sup>. The result showed very low fat percentage in both malnourished groups with mean value of  $12.77 \pm 4.32$  in group C (PEM) and  $11.35 \pm 3.85$  in group D (PEM with ARI) respectively. Wellnourished two groups (control and ARI) have almost two times higher fat percentage than malnourished groups having percentage of fat of  $23.10 \pm 8.31$  and  $22.68 \pm 7.68$  in control (group A) and in ARI (group B) respectively.

Body composition thus proves that it is the fat which is significantly reduced in malnourished children. Similar results were also obtained by another study on malnourished children (Islam S 1991)<sup>238</sup> where it was observed that fat percentage in children were inversely related to degree of malnutrition, that is more the percentage of fat less the malnutrition. Severe malnutrition having least fat.

Body composition results also revealed that % TBW and % FFBM (TBW plus protein, extracellular and intracellular water, osseous and non osseous tissue) are less in group A & B (well nourished control and ARI) and they are raised in malnourished group (Group C & D). Body solid therefore were found higher in welnourished group (mean value 39.17%, 37.78% in group A & B respectively) than malnourished group (32.23% & 32.30%) in group C & D respectively.

## OUTCOME OF ZINC ANALYSIS

Mean serum zinc in parts per million (ppm) in 4 groups are shown in Fig.6 & table XII. Mean serum zinc in PEM ( $1.31 \pm 0.55$ ), ARI ( $1.26 \pm 0.60$ ) and in ARI with PEM ( $0.90 \pm 0.51$ ) were found significantly lower ( $p < 0.05$ ) than welnourished control having mean serum zinc of  $1.76 \pm 0.98$ . Serum zinc in malnourished children associated with ARI was found even significantly further low ( $P < 0.05$ ) than serum zinc of malnourished children without ARI. Statistically, considering serum zinc to be low if it was  $< 1$  ppm, serum zinc in welnourished control group was found four, five and six times higher than serum zinc in children with pneumonia (ARI), malnourished children (PEM) and malnourished children associated with pneumonia (OR 4.5 & 6 respectively with  $p < 0.05$ ). Serum zinc was again found statistically two times low, when pneumonia affected malnourished children than pneumonia, occurring in healthy welnourished children (OR 2, 95% CI

1.65, 4.58  $P < 0.05$ ). Serum zinc of less than 1 ppm ( $< 15.3 \mu\text{mol/L}$ ) were found in more children in ARI (40%) malnourished (43%) and in malnourished children associated with pneumonia (56%) than control (13%) children.

The mean hair zinc concentration in ppm ( $\mu\text{gm/gm}$ ), in 4 groups are shown in the table XIII & Fig. 7. The mean hair zinc concentrations in ppm in ARI ( $158 \pm 48$ ), PEM ( $174 \pm 75$ ) and in PEM associated with ARI were ( $177 \pm 70$ ) significantly lower than wellnourished control ( $249 \pm 154$ ) group. However unlike serum zinc, no significant difference in zinc concentration were found among ARI, PEM and PEM associated with ARI. Hair zinc concentrations in ARI, PEM and in PEM associated with ARI were found statistically three times (OR 3,  $P < 0.05$ ) lower than wellnourished control group. However unlike serum zinc, no difference in odds ratio were found between control and three groups (ARI, PEM, ARI with PEM) and among later three groups. Hair zinc concentration below 150 ppm were found in more cases in ARI (46%), PEM (47%) and in PEM associated with ARI (47%) than wellnourished control (21%) group.

Statistical analysis using multivariate logistic relation model, using serum zinc, age and sex and hair zinc, age, sex and combined serum zinc, hair zinc age and sex in malnutrition (PEM) group, ARI group and in malnutrition associated with ARI response profile were seen. All three models mentioned above were statistically significant as shown in three tables. The p values of the models were  $p = 0.0001$ ,  $0.0001$  and  $0.0001$  in PEM, ARI and in PEM with ARI response profile respectively. Table XIV shows serum zinc has significant negative (as shown by negative parameter estimate) association with malnutrition ( $p = 0.0016$ ), ARI ( $p = 0.0139$ ) and with PEM associated with ARI ( $p = 0.0034$ ). That is low serum zinc are significantly associated with PEM, ARI and with



PEM associated with ARI. Table XV showing hair zinc, age and sex as variable in PEM, ARI and PEM with ARI response profile the model used also found statistically significant with p value being 0.0020, 0.0001 and 0.0010 respectively. Hair zinc was also found negatively, although statistically not significantly associated with PEM ( $p=0.1282$ ), ARI ( $p=0.8845$ ) and with PEM associated with ARI ( $p=0.4471$ ). That is low hair zinc are associated with malnutrition, ARI and with malnutrition associated with ARI. Table XVI using combined serum and hair zinc, age and sex, in PEM, ARI and ARI with PEM response profile, the model used was again found statistically significant ( $p=0.0001$ , 0.0001 and 0.0010 in PEM, ARI and PEM with ARI respectively). Both serum zinc and hair zinc were found negatively associated with PEM, ARI and with PEM associated with ARI, but only serum zinc was found to have significant negative ( $p=0.0020$ ) association with malnutrition (PEM) and with malnutrition associated with ARI ( $p=0.0138$ ).

In all three groups age and sex were negatively associated with all three groups, that is lower age groups and female children were more associated with PEM, ARI and with PEM associated with ARI. However statistically significant association with PEM, ARI and PEM with ARI were found with lower age group only.

Table XVII Shows the correlation of serum and hair zinc with anthropometric measurement. Both serum zinc and hair zinc had statistically significant positive correlation with weight for age and height for age. However serum zinc had stronger correlation (Fig. 8, table XVII) with weight for age ( $r\ 0.4006$ ,  $p<0.001$ ) whereas hair zinc had more strong positive correlation (Fig. 9, table XVII) with height for age ( $r=0.314$ ,  $P<0.01$ ). However hair zinc (Fig.10, table XVII) also showed significantly positive

correlation ( $r=.2887$ ,  $p<0.01$ ) with weight for age, and serum zinc (Fig.11, table XVII) also showed significantly positive correlation with height for age ( $r=.2991$ ,  $p=0.043$ ). Weight for height however had shown no statistically significant correlation with serum zinc or hair zinc.

### **CORRELATION OF SERUM AND HAIR ZINC WITH AGE AND WITH EACH OTHER.**

The result (Fig.12, table XVIII) showed significantly ( $r=0.3821$ ,  $p<0.01$ ) positive correlation of hair zinc with increasing age in months that is zinc concentration increase significantly in hair with increasing age in months. Serum zinc had negative but statistically not significant ( $r=-0.0575$ ,  $p >0.05$  NS) relationship with increasing age that is serum zinc level decreases insignificantly as the child age increases. The results also showed negative but statistically insignificant, correlation between serum zinc and hair zinc that is as serum zinc decreases as hair zinc increases ( $r -0.0925$ ,  $P> 0.05$  NS).

### **Zinc concentrations in different types of malnutrition**

Table XIX shows the hair and serum zinc concentration in marasmus, kwashiorkor and in marasmic kwashiorkor. There was no significant difference in hair zinc concentration in three types of severe malnutrition. Serum zinc concentration were found lower in marasmic kwashiorkor ( $0.87\pm0.04$  ppm) and in marasmus ( $1.16\pm0.50$  ppm) than pure kwashiorkor ( $1.64\pm0.72$ ).

### **Serum Proteins and Haemoglobin in Malnourished (PEM) and in PEM associated with ARI.**

Table XX shows almost identical decreased serum protein and albumin (gm/dl) in both PEM ( $4.41 \pm 0.64$ ,  $1.90 \pm 0.37$ ) and in PEM with ARI ( $4.28 \pm 0.53$ ,  $1.78 \pm 0.42$ ) with change in albumin globulin ratio. Haemoglobin concentration in gm/dl were also found low ( $6.02 \pm 1.15$  and  $6.78 \pm 1.20$  respectively) in two groups.

### **Correlations of serum and hair zinc with serum proteins and haemoglobin.**

Significant positive correlation were found with hair zinc and serum albumin ( $r=0.2667$ ,  $P=0.008$ ), that is hair zinc increases as serum albumin increase. There was also significant positive correlation with serum total protein and haemoglobin ( $r=0.4699$ ,  $p=0.001$ ) and serum albumin with haemoglobin ( $r=0.3336$ ,  $p=0.002$ ) (Table XXI).



## **DISCUSSION**

## DISCUSSION :

The study clearly demonstrates that both severe malnutrition and ARI were associated with low zinc status which was assessed by determining zinc concentration in both hair and serum and in both circumstances the results were found statistically significant. Zinc status was found further decreased when ARI (Pneumonia) took place in severely malnourished children. Detection of mild to moderate zinc deficiency and confirmation by biochemical data are difficult. In contrast to iron and iodine deficiency, zinc has not yet established itself in clinical medicine except as a specific treatment of Acrodermatitis enteropathica despite enthusiastic reports by interested clinicians and circumstantial evidence to suggest that zinc deficiency, often in combination with other deficiencies is a common clinical and public health problem. This is because of the considerable confusion that still surrounds the clinical features of mild zinc deficiency which are nonspecific and we lack insights into which of the many zinc pool is the most vulnerable to deficiency and the mechanism by which this pool exerts its effects (Golden MHN 1982)<sup>37</sup>. The clinical symptoms such as diminished growth velocity and probable increased susceptibility to infections are not characteristics. The advent of atomic absorption spectrophotometry, coupled with the availability of polyethylene materials that minimize contamination, has led to the acquisition of a large amount of accurate data respecting the zinc content of biological fluids and tissues. Because of the ease of acquisition, plasma and hair are of special interest. Many clinical disorders are known to be accompanied by a decreased plasma zinc content (Halsted JA 1970)<sup>239</sup>. In view of increasing evidence that zinc deficiency occurs in man and leads to abnormalities of growth and development and many other clinical problems it is important to ascertain whether lowered plasma zinc concentration signifies zinc deficiency of clinical

importance or merely a redistribution of zinc in body compartments. There is still uncertainty and difficulty in defining or assessing zinc status in individual patient. Sufficiently sensitive unambiguous indices of homeostatic adaptation of marginal changes, as would be ideal to detect incipient deficiency or excess are not yet available (Aggett PJ 1993)<sup>172</sup>. Although currently, careful interpretation of serum zinc concentration in relation to metabolic state of body is probably the best biochemical indicator of Zinc status (Aggett PJ 1993)<sup>172</sup>, zinc deficiency can be demonstrated only by observing the effects of supplementation under specific conditions with appropriate control (Golden and Benette 1988)<sup>39</sup>. A lowered plasma or serum zinc concentration would be more likely to signify zinc deficiency if it could be correlated with corresponding depressed zinc level in another tissue such as hair (Mcbean LD 1971)<sup>46</sup>. No study has been reported in which hair and blood samples have been clearly obtained from a large number of same individuals in similar age group of severely malnourished children suffering from pneumonia. Therefore, the study is unique and first of its kind not only in Bangladesh but also probably in the world, since no such article published upto date. Blood and urine analysis of trace elements although are the more traditional approach to evaluate zinc and other trace elements, they are very labile pool and fluctuate rapidly in response to changing physiological and environmental condition (Katz and Chatt A, 1994)<sup>240</sup>. The most labile zinc pool are those in the plasma and in the liver (Jackson MJ 1989)<sup>241</sup>. Plasma zinc is frequently within normal range in mild zinc deficiency or on the other hand may be reduced without body zinc depletion during infections or stress. Serum zinc in compensated metabolic phase or even in gross clinical deficiency may remain normal by zinc homeostasis mechanism and by release of zinc from tissue breakdown (Aggett PJ 1994)<sup>47</sup>. The release of zinc from tissue breakdown can maintain plasma/serum zinc levels within the reference range although the patient



has clinical and biochemical features of zinc deficiency (Conrad ME 1991)<sup>242</sup>. Therefore, a lowered plasma zinc concentration would be more likely to signify zinc deficiency if it could be correlated with a corresponding depressed zinc level in another tissue such as hair (McBean LD 1971)<sup>46</sup>. On the other hand zinc in hair is more stable. The length of hair sampled could deplete store of 3-4 months (Dorea JG 1985)<sup>235</sup> while in contrast plasma zinc reflects zinc nutrition at the moment and still could be influenced by meal spacing (Foley BS et al 1968)<sup>243</sup>. Therefore, hair and plasma zinc can represent past and present zinc status respectively (Lee HH et al 1989)<sup>157</sup>. The popularity of using hair as a biopsy tissue for trace elements resides in the fact that it can be collected easily and in nontraumatic way, can be easily transported and stored and it contains normally relatively high concentrations of many minerals and trace elements which facilitates analytical procedures. However hair zinc detection appears to be useful in chronic mild zinc deficiency. In severe zinc deprivation there may be paradoxical increase in zinc content of hair (Hemp JM et al. 1991)<sup>168</sup>. In animal experiments hair zinc level are shown to be not always reduced during severe zinc deficiency (Kollmar WE 1986)<sup>244</sup>. The same can happen in patients with severe malnutrition (Hurry VJ 1982)<sup>245</sup>, and in Kwashiorkor (Bradfield RB 1969, Erten J 1978, Hambidge KM 1974)<sup>246,247,248</sup>. This phenomenon is due to reduced hair growth during malnutrition (Bradfield RB 1966, Hsu JM 1978)<sup>249,250</sup>. Hambidge et al found a significant decrease in hair zinc levels in malnourished children only during recovery (Hambidge KM 1972)<sup>36</sup>. Zinc is transferred to hair in proportion to its availability in the central compartment. In dietary Zinc deficiency the level of this compartment may be kept normal in circumstances where homeostatic mechanisms counterbalance the low supply in the diet. Thus the level in the hair reflect insufficient internal availability rather than the level in the diet. Therefore hair zinc analysis does not appear to be suitable for indicating dietary adequacy or

marginal deficiency of zinc in adults. Due to relatively high demand for zinc in infancy combined with relatively low body stores the levels in hair of infants or of growing individuals may be more susceptible to low dietary supply than those of adults. Low levels in hair of infants are obviously a relatively reliable indicator of state of zinc deficiency while normal levels may not always indicate adequate supply (NAHRES-22 Vienna 1994)<sup>48</sup>. State of zinc deficiency becomes even more obvious and reliable when low hair zinc is coupled with low serum zinc in same individual (McBean et al 1971)<sup>46</sup>. In the study both serum and hair zinc were found significantly low in malnourished children and in children suffering from pneumonia and was found further diminished when pneumonia attacked severely malnourished children.

The present data have demonstrated low zinc status, both in hair and serum with acute respiratory tract infections irrespective of nutritional status, in comparison to control group without ARI. Hair zinc was also found low in ARI by studies done by Lombeck (Lombeck I 1988)<sup>251</sup> in Germany and by van Wouwe JP (Van Wouwe JP 1986)<sup>252</sup> in Netherlands. It is clear that the rapid decrease of serum zinc may occurs in acute infections like ARI, and may indicate a change in distribution but not a reduced body status. However it is unusual for hair zinc to be reduced in acute infections as hair zinc is more stable. Therefore low serum zinc coupled with reduced hair zinc clearly indicates low zinc status in children suffering from pneumonia. The pathways by which hair zinc levels may be lowered during ARI is not known. It may be speculated that altered metallothionein synthesis in the gut and liver may affect zinc bioavailability in various tissues. Because of significant association of low hair zinc with ARI in children, the need to discover whether or not marginal zinc deficiency, contribute to the disease frequency is urgent. Although it is clear that serum zinc may be lowered in acute infections like



ARI as shown in my study it was also found low in recurrent upper respiratory tract infections (Bondestam M 1985)<sup>253</sup> in Sweden and in chronic respiratory infections like pulmonary tuberculosis in India (Taneja DP 1990)<sup>254</sup>.

Trace elements deficiency including zinc deficiency are being increasingly recognized in severely malnourished children (Castillo-Duran C 1987)<sup>255</sup>. Golden et al (Golden MHN 1981)<sup>90</sup> demonstrated in severely malnourished children that inadequate zinc intake may be a limiting factor for growth. Oral supplementation in that study was associated with a significant acceleration in linear growth and improved zinc nutriture. Therefore, combined severe malnutrition with acute respiratory infections, two zinc lowering clinical conditions are expected to have additive or confounding effect in lowering zinc status of the body, as we have found in our study. Statistical analysis by both bivariate and multivariate linear logistic relation model, with serum zinc, hair zinc, age and sex as variables in PEM, ARI and PEM with ARI response profile showing negative association of both serum and hair zinc with PEM, ARI and with PEM associated with ARI suggest the scientific validity of the study and strongly supports that the results are less likely to be erroneous and expected to be widely accepted.

The result also showed that children having ARI (with or without severe malnutrition) have lower age group than control. Although all groups of children were under five years of age, we found difficulty in matching PEM and ARI groups with control in age and sex wise and ARI and PEM children had low mean age than control. This is the usual findings in ARI as small children are more vulnerable to pneumonia and require hospital admissions more for treatment than older children. The result therefore showed that the 4 groups are not welmatched in age wise, with control having higher mean age in control



than PEM, and ARI groups. This may be a drawback of our study as variance of age may affect zinc concentration in hair in a controversial way. In general, new born has a higher zinc concentration of hair than that of older children (Hambidge et al 1972)<sup>36</sup>. Zinc concentration in hair then decreases from birth to twelve months of age (Hambidge et al 1972, Gibson RS 1979)<sup>36,256</sup> and then gradually increases upto 13-17 years when it reaches adult level (Hambidge KM 1972, Bergmann KE 1979)<sup>36,257</sup>. Gentle, Trentalange and Coleman found a startling linear increase in hair zinc with age accounting for 49 percent of the total variance in hair zinc values (Gentle P et al 1981)<sup>258</sup>. Petering, Yeager and Witherup in 1971 also found a significant positive association between increase in age and hair zinc from 2 to 12 years (Petering HG 1971)<sup>259</sup>. However in disagreement with both Gentle (1981) and Petering (1971), Moser, Krebs and Blyler (1982) who studied 42 children from 3 to 9 years of age found no association between age and hair zinc concentration (Moser PB et al 1982)<sup>260</sup>. The use of Log intervals by Petering et al (1971) may explain the divergence with Moser's findings. Had Petering computed a correlation coefficient as done by Moser, the non linear relationship between the variables would have emerged as non significant correlation. It seems that zinc concentration in hair changes with age although not in any predictable manner. Taking into consideration the larger inter individual variability of hair zinc, it seems unlikely that age alone would account for half the variable in zinc values as was alleged by Gentle et al (1981). Similarly influence of sex on concentration of hair zinc is inconclusive. While Hambidge et al found significantly higher level of zinc in girl's hair than boys between 33 and 90 months of age (Hambidge KM 1979)<sup>261</sup>, Erten et al (1981) observed that sex had no influence on hair zinc at intervals of 5 years from 0 to 15 years (Erten J 1978)<sup>262</sup>. Klevay M et al found that although hair zinc varies with age, but no difference of zinc concentration due to sex difference (Klevay LM 1970)<sup>263</sup>. In our study however

we found a significant positive correlationship ( $r=0.3440$ ,  $p<.001$ ) between hair zinc and age in months, which was linear from 2 years onward. Serum zinc was found negative but statistically insignificant correlation with age in our study ( $r=-0.0375$ ,  $p=0.771$ ). The depression in serum zinc in children as age increases, is due to fact that as the child grows, the demand for zinc in tissues, particularly to muscles increase and it is supplied and taken up from serum, the fall in serum being greater in those infants who grow fastest and is more marked in males. This phenomena is observed not only in growing healthy child but also in recovery phase of malnutrition. There is direct relationship between the fall in plasma zinc and the rate of weight gain, emphasizing the dominant role growth has on zinc requirement (Golden BE et al 1981)<sup>90</sup>. Typically a recovering malnourished child may gain weight at a rate of 15g/kg/day. If this were lean tissue with a zinc content similar to normal mixed muscle a 5kg - child would need to retain an additional 127 micromol (8mg) zinc per day . It is obvious that during rehabilitation very large amount of zinc are required to replete depleted tissues and to permit growth. None of the currently use diets supply any where approaching the amounts of zinc required to return the child to normal. It is perhaps for these reasons that anthropometrically recovered children still have thin atrophic muscle fibre (Hansen-Smith FM, et al 1979)<sup>87</sup> Growth of muscle fibres during recovery from severe malnutrition in Jamaican infants have a much reduced zinc concentration (Cheek DB 1970)<sup>31</sup>. Malnourished children who are given maintenance diets which does not permit growth and supply only 1.5 micromol zinc/kg/day (less than content of 1 gm of muscle) maintain their plasma zinc concentration (Golden BE 1981)<sup>90</sup>. When they are given an increased diet so that they can gain weight rapidly, even provision of the recommended dietary allowance does not prevent a profound fall in plasma zinc concentration. This remains low throughout recovery and returns to normal only when the children cease



gaining weight rapidly. Chandra et al also observed a decline in plasma zinc concentration in children with ages of growth spurt in healthy children and in anabolic phase of recovery from malnutrition (Chandra RK 1983)<sup>25</sup>. This is presumably because zinc is being taken up from plasma pool more rapidly than it is replaced from intestine or from other compartments and distributed to other tissues like hair, bones, muscles etc. Therefore as serum zinc falls with age, hair zinc increases with age. In my study this inverse relationship between hair and serum zinc was also found. However it was not statistically significant ( $p=0.0771$ ). A lack of significant correlation between the zinc content of these two tissues has been reported in children by McBean et al and Chase et al (McBean LD 1971, Chase HP 1980)<sup>46,264</sup>. Klevay et al found no correlation between zinc content of hair and of plasma measured on a random sample of the Panamanian population (Klevay LM. 1970)<sup>263</sup>. Eminians et al also found no significant correlation between hair and serum zinc concentration in Iranian Children (Eminians J.G. 1967)<sup>265</sup>. Eminians et al rather found a statistically insignificant positive correlation coefficient between zinc content of paired plasma and hair sample with an absolute value of 0.05 which was not statistically significant ( $p>0.05$ ). Plasma zinc concentrations in healthy infants match those of adults. A higher concentration has been noted in breastfed infants at 6 months than in those fed formula. In preterm neonates there is a progressive decline in serum or plasma zinc concentration with a nadir of  $9.1 \pm 2.6$  micromol/l at about 6-12 weeks of age, then it increases subsequently (Golden MHN & Benette 1988, Golden MHN 1982)<sup>39,37</sup>. This depression correlates with the rate of weight gain, being greater in those infants who grow fastest and is more marked in male children.

Correlationship of serum and hair zinc with various anthropometric measurement in my study showed serum and hair zinc to have significant positive correlationship with



weight for age (W/A) and height for age (H/A) but statistically insignificant negative correlation with weight for height (W/H). However serum zinc showed stronger correlations with weight for age ( $r=0.4006$ ,  $p<.001$ ) than hair zinc ( $r=0.2887$ ,  $p<.001$ ) and hair zinc have showed stronger relationship with H/A ( $r=.314$ ,  $p<.001$ ) than serum zinc ( $r=.2991$ ,  $p=0.043$ ). Just as hair and plasma zinc represent past and present zinc status respectively, H/A, and W/A represent past and present over all nutritional status. This indicate that children with higher stature also had higher hair zinc levels and in children with chronic zinc deficiency has lower hair zinc status and have short stature (Hambidge KM 1972, Bergman KE 1979)<sup>36,257</sup>. Although we did not find positive relationship between serum or hair zinc with weight for height, we found stronger correlation between serum zinc and weight for age ( $r=0.4006$ ,  $p<.001$ ) and relatively weaker relationship between hair zinc and weight for age ( $r=.2887$ ,  $p<.01$ ). Similar correlation was also found in Buzina's Study (Buzina R 1980)<sup>266</sup>. Buzinia concluded that there was increase incidence of growth retardation in children with below average hair and plasma zinc values. Castillo-Duran et al found enhanced weight gain without significantly modifying food intake with zinc supplementation during the recovery phase of marasmus (Castillo - Duran C 1987)<sup>255</sup>. In Jamaica, an adequate zinc intake has been found to be necessary for optimal weight gain in infants during the recovery state of Protein-energy malnutrition (Golden MHN 1981)<sup>90</sup>. If zinc deficiency persists, it may contribute to the poor linear growth in later childhood which is characteristic of many populations where Protein energy malnutrition is common (Sandstead HH 1981)<sup>267</sup>. However anthropometric relationship with hair or serum zinc deserve explanationns, as other investigators have found no significant correlations between anthropometric measurements and hair zinc in children (O'Leary MJ 1980)<sup>268</sup> or between anthropometric measurement and plasma zinc (Griger JL 1978)<sup>269</sup>.

In addition to anthropometric assessment of nutritional status in four groups studied, the study also assessed the body composition by using Bioelectric impedance analysis (BIA). Standard anthropometric measurements have utility values to the extent that most children suffering from PEM can be identified. Nevertheless there also occurs gross errors following such methods (Bai Ki et al 1977)<sup>270</sup>. Different studies showed that faults remained in most of the approaches, since such measurement cannot reach high scientific precision due to lack of proper standardisation; interobserver errors and failure to determine actual age due to lack of education and maintenance of proper birth records (Gumery M et al 1980, Ramchandran K, et al 1978, Agarwal CM et al 1980)<sup>271,272,273</sup>. Bioelectric method is superior to anthropometry in terms of accuracy and precision. It has gained its popularity due to fact that body composition can be analyzed rapidly, easily and in a noninvasive way. Total body water (TBW) is an indicator of fat free mass from which fat mass (FM) can be calculated. However TBW and FM cannot be measured easily by BIA method in malnourished children. A linear regression equation was used to calculate (Fjeld CR 1990)<sup>224</sup> the TBW. Fat free body mass (FFM) or Lean body mass was then calculated using a specific formulas and age specific constants of body density. The Fat Mass and percentage of Fat Mass (% FM) were calculated using standard formulas. The FFM and FM of boys from 9-18 months old is approximately 77% and 23% respectively (Fomon JJ 1982)<sup>225</sup> TBW% and FFM% are increased and corresponding Fat % is decreased in malnourished children. The FM % is positively related with nutritional status of the children, severely malnourished children have least FM%. In our study Fat % in malnourished groups (PEM & PEM with ARI) were found lower ( $12.77\% \pm 4.32$  and  $11.35\% \pm 3.85$  respectively) than welnourished groups of control and ARI without malnutrition ( $23.10\% \pm 8.31$  and  $22.68\% \pm 7.68$  respectively). Islam S et al found similar low Fat % in malnourished children in Dhaka Shishu Hospital



(Islam S 1991)<sup>238</sup> and endeavored to classify malnutrition on the basis of Fat % in the body. He found 1st degree, 2nd degree and 3rd degree malnutrition had <22.5%, < 20% and <13% Fat respectively. Thinking of classifying malnutrition on the basis of Fat content in the children was hypothetical but logical in the sense that store of fat in the body becomes used up first to maintain energy level and metabolic needs of the body when a child starts suffering from energy deficiency. It was therefore thought that the quantity and percentage of Fat will be proportional to the exhaustion of Fat store in different degree of malnutrition. The most severely affected child will have the least amount of Fat store and so on.

382493

No study showing zinc status in severely malnourished children suffering from ARI, measuring both plasma/serum and hair zinc obtained at the same time in the same individual and in large enough numbers to be meaningful has been done so far. This study is therefore first of his kind. The reported literature on relationship of zinc and respiratory infection in children is limited. Hair zinc was found low in recurrent upper respiratory tract infections in Kinder gartens children in North-Rhine-Westphalia, Germany (Lombeck I 1988)<sup>251</sup> and in Netherland where Van Wouwe et al (Van Wouwe JA 1980)<sup>252</sup> have also shown reduced hair zinc level in 20 children with chronic respiratory tract infection compared with 35 control, and low serum zinc in children suffering from upper respiratory tract infection in study done by Bondestam et al (Bondestam M 1985)<sup>253</sup>. It is still unclear whether the zinc content is low because the children suffer from many infections or whether they suffer from frequent infections due to low zinc status. Acute infections may reduce serum zinc because of redistribution of serum zinc but not a reduced body zinc status. However hair zinc is stable and do not fluctuate readily in acute stressful condition like ARI. The need to discover whether or



not marginal zinc deficiency contributes to disease frequency is urgent. It is tempting to believe that zinc deficiency is attributable to diseases frequency particularly infectious diseases including ARI (pneumonia). This is more relevant as far as pneumonia in malnourished children is concerned. As mentioned earlier malnourished children are vulnerable to have increased incidence and severity of ARI including pneumonia (James JW 1972, Zaman K 1996)<sup>14,15</sup>. The postulated mechanism for this effect is reduction in immunological capacity specially in cellular immunity which occurs in malnourished children (McMurry DN 1981, Chandra RK 1991)<sup>274,275</sup>. The reduced immunological competence in malnourished children may be attributable to zinc deficiency, because this is associated with impaired cellular immune status (Fraker PJ 1987)<sup>276</sup> and effect that reversed by zinc supplementation (Sazawal S 1997, Castillo-Duran C 1987, Roy SK, 1999)<sup>121,255,277</sup>. In experimental models isolated Protein energy malnutrition does not result in impaired cellular immunity (Good RA 1979)<sup>26</sup>.

Impaired cell mediated immunity (CMI) in malnourished children was found to be associated with increased ARI incidence in a study done in Bangladesh by Zaman K et al (Zaman K 1996)<sup>15</sup>. Zaman et al found in this study that a one unit decline in the weight for height Z score was associated with a 52% increase in rise of ARI and anergic children had 94% higher risk of developing ALRI than immunocompetent children. The degree of association persisted even after correcting age, sex, nutritional status and maternal education. It can be assumed that many of these children's immunodeficiencies including zinc are attributable to single or multiple micronutrient deficiencies including zinc (Neumen CG 1975)<sup>278</sup>. These deficiencies may lead to impaired immunity and reduced integrity of mucosal surface which may in turn increase susceptibility to infections in the host (Mim CA 1991)<sup>279</sup>. However zinc deficiency were found in studies to be

associated with impaired cell mediated immunity in malnourished children. Sempertegui F et al found reduced delayed type cutaneous hypersensitivity (DTH) in malnourished children with low serum zinc associated with increased respiratory symptoms (Sempertegui F 1996)<sup>280</sup>. After zinc supplementation in their study children improved their cutaneous DTH response. Since no other dietary change were made, it is likely that this improved DTH response was in fact secondary to zinc repletion. Low zinc are associated with ongoing sepsis in malnourished children were evidenced by increased C-reactive protein (CRP) and alpha-1 acid protein in these children, which is an indirect measure of infection and inflammation (Sempertegui F 1996)<sup>280</sup>. While detection and treatment of ALRI can reduce child mortality in developing countries, additional preventive interventions are needed. Routine immunization for measles and pertussis can play modest role and new vaccines for other infective diseases, such as pneumococcal pneumonia and respiratory syncytial virus infection may be very useful but immunocompetence by zinc supplement may be of particular importance, in decreasing incidence morbidity and mortality of ARI in malnourished children.

The stress of infections is known to affect nutritional well-being and compromised nutrition contributes to alteration in immunological function, although precise mechanism involved are poorly understood (Chandra RK. 1980)<sup>281</sup>. There is currently a widely expanding area of research concerning the role of trace elements in immune function, although the focus so far has been on individual elements. (Underwood EJ 1977)<sup>62</sup>. Thus several investigations in animals points to the possible implication of zinc in immune deficiency status such as T-Cell defects (Gross RL 1979)<sup>282</sup>. Zinc appears to interfere both lymphocyte and neutrophil function (Alford RH 1970, Chvapil M 1977)<sup>283,284</sup>. Compromised immunocompetence has been found in malnutrition (Kielman



1976)<sup>285</sup> and in single micronutrient deficiency such as those of vitamin A, zinc, folic acid, iron or iodine (Beisel WR 1982)<sup>286</sup>. In regard to immunity and infectious disease morbidity, zinc deficiency may be particularly important. Zinc deficiency has been shown to be common in developing country children with high rates of diarrhoea and malnutrition (Sazawal S. 1995)<sup>287</sup>. Animal experiments have clearly demonstrated that selective zinc deficiency results in diminution of thymus size and function as well as impairment in T-Cell dependent immune function (Fraker PJ. 1979, Fraker PJ 1982)<sup>288,289</sup>. Decreased immunocompetence has been shown to be a risk factor for diarrhoeal and respiratory morbidity and zinc supplementation has been shown to reduce the morbidity.(Baqui AH 1993, Black RE 1989)<sup>290,291</sup>. Golden et al found improvement in cell mediated immune response (CMI) to candida antigen in malnourished children by application of 1% zinc sulphate ointment (Golden MHN 1978)<sup>100</sup>. An effect of zinc supplementation on delayed cutaneous hypersensitivity in acrodermatitis enteropathica and in marasmic children during rehabilitation has also been documented (Chandra RK 1980 and Schlesinger L 1992)<sup>292,182</sup>. Zinc deficiency has been shown to be associated with atrophy of lymphoid tissue, reduced lymphocyte count and proportion of CD4 T-helper cells (Kay RG 1975, Coello-Ramirez P 1982, Luecke RM 1978)<sup>293,294,295</sup>. Decreased lymphocyte proliferation and reduced cytotoxic activity has also been found in zinc deficiency status (Chandra RK 1980)<sup>292</sup>. All of these respond dramatically to repletion of zinc (Frost P, 1977)<sup>296</sup>.

The beneficial influence of zinc may be due to the correction of latent zinc deficiency or to immunostimulation of zinc or both. The possibility of an immunostimulatory effect of zinc supplement independent of zinc depletion, is suggested by several observations: in healthy young adults oral administration of zinc increases in vitro lymphocyte sub



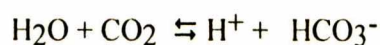
population in patients' with lymphoblastic leukemia and addition of zinc to drinking water of mice associated with a time and dose dependent increase in mitogen stimulation of their spleen lymphocyte in vitro (Wazewska Czyzewska M. 1978)<sup>297</sup>. The observation of and increase in the number of circulatory T-lymphocytes especially CD4 cells, after zinc supplementation may be explained by direct effect of zinc ion on the lymphocyte membrane affecting maturation and differentiation of T-lymphocyte or by stimulation of thymus endocrine function. There is marked atrophy of the thymus in children with protein energy malnutrition (PEM) with defect in CMI. (Rao KMK 1979, Muger JW 1971)<sup>298,299</sup>. The T-lymphocyte immaturity can be reversed within vitro incubation with thymulin. These changes may in fact be an effect of zinc deficiency. Experimental studies show that zinc deficiency without PEM causes profound defects in cellular immunity with thymic hypoplasia (Schloen LH 1979)<sup>300</sup>. Recent work has made it apparent that thymic epithelial cells are source of a number of peptide and hormones which participate in a cascade of multimolecular and multicellular events including T Cell maturation (Hadden JW 1995)<sup>301</sup>. Once T-Lymphocytes leave the thymus there differentiation and maturation is thought to be regulated by zinc thymulin and deficiency of zinc thymulin has been associated with secondary cellular immune deficiency and with immune senescence (Hadden JW 1995)<sup>301</sup>. Thus terminal deoxynucleotidyl transferase or zinc-thymulin have been suggested as possible mechanism by which zinc may be affecting T-cell development and function (Hadden JW 1995)<sup>301</sup>.

Studies done in various parts of the world found that vitamin A, particularly  $\beta$  Carotene, which has antioxidant property, helps in the management of ARI (Martin AA 1994)<sup>302</sup>. Vitamin A have been found more useful in pneumonia associated with measles and in pneumonia associated with PEM (Ferguson EL, 1993)<sup>303</sup>. Zinc supplementation may

potentiate the positive effect of vitamin A in the management of ARI, since for bioavailability and for optimum function of vitamin A, zinc is essential. For mobilization of vitamin A from liver to target organ, require a specific protein, called retinol binding protein (RBP). Many studies have shown positive linear correlation between zinc and RBP (Michaelsson C 1976)<sup>304</sup>. Besides its potential to aggravate severe protein energy malnutrition, zinc deficiency can precipitate severe vitamin A deficiency and thereby might cause blindness by interfering with retinol binding protein (RBP) synthesis (Bhaskaram P. 1995)<sup>305</sup>. Because of its importance in protein synthesis adequacy of zinc status particularly acquires clinical significance in population where malnutrition is wide spread. Plasma vitamin A values of zinc deficient rats were considerably lower than in zinc sufficient control (Sunderesan PR 1977)<sup>306</sup>. PEM is probably the most common cause of zinc deficiency in developing countries like Bangladesh.

Therefore children suffering from ARI associated with low zinc in PEM, vitamin A supplementation alone, may not help in the course and outcome of pneumonia as vitamin A even adequately present may not be bioavailable and well utilized due to inadequate RBP, a zinc containing protein. Therefore research involving administration of zinc together with vitamin A to children suffering from ARI, in malnourished children may be rewarding.

The most conspicuous biochemical role of zinc lies in its association with various enzyme. The first zinc metalloenzyme, recognized is carbonic anhydrase (Malek MA 1988)<sup>307</sup>. Reversible hydration of carbondioxide is catalyzed by this enzyme and is so essential for respiration (Malek MA 1988)<sup>307</sup>.



This enzyme contains one atom of zinc per mole and has a molecular weight of 30,000 with some 260 aminoacids (Cohen JJ 1966)<sup>308</sup>.

Prasad A.S et al found a significant positive correlation of carbonic anhydrase with erythrocyte zinc (Prasad AS 1975)<sup>44</sup>. Prasad AS et al from the study suggested that synthesis of specific carbonic anhydrase apoenzyme depends on the activity of zinc and this apoenzyme does not accumulate if zinc is lacking. This was consistent with their previous studies with respect to other zinc dependent enzyme in which they were unable to demonstrate in vitro stimulation of enzyme activities with zinc in zinc deficient tissues (Prasad AS. 1971)<sup>309</sup>. The mechanism by which zinc influence specific apoenzyme synthesis is unclear.

Acute respiratory tract infection is characterized by fast breathing and chest indrawing (WHO 1991)<sup>220</sup>. Therefore for increased respiratory effort during ARI(Pneumonia), carbonic anhydrase activity has to be increased to wash out more CO<sub>2</sub> during expiration and in fact most pneumonias are characterized by hypoxaemia and hypocarbia with respiratory alkalosis (Golden MHN 1978 and Cohen JJ 1966)<sup>100,308</sup> and only occasionally severe pneumonia may be associated with hypercapnia and respiratory acidosis, which is type II respiratory failure and more serious condition (Richard E, Behrman, et al. Nelson Text book of Paediatrics.1987)<sup>310</sup>. Kawakami have found inverse relationship of activity of carbonic anhydrase with PCO<sub>2</sub>, that is decreased PCO<sub>2</sub> (more frequently found in pneumonia) is associated with increased carbonic anhydrase level and viceversa (Kawakami Y et al 1978)<sup>311</sup>. Therefore in acute respiratory tract infection, thereby in majority of children with pneumonia, activity of carbonic anhydrase has to be increased to wash out carbondioxide by increasing ventilatory rate. For increased activity of carbonic anhydrase, a zinc metalloenzyme, there will be increased demand of



zinc. However in malnourished children there is increased likelihood that due to low zinc status this demand may not be met. In fact in malnourished animal model carbonic anhydrase activity was found decreased (Kirchgessner M 1975)<sup>312</sup>. Consequently the patient cannot breathe out CO<sub>2</sub> and it accumulates in blood causing hypercapnia giving rise to Type II respiratory failure characterized by hypoxia and hypercapnea, which is more serious condition, decreases patient's ventilatory effort causing more difficulty in breathing, which requires artificial ventilation by intubation and by assisted ventilation in intensive care unit (ICU) which is rarely available in developing countries and at the same time more expensive.

Therefore in severely malnourished children with zinc deficiency, ARI (Pneumonia) may cause respiratory insufficiency quickly due to rapid exhaustion of carbonic anhydrase which may more readily lead to respiratory failure (Type II) which is more difficult to manage and thereby increasing the mortality of malnourished children suffering from pneumonia.

However, zinc supplementation in the presence of ongoing sepsis and in an already compromised immune system may not necessarily be beneficial. Zinc has been associated with impairment of certain immune responses both in vitro (Mustafa MG 1971, Stankova L 1997, Chvapil M 1977)<sup>313,314,315</sup> and in animal experiments (Chvapil M 1976)<sup>125</sup>. One hundred fifty milligrams elemental zinc twice a week for 6 wk given to 11 healthy adult men was associated with reduction in lymphocyte stimulation response to phytohemagglutinin as well as chemotaxis and phagocytosis of bacteria by polymorphonuclear leukocytes (Chandra RK 1984)<sup>316</sup>. Marasmic infants fed a zinc-fortified liquid diet (15 mg Zn/L) showed reduced phagocytic and fungicidal monocytic

activity and a significantly increase number and duration of impetigo episodes (Schlesinger L 1993)<sup>317</sup>. Serum concentration of zinc decrease sharply in inflammation and infection and may reflect a natural protective mechanism because lower concentrations of zinc are associated with both optimal phagocytic function and depressed microbial virulence (Sugarman B 1983)<sup>318</sup>. This decrease is accounted for largely by a redistribution to the liver (Powanda MC 1973, Pekarek RS 1972)<sup>319,320</sup>, serving to favour the immune system in the production of acute phase proteins and hemopoiesis (Chvapil M 1976)<sup>125</sup>. Interleukin 1 has been implicated in this process (Cousins RJ 1988, Coyle P 1993, Klasing KC 1984, Morimoto A 1989)<sup>166,321,322,323</sup>. Zinc is important for microbial virulence with near physiologic concentration of zinc being necessary for toxin production in *Clostridium perfringens* (Murata R 1969)<sup>324</sup> and *Pseudomonas aeruginosa* (Jeasen SE 1980)<sup>325</sup> and in the adherence of enterobacter to epithelial suface (Sugarman B 1980)<sup>326</sup>.

Serum Iron concentrations also fall during infection as a result, again, of redistribution to the liver, reticuloendothelial system, and bone marrow (Biesel WR 1976)<sup>327</sup>. Early iron supplementation of children with severe protein-energy malnutrition led to increased mortality (Smith IF 1989)<sup>328</sup>, probably because of increased microbial virulence and free radical generation.

As well as possibly having a directly detrimental effect on the immune system during sepsis, high-dose zinc supplementation could aggravate deficiencies of other minerals by decreasing their intestinal absorption. Zinc inhibits copper absorption and copper deficiency can co-exist with severe protein-energy malnutrition (Cordano A 1964, Hemalatha P 1993)<sup>329,330</sup>. Copper deficiency is also associated with an impaired

immune response, specifically, a reduction in the antibody response to T cell-dependent antigens and a decrease in the microbiocidal activity of phagocytes (Vyas D 1983, Babu U 1989)<sup>331,332</sup>. Metallothionein, an intracellular polypeptide, plays an important regulatory role in the metabolism of ingested zinc and copper. It binds copper more avidly than zinc and, therefore, giving supplementary zinc to a malnourished child may induce the synthesis of metallothionein and aggravate a preexisting copper deficiency. Zinc induced copper deficiency has been documented (Botash AS 1992, Sandstorm B 1994, Honkanen V 1991, Porter KG 1977, Prasad AS 1978)<sup>333,334,335,336,337</sup> although high concentrations of zinc for prolonged periods are usually required to manifest it. Prasad et al (Prasad AS 1978)<sup>337</sup> described how 150 mg zinc/d for 23 months precipitated severe copper deficiency in patients with sickle cell disease and Porter et al (Porter KG 1977)<sup>336</sup> described how the same level of zinc supplementation for 13 months again precipitated copper deficiency. However an intake of 18.5 mg zinc/d for just two weeks reduced apparent copper retention in a group of healthy, well-nourished men (Festa MD 1985)<sup>338</sup>.

In many studies (Hemalatha P 1993, Ronaghy HA 1974, Sachdev HPS 1988, Nakamura T 1993, Shrivastava SP 1993)<sup>330,339,340,341,342</sup> where zinc were supplemented in 1 to 10 mg per kg per day, no side effects related to copper status were reported. Many studies including studies done by Hemalatha et al (Hemalatha P 1993)<sup>331</sup> and Walravens et al (Walravens PA 1983)<sup>101</sup>, examined biochemical markers of copper status and none showed an effect as measured by serum or plasma copper and ceruloplasmin concentrations or superoxide dismutase activity. Study done by Doherty et al (Doherty CP 1998)<sup>343</sup> on effect of zinc supplementation on severely malnourished children, found neither the use of higher dose of zinc initially nor prolongation of the period of supplementation resulted in a significant anthropometric benefit in severely



malnourished children, and in deed the higher dose was associated with increased mortality (Doherty CP, Sarkar MA, Shakur MS et al .1998)<sup>343</sup>. In the study 141 children between the ages of 6 months and 3 year were enrolled after admission to a nutritional rehabilitation unit in Dhaka Shishu (children) hospital, Bangladesh and randomly assigned to receive elemental zinc by mouth, 1.5mg per kg per day for 15 days, 6mg per kg per day for 15 days and 6mg per kg per day for 30 days and thereafter they were followed for a total of 90 days. Anthropometric measurements were taken and change in height for age, weight for age and weight for height Z scores were recorded. Higher zinc doses were not associated with significant change in any anthropometric measurement, but mortality was significantly greater in children who received high dose zinc (6 mg/kg/day) initially as opposed to those who received low dose zinc supplementation (1.5mg/kg/day), Yates-corrected chi square p value of 0.033 and risk ratio of 4.53, 95% CI: 1.09< risk ratio < 18.8. We therefore concluded that there is no benefit of using high dose zinc supplementation regimen and that they could contribute to increased mortality in severely malnourished children.

From the above mentioned studies in summary, we can conclude that zinc is necessary in trace amounts for microorganisms (usually micromolar concentration) and in daily milligram oral quantities for human. Zinc is essential because it is part of many metalloenzymes that help regulate almost all aspects of cellular metabolism (Riordan JF 1976, Vallee V 1959)<sup>344,345</sup>. Normally it also plays a role in membrane stabilization and other cellular function (Chvapil M 1976)<sup>125</sup>. Work with zinc must be performed carefully and results may not be as precise as they first appear, because zinc is present in trace amounts in many media and can bind to a variety of compounds including many commonly used buffers (Falchuk KH 1975, Torriani A 1968, Feeney RE 1947, Collier

HB 1979)<sup>346,347,348,349</sup>. Unfortunately this often has not been taken into account in reported works. In zinc deficiency T lymphocyte function declines significantly, this probably occurs because nucleic acid production decreases (Farnandes G 1979, Fraker PJ 1978)<sup>135,148</sup>. Often minor alterations in immune functions may occur, but the commonly associated protein calorie malnutrition makes specific identification of zinc mediated defects difficult to establish. Supplementation with zinc improves T-lymphocyte function (Frakar PJ 1978)<sup>148</sup> but phagocytic cells are less active probably because the membrane is stabilized (Chvapil M 1977)<sup>350</sup>. Chronic zinc deficiency can also be associated with dysfunction of many organ system (Hsu J 1980)<sup>126</sup>. However having said that too much zinc can be lethal (Failla ML 1977)<sup>91</sup>. The result was also found recently by a recently concluded study in Dhaka shishu Hospital, Bangladesh (Doherty C P 1998)<sup>343</sup>. At zinc concentration close to those normally found in human serum bacteria display charecteristics associated with increased virulence (Murata R 1969, Sato H 1978, Soda S 1969, Jeasen SE 1980)<sup>324,351,352,325</sup>. The requirement of indigeneous and infecting microbes for zinc from mammalian hosts may be carefully regulated, and indiscriminate zinc replenishment could be associated with an increased incidence of infections. The association warrants more study, as does the normal functioning of zinc in all living organisms

A review of the literature on estimation of zinc in different body compartments in malnourished children and in ARI reveals conflicting results due to the rarity of studies where zinc status were assessed by determining zinc concentration in more than one compartment in children suffering from ALRI (Pneumonia). Infact no such study has been done on severely malnourished children suffering from ALRI. The study is unique in the fact that serum zinc and hair zinc sample were obtained and estimated at the same



time for the same individuals in all four groups and in large enough numbers to be meaningful. Both ARI and malnourished children were found to have significantly low zinc status and it is least likely to be conflicting as it was found low in both serum and hair. Zinc status in the study was found more decreased when malnourished children suffered from pneumonia. Although until now the best way to detect zinc deficiency is to observe the clinical response by zinc supplement under specific conditions with appropriate control, in many studies it have been found that beneficial effect of zinc supplement are observed only when pretreatment value of zinc were found low (Sanstead HH 1970, Hallbook T 1972)<sup>52,53</sup>. Therefore, from the study result we can predict that zinc supplemented malnourished children are less likely to be affected by pneumonia in terms of frequency as well as severity, thereby decreasing overall mortality from malnutrition and pneumonia. From the outcome of the study result, interventional case control study with zinc supplementation may be undertaken in Bangladesh to observe the effect of zinc supplementation in reducing incidence of ARI in vulnerable and susceptible children like malnourished children of Bangladesh where both ARI and malnutrition are major cause of infant mortality. Very recently Sazawal S et al in their study in India found that zinc supplementation reduces acute lower respiratory infections in infants and preschool children by 45% and it was statistically significant (Sazawal S, Black RF, Jalla S et al 1998)<sup>353</sup>. From their study results along with previously documented effects of zinc on diarrhoea, immunity and growth, they have indicated that intervention to improve zinc intake should deserve more attention as means to improve child health. Such interventions might include supplementation with zinc and possibly other micro-nutrients as well as dietary modifications or fortifications of staple foods.



## **CONCLUSION**

## **CONCLUSION**

Zinc status is low both in severely malnourished children and in children suffering from pneumonia, as assessed by determining zinc concentration in serum and in hair in same individuals. Zinc status is further decreased when malnutrition is associated with pneumonia. There remains a diagnostic dilemma of assessing zinc status in severely malnourished children where zinc status is assessed by determining zinc concentration in single body compartment which according to previously documented literature review may be unreliable in severely malnourished children who are susceptible to various stressful conditions like acute infections including pneumonia, which may influence zinc concentration in a body compartment in various ways. The diagnostic dilemma of assessing zinc status in malnourished children and in children suffering from pneumonia can be removed by the studies which show decreased zinc concentration both in serum and hair like this study, which provides more reliable index of zinc status, as according to previous documented studies assessment of zinc status by determining zinc concentration in multiple body compartments provide better and more accurate state of zinc status of the body. The study also revealed that children of lower age groups and female children are more vulnerable to severe malnutrition and pneumonia. Since previous documented studies in Bangladesh (Zaman K 1996)<sup>15</sup> found increased association of lower respiratory tract infection and impaired cell mediated immunity in malnourished children, we can expect from the study results that intervention to improve nutritional status and immunocompetence by zinc supplement will be particularly important, as zinc which improves cell mediated immunity is deficient in malnourished children. Taken into consideration the increased frequency of pneumonia and malnutrition in developing country children, the magnitude of the impact could be

substantial, if successful interventions could be implemented, although such interventions in no way simple. Improving dietary quality, especially zinc intake in weaning age children deserve special attention, not only for their possible effects in reducing ARI, but also because of known benefits in reducing diarrhoea, improving growth and enhancing development (Mora et al 1981, Hussain et al 1991)<sup>354,355</sup> and decreasing overall high infant and child mortality in developing countries.



## **LIST OF FIGURES**

## LIST OF FIGURE

- Fig-1 Age distribution in 4 groups
- Fig-2 Sex distribution in 4 groups, showing male female ratio approximately 1.3:1 in all groups
- Fig-3 Mean weight for age (W/A), weight for height (W/H) and height for age (H/A) in 4 groups; showing normal anthropometric status in Group A (control) and Group B (ARI) and severely under weight (W/A <60% of NCHS median), severely wasted (W/H <70% of NCHS median) and severely stunted (H/A <85% of NCHS median) in group C (PEM) and in group D (PEM + ARI)
- Fig-4 Showing distribution of marasmus, kwashiorkor and marasmic kwashiorkor in group C (PEM) and in group D (PEM with ARI), showing higher frequency of marasmus (non oedematous PEM) than kwashiorkor and marasmic kwashiorkor (oedematous PEM)
- Fig-5 Percentage of total body water (%TBW), Fat free body mass (%FFBM), Fat mass (%FM) and % of body solid in 4 groups obtained by body composition analysis, showing decreased % Fat in malnourished groups (PEM & ARI with PEM) than wellnourished group (Control and ARI without PEM)
- Fig-6 Distribution of serum zinc concentration among four groups of children, showing significant hypozincaemia (<1 ppm) in ARI (n=14, 40%), PEM (n=20, 43%) and in ARI+PEM (n=18, 56%) than control (n=5, 13%),  $p < 0.05$ )
- Fig-7 Distribution of hair zinc concentration among four groups of children, showing significant low hair zinc (<150 ppm) in ARI (n=16, 46%), PEM (n=22, 47%) and in ARI with PEM (n=15, 47%), than control (n=8, 21%),  $p < 0.05$
- Fig-8 Relationship of weight for age with serum zinc showing strongly positive correlation of serum zinc with W/A ( $r=0.40006$ ,  $p < 0.001$ )

- Fig-9 Mean hair zinc level in children with different height for age showing significantly positive correlation of hair zinc with height for age ( $r=.314$ ,  $p<0.01$ )
- Fig-10 Relationship of weight for age with hair zinc, showing significant positive correlation of weight for age with hair zinc ( $r=.2887$ ,  $p<0.01$ )
- Fig-11 Mean serum zinc level in children with different Height for age, showing significantly positive correlation ( $r=.2991$ ,  $p<0.043$ ) of H/A with serum zinc
- Fig-12 Correlation of hair zinc and age in months showing significantly positive correlation ( $r=0.3821$ ,  $p<0.01$ ) of hair zinc with increasing age in months



### Age (in month) distribution in 4 groups

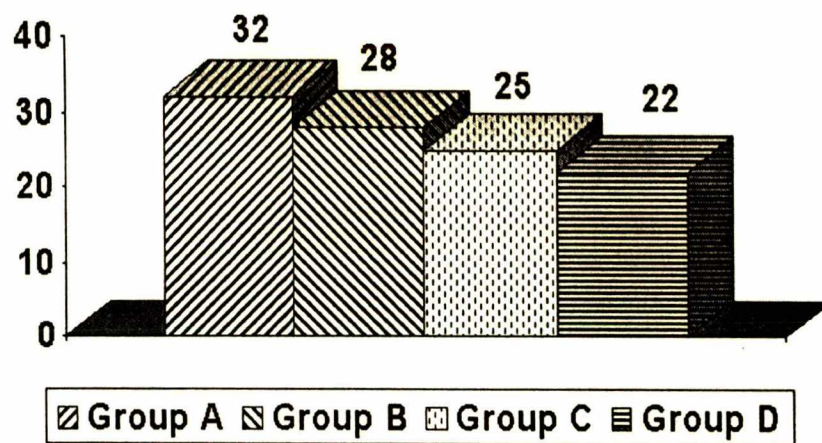


Figure 1: Age distribution in 4 groups.

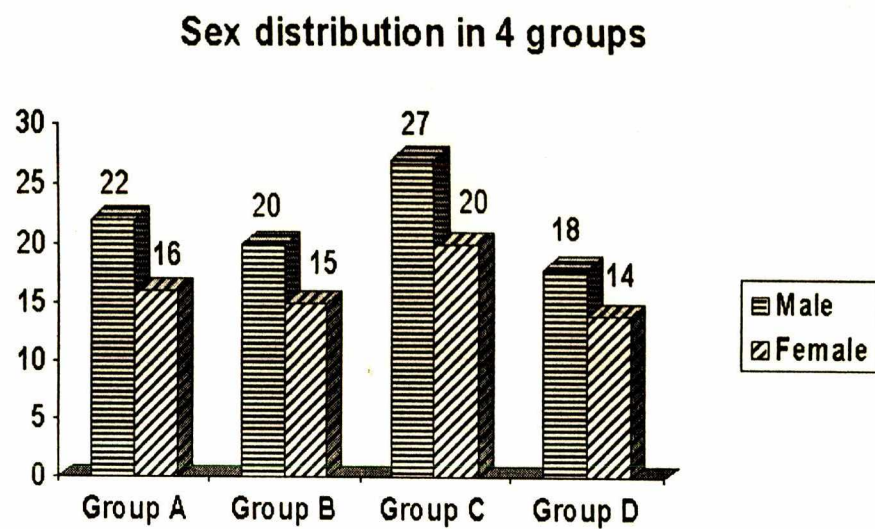


Figure 2: Sex distribution in 4 groups, showing male female ratio approximately 1.3:1 in all groups.

### Anthropometric status in 4 groups

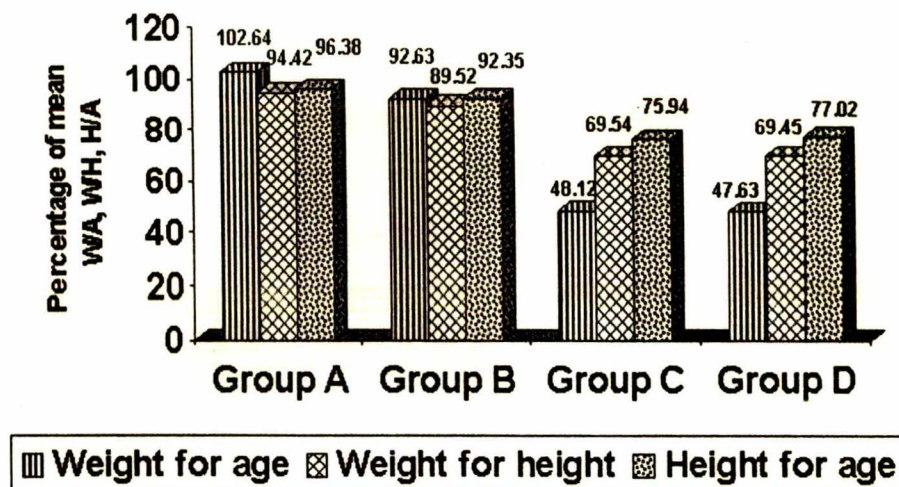


Figure 3: Mean weight for age (W/A), weight for height (W/H) and height for age (H/A) in 4 groups; showing normal anthropometric status in Group A (control) and Group B (ARI) and severely under weight (W/A <60% of NCHS median), severely wasted (W/H <70% of NCHS median) and severely stunted (H/A <85% of NCHS median) in group C (PEM) and in group D (PEM + ARI).



Distribution of Marasmus, Kwashiorkor and  
Marasmic Kwashiorkor in malnourished group  
(PEM & PEM+ARI)

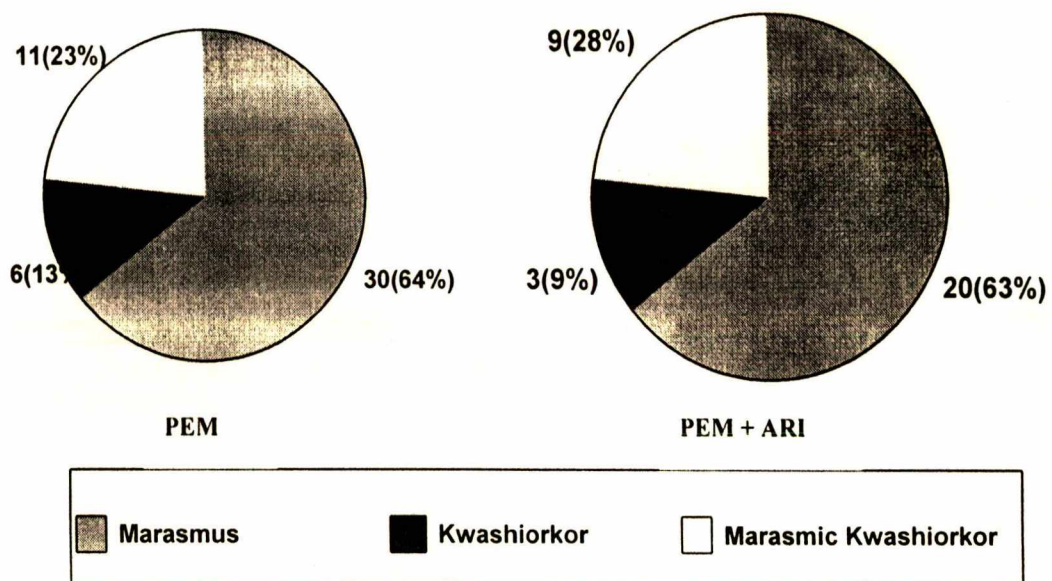


Figure 4 : Showing distribution of marasmus, kwashiorkor and marasmic kwashiorkor in group C (PEM) and in group D (PEM with ARI), showing higher frequency of marasmus (non oedematous PEM) than kwashiorkor and marasmic kwashiorkor (oedematous PEM).

### Body composition Analysis in 4 groups

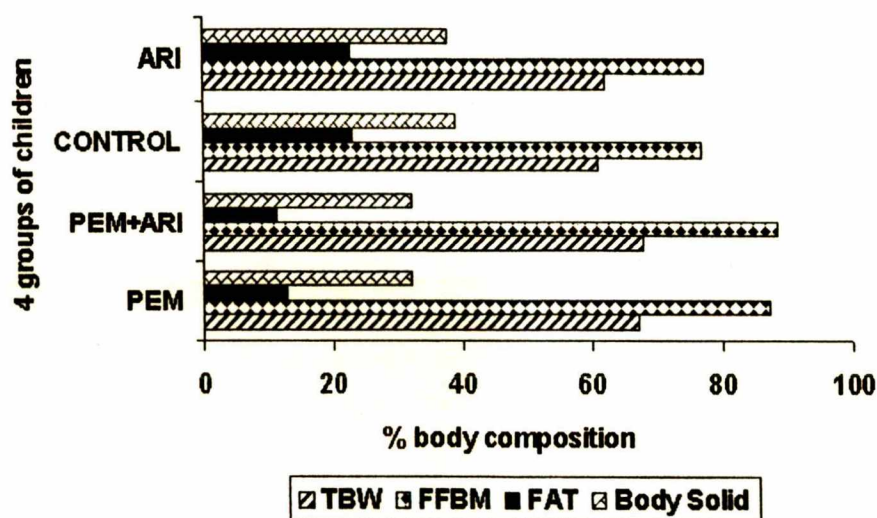


Figure 5: Percentage of total body water (%TBW), Fat free body mass (%FFBM), Fat mass (%FM) and % of body solid in 4 groups obtained by body composition analysis, showing decreased % Fat in malnourished groups (PEM & ARI with PEM) than welnourished group (Control and ARI without PEM).

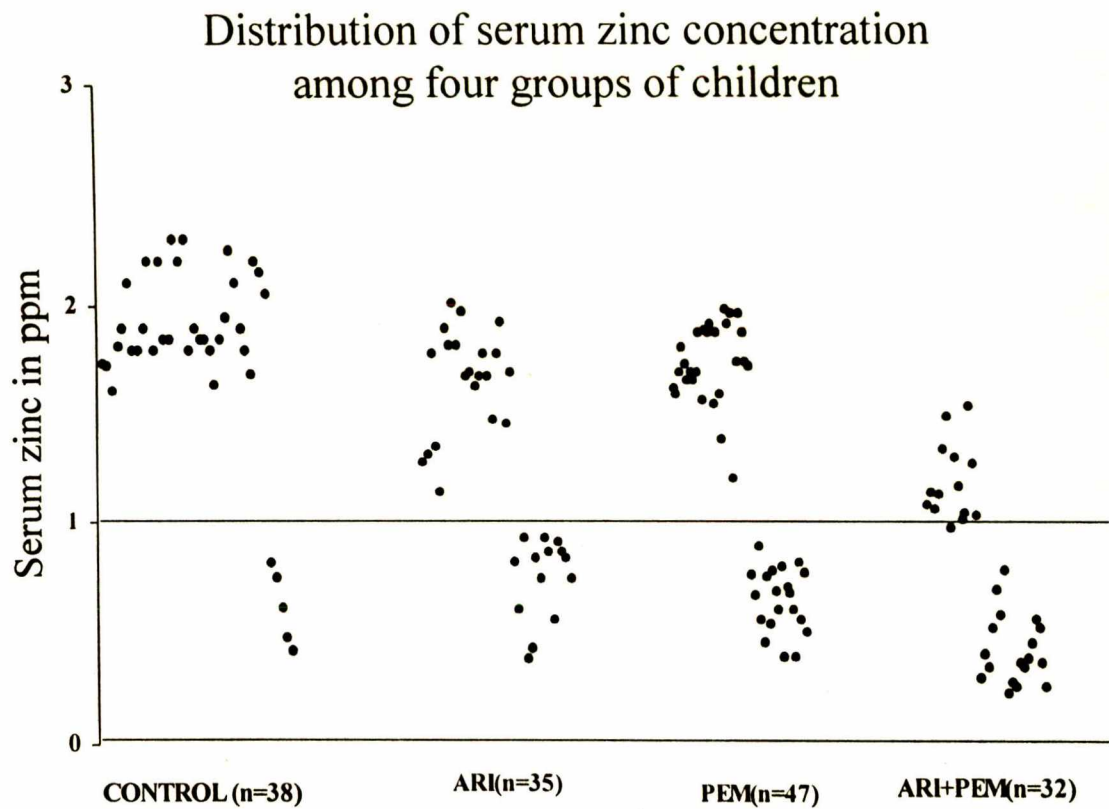


Figure 6: Distribution of serum zinc concentration among four groups of children, showing significant hypozincaemia ( $<1$  ppm) in ARI ( $n=14$ , 40%), PEM ( $n=20$ , 43%) and in ARI+PEM ( $n=18$ , 56%) than control ( $n=5$ , 13%),  $p<0.05$ ).



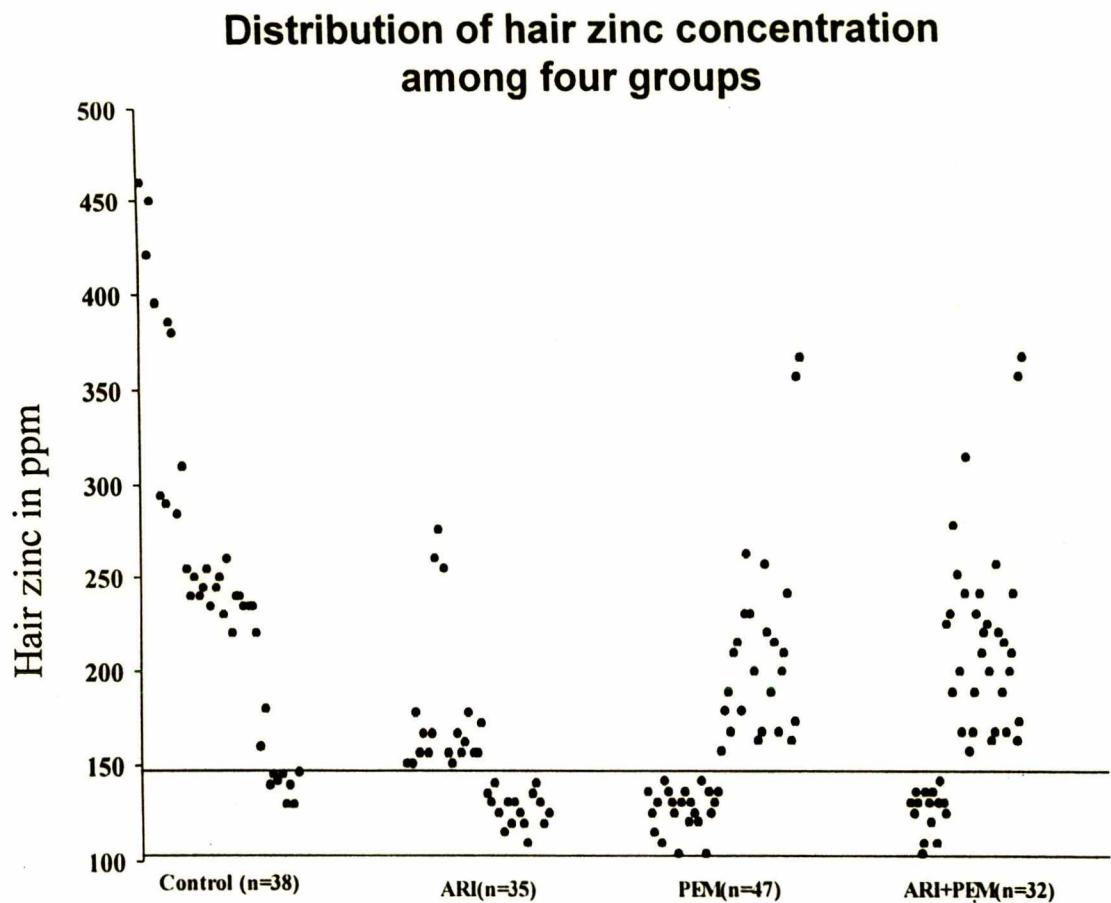


Figure 7: Distribution of hair zinc concentration among four groups of children, showing significant low hair zinc (<150 ppm) in ARI (n=16, 46%), PEM (n=22, 47%) and in ARI with PEM (n=15, 47%), than control (n=8, 21%),  $p < 0.05$ .

### Relationship of serum zinc with weight for age

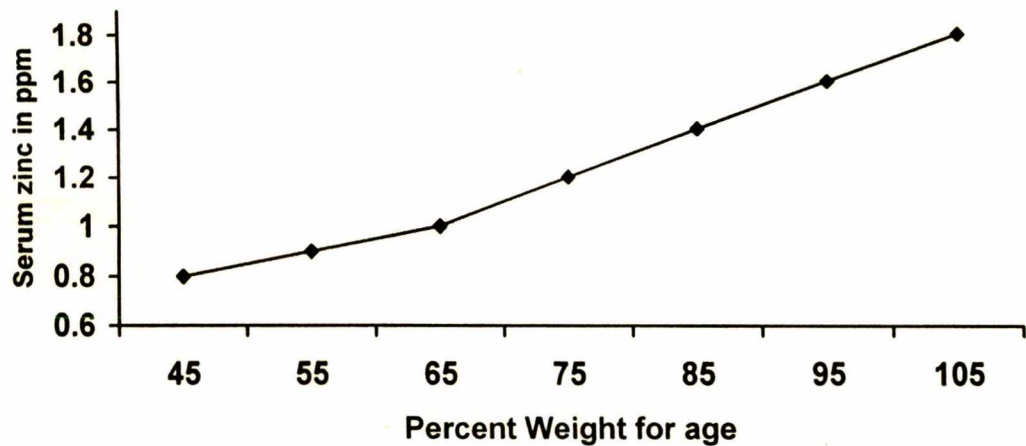


Figure 8: Relationship of weight for age with serum zinc showing strongly positive correlationship of serum zinc with W/A ( $r=0.40006$ ,  $p<0.001$ ).

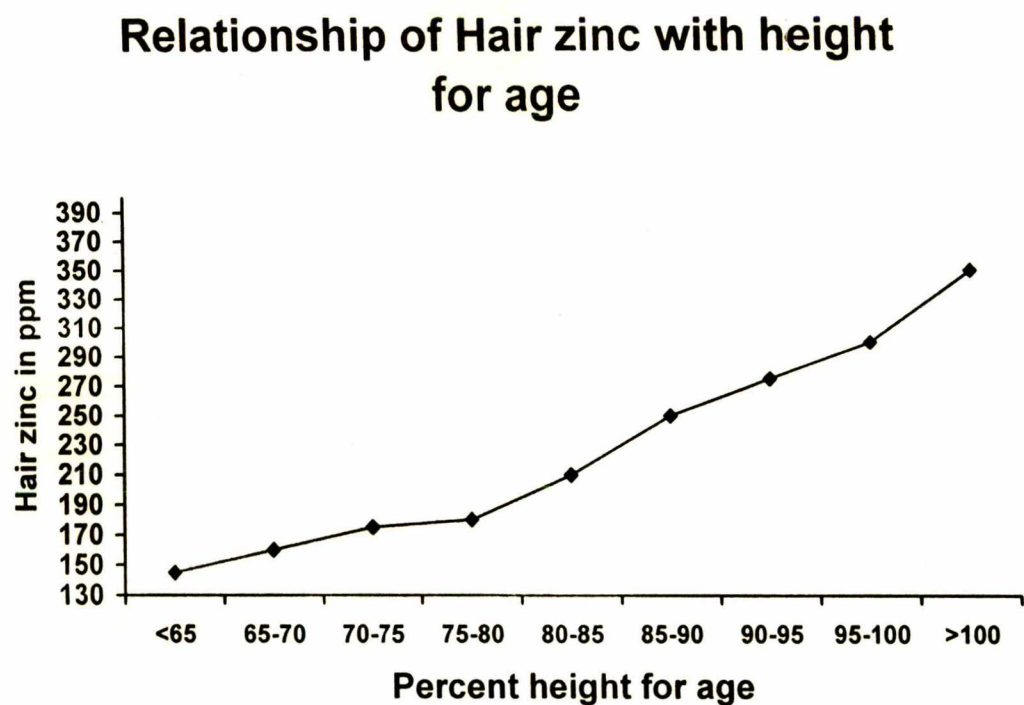


Figure 9: Mean hair zinc level in children with different height for age showing significantly positive correlationship of hair zinc with height for age ( $r=.314$ ,  $p<0.01$ ).



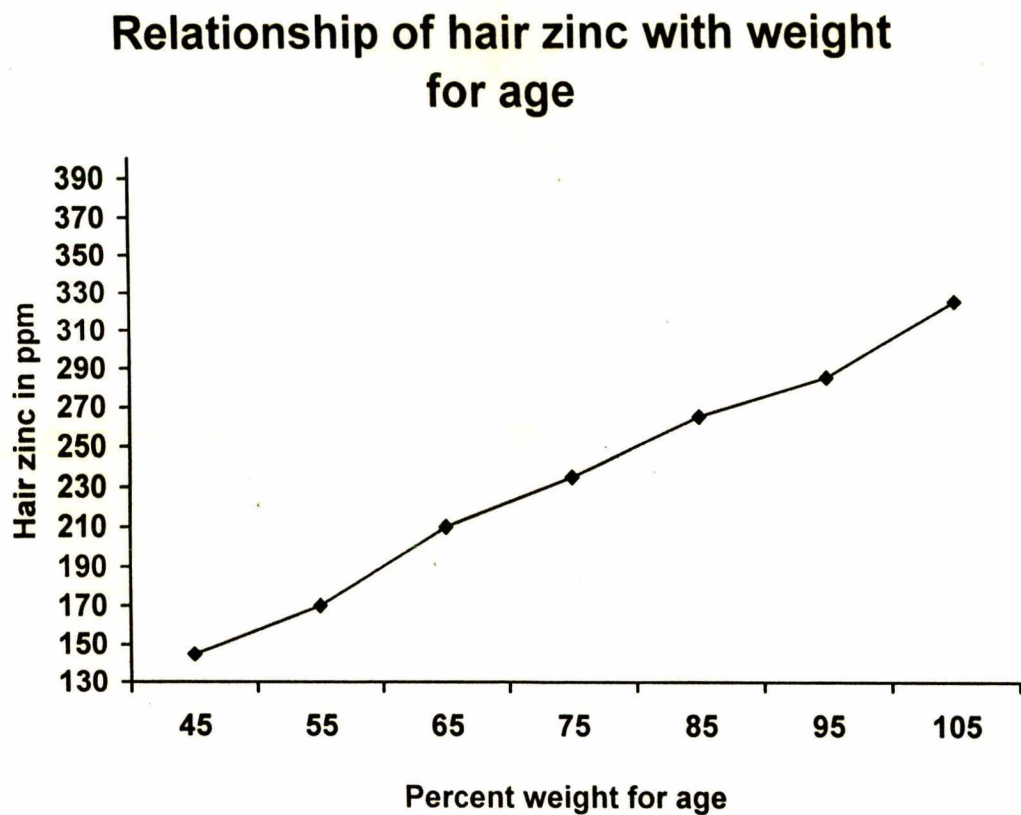


Figure 10: Relationship of weight for age with hair zinc, showing significant positive correlation of weight for age with hair zinc ( $r=.2887$ ,  $p<0.01$ ).

### Relationship of serum zinc concentration with height for age

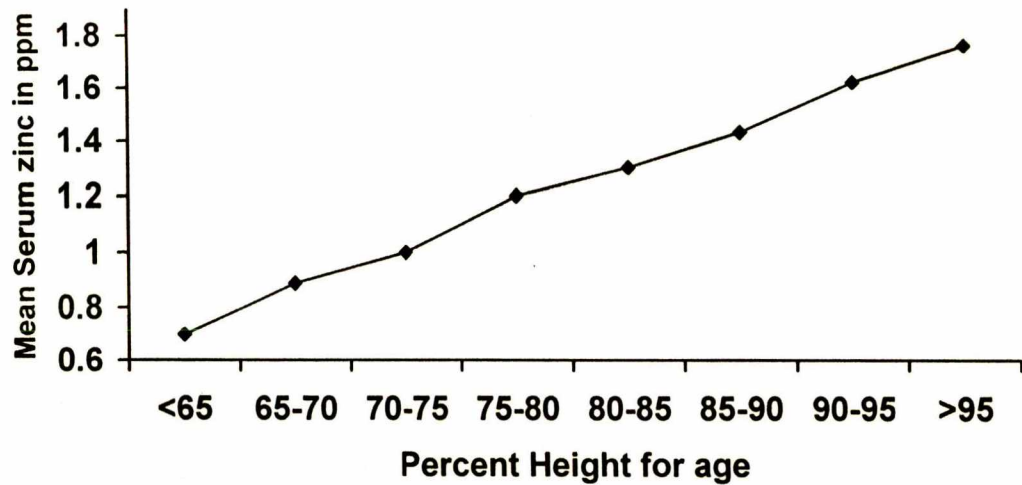


Figure 11: Mean serum zinc level in children with different Height for age, showing significantly positive correlationship ( $r=.2991$ ,  $p<0.043$ ) of H/A with serum zinc.

### Correlation of hair zinc with age in months

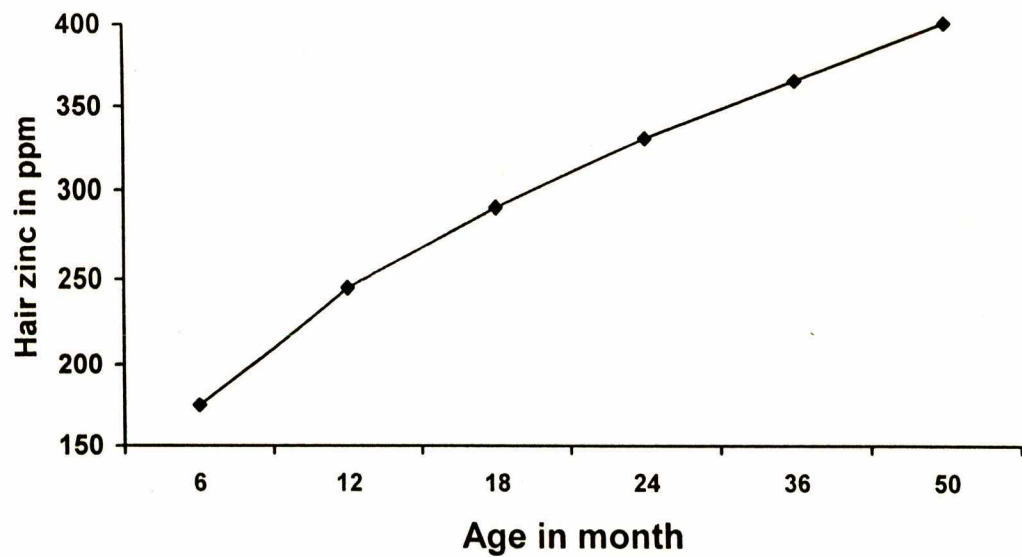


Figure 12: Correlation of hair zinc and age in months showing significantly positive correlation ( $r=0.3821$ ,  $p<0.01$ ) of hair zinc with increasing age in months.



## **LIST OF TABLES**

## LIST OF TABLES

|            |                                                                                                                                                                                                                                                                                                                                                                                            |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Table-I    | Relative sizes of tissue zinc pools in adults                                                                                                                                                                                                                                                                                                                                              |
| Table-II   | Factors which will influence the intestinal uptake and transfer of zinc                                                                                                                                                                                                                                                                                                                    |
| Table-III  | Mechanisms and conditions which predispose to dietary and systemic deprivation of zinc                                                                                                                                                                                                                                                                                                     |
| Table-IV   | A postulated scheme of the spectrum of zinc "status" and its associated pathophysiological phenomena                                                                                                                                                                                                                                                                                       |
| Table-V    | Age (in month) and Sex distribution in 4 groups, showing welmatched male and female ratio of approximately 1:3 in all groups                                                                                                                                                                                                                                                               |
| Table-VI   | Anthropometric status showing mean weight for age (W/A), weight for height (W/H) and height for age (H/A) in 4 groups, showing normal anthropometric status in Group A(Control) and Group B (ARI) and severely under weight (W/A <60% of NCHS median), showing wasted (W/A <70% of NCHS median) and severely stunted (H/A <85% of NCHS median) in Group C (PEM) and in Group D (PEM & ARI) |
| Table-VII  | Distribution of marasmus, kwashiorkor and marasmic kwashiorkor in group C&D, showing out of 79 severely malnourished children (47 in group C and 32 in group D), 50 (30 in group C and 20 in group D) i.e. > 60% were marasmus and remaining 29 (17 in group C and 12 in group D i.e. < 40% were kwashiorkor and marasmic kwashiorkor (oedematous malnutrition)                            |
| Table-VIII | Body Composition analysis of PEM showing decreased % FAT and increased % TBW and % FFBM                                                                                                                                                                                                                                                                                                    |
| Table-IX   | Body Composition of PEM + ARI, showing decreased % FAT and increased % TBW and % FFBM                                                                                                                                                                                                                                                                                                      |
| Table-X    | Body Composition analysis of Control(welnourished), showing normal % FAT and normal % TBW and % FFBM                                                                                                                                                                                                                                                                                       |

|             |                                                                                                                                                                                                                                                                                                                                                              |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Table-XI    | Body Composition of ARI (without malnutrition) showing normal % FAT and normal % TBW and % FFBM, which are almost similar to body composition of wellnourished control                                                                                                                                                                                       |
| Table-XII   | Serum zinc in ppm                                                                                                                                                                                                                                                                                                                                            |
| Table-XIII  | Hair Zinc in ppm                                                                                                                                                                                                                                                                                                                                             |
| Table-XIV   | Statistical analysis, using multivariate logistic relation model (logistic procedure) in PEM, ARI & PEM + ARI response profile using serum zinc , age and sex as variable, showing significantly negative association of serum zinc with PEM, ARI and ARI with PEM                                                                                           |
| Table-XV    | Statistical analysis using multivariate logistic relation model in PEM, ARI and ARI with PEM response profile, using hair zinc, age and sex as variables showing negative association of hair zinc (statistically not significant) with PEM, ARI and ARI with PEM                                                                                            |
| Table-XVI   | Statistical analysis using multivariate logistic relation model in PEM, ARI and ARI with PEM response profile, using serum zinc, hair zinc, age and sex as variables showing significantly negative association of serum zinc with PEM and ARI with PEM and negative association (statistically not significant) of hair zinc with PEM, ARI and ARI with PEM |
| Table-XVII  | Correlation of serum & hair zinc with various anthropometric measurement                                                                                                                                                                                                                                                                                     |
| Table-XVIII | Correlation of serum and hair zinc with age and with each other                                                                                                                                                                                                                                                                                              |
| Table-XIX   | Zinc status in various types of PEM (combined PEM and ARI with PEM)                                                                                                                                                                                                                                                                                          |
| Table-XX    | Serum protein and haemoglobin (Hb) in two malnourished (PEM and PEM+ARI) groups.                                                                                                                                                                                                                                                                             |
| Table-XXI   | Correlation of Serum and Hair Zinc with Serum Proteins and Haemoglobin                                                                                                                                                                                                                                                                                       |



**Table – V**  
**Age (in month) and Sex distribution in 4 groups, showing welmatched male and female ratio of approximately 1:3 in all groups.**

|               | <b>Group A<br/>(Control)<br/>n = 38</b> | <b>Group B<br/>(ARI)<br/>n = 35</b> | <b>Group C<br/>(PEM)<br/>n = 47</b> | <b>Group D<br/>(ARI + PEM)<br/>n = 32</b> |
|---------------|-----------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------------|
| <b>Age</b>    | <b>32±6.52</b>                          | <b>28±6.54</b>                      | <b>25±5.62</b>                      | <b>22±4.25</b>                            |
| <b>Sex</b>    |                                         |                                     |                                     |                                           |
| <b>Male</b>   | 22                                      | 20                                  | 27                                  | 18                                        |
| <b>Female</b> | 16                                      | 15                                  | 20                                  | 14                                        |

Table – VI

Anthropometric status showing mean weight for age (W/A), weight for height (W/H) and height for age (H/A) in 4 groups, showing normal anthropometric status in Group A(Control) and Group B (ARI) and severely under weight (W/A <60% of NCHS median), severely wasted (W/A <70% of NCHS median) and severely stunted (H/A <85% of NCHS median) in Group C (PEM) and in Group D (PEM & ARI)

|                 | Weight for age | Weight for height | Height for age |
|-----------------|----------------|-------------------|----------------|
| Gr. A (Control) | 102.64±10.54   | 94.42±8.35        | 96.38±9.90     |
| Gr. B (ARI)     | 92.63±8.62     | 89.52±8.29        | 92.35±9.35     |
| Gr. C (PEM)     | 48.12±12.20    | 69.54±8.23        | 75.94±8.04     |
| Gr. D (ARI+PEM) | 47.63±10.42    | 69.45±8.10        | 77.02±7.82     |

**Table VII**

**Distribution of marasmus, kwashiorkor and marasmic kwashiorkor in group C&D, showing out of 79 severely malnourished children (47 in group C and 32 in group D), 50 (30 in group C and 20 in group D) i.e. > 60% were marasmus and remaining 29 (17 in group C and 12 in group D i.e. < 40% were kwashiorkor and marasmic kwashiorkor (oedematous malnutrition).**

|                      | <b>Group C (PEM)<br/>N= 47</b> | <b>Group D( PEM + ARI)<br/>n=32</b> |
|----------------------|--------------------------------|-------------------------------------|
| Marasmus             | 30                             | 20                                  |
| Kwashiorkor          | 6                              | 3                                   |
| Marasmus Kwashiorkor | 11                             | 9                                   |



**Table VIII**  
**Body Composition analysis of PEM showing decreased % FAT and increased % TBW and % FFBM.**

| <b>% TBW</b> | <b>%FFBM</b> | <b>%FAT</b> | <b>%Body Solid</b> | <b>BMI</b> | <b>Impedence</b> |
|--------------|--------------|-------------|--------------------|------------|------------------|
| 67.17±4.32   | 87.23±8.72   | 12.77±4.32  | 32.23±5.62         | 13.1±1.88  | 844±168          |

**Table IX**  
**Body Composition of PEM + ARI, showing decreased % FAT and increased % TBW and % FFBM**

| <b>% TBW</b> | <b>%FFBM</b> | <b>%FAT</b> | <b>%Body Solid</b> | <b>BMI</b> | <b>Impedence</b> |
|--------------|--------------|-------------|--------------------|------------|------------------|
| 67.70±4.48   | 88.35±7.81   | 11.35±3.85  | 32.30±4.84         | 12.2±1.72  | 896±138          |

**Table X**  
**Body Composition analysis of Control(welnourished), showing normal % FAT and normal % TBW and % FFBM.**

| <b>% TBW</b> | <b>%FFBM</b> | <b>%FAT</b> | <b>%Body Solid</b> | <b>BMI</b> | <b>Impedence</b> |
|--------------|--------------|-------------|--------------------|------------|------------------|
| 60.83±3.56   | 76.85±8.29   | 23.10±8.31  | 39.17±3.38         | 19.8±1.80  | 802±141          |

**Table XI**  
**Body Composition of ARI (without malnutrition) showing normal % FAT and normal % TBW and % FFBM, which are almost similar to body composition of welnourished control.**

| <b>% TBW</b> | <b>%FFBM</b> | <b>%FAT</b> | <b>%Body Solid</b> | <b>BMI</b> | <b>Impedence</b> |
|--------------|--------------|-------------|--------------------|------------|------------------|
| 62.22±4.56   | 77.32±7.31   | 22.68±7.68  | 37.78±4.42         | 15.18±1.70 | 779±132          |

**Table – XII**  
**Serum zinc in ppm**

|                   | Serum Zinc in ppm |           | Serum Zinc (<1 ppm) |
|-------------------|-------------------|-----------|---------------------|
| Gr. A (Control)   | n=38              | 1.76±.98  | 5 (13%)             |
| Gr. B (ARI)       | n=35              | 1.26±.60  | 14 (40%)            |
| Gr. C (PEM)       | n=47              | 1.31±0.55 | 20 (43%)            |
| Gr. D (ARI + PEM) | n=32              | 0.90±0.51 | 18 (56%)            |

p<0.05 Between A & B, C & D,

Duncan Multiple Range Test (DMRT)

p<0.05 Between C & D

|                     |                              |        |
|---------------------|------------------------------|--------|
| OR between A & B 4, | 95% CI of OR (1.81, 18.896)  | p<0.05 |
| OR between A & C 5, | 95% CI of OR (1.132, 21.284) | p<0.05 |
| OR between A & D 6, | 95% CI of OR (1.83, 19.628)  | p<0.05 |
| OR between B & D 2, | 95% CI of OR (1.650, 4.578)  | p<0.05 |

OR = Odds ratio

CI = Confidence interval.

To convert ppm to µgm/dl multiply by 100 i.e. 1.76 ppm = 17.60 µgm/dl

To convert ppm to µmol/l multiply by 15.3 i.e. 1.76 ppm = 26.92 µmol/l



**Table – XIII**  
**Hair Zinc in ppm**

|                   |      | Hair Zinc in $\mu\text{gm/gm}$ | Hair Zinc (<150 ppm) |
|-------------------|------|--------------------------------|----------------------|
| Gr. A (Control)   | n=38 | 249 $\pm$ 154                  | 8 (21%)              |
| Gr. B (ARI)       | n=35 | 158 $\pm$ 48                   | 16 (46%)             |
| Gr. C (PEM)       | n=47 | 174 $\pm$ 75                   | 22 (47%)             |
| Gr. D (ARI + PEM) | n=32 | 177 $\pm$ 70                   | 15 (47%)             |

p<0.05 Between A & B, C & D Tukey's and DMRT

|                     |                              |        |
|---------------------|------------------------------|--------|
| OR between A & B 3, | 95% CI of OR (1.457, 10.044) | p<0.05 |
| OR between A & C 3, | 95% CI of OR (1.119, 8.32)   | p<0.05 |
| OR between A & D 3, | 95% CI of OR (1.013, 15.04)  | p<0.05 |

**Table XIV**

Statistical analysis, using multivariate logistic relation model (logistic procedure) in PEM, ARI & PEM + ARI response profile using serum zinc , age and sex as variable, showing significantly negative association of serum zinc with PEM, ARI and ARI with PEM.

| Serum zinc, age, sex |                            |                            |                            |
|----------------------|----------------------------|----------------------------|----------------------------|
|                      | PEM (p=0.0001)             | ARI (p=0.0001)             | ARI +<br>PEM(p=0.0010)     |
| Serum Zinc           | PE= -2.933<br>(p=0.0016)** | PE= -3.6640<br>(p=0.0139)* | PE -3.694<br>(p=0.0034)*   |
| Age in month         | PE= -0.127<br>(p=0.0026)*  | PE= -0.1935<br>(p=0.05)*   | PE= -0.1695<br>(p=0.0041)* |
| Sex                  | PE= -1.931<br>(p=0.054)    | PE= -1.0671<br>(p=0.434)   | PE= -1.4770<br>(p=0.2944)  |

\* Significant

PE= Parameter Estimate

**Table XV**

Statistical analysis using multivariate logistic relation model in PEM, ARI and ARI with PEM response profile, using hair zinc, age and sex as variables showing negative association of hair zinc (statistically not significant) with PEM, ARI and ARI with PEM.

|              | PEM (p=0.0020)             | ARI (p=0.0001)             | ARI +<br>PEM(p=0.0010)     |
|--------------|----------------------------|----------------------------|----------------------------|
| Hair Zinc    | PE= -0.00606<br>(p=0.1282) | PE= -0.00328<br>(p=0.8845) | PE= -0.00357<br>(p=0.4471) |
| Age in month | PE= -0.0569<br>(p=0.022)*  | PE= -0.3919<br>(p=0.0406)* | PE= -0.0937<br>(p=0.0040)* |
| Sex          | PE= -1.4505<br>(p=0.0740)  | PE= -0.9117<br>(p=0.6428)  | PE= -1.2599<br>(p=0.226)   |



**Table XVI**

Statistical analysis using multivariate logistic relation model in PEM, ARI and ARI with PEM response profile, using serum zinc, hair zinc, age and sex as variables showing significantly negative association of serum zinc with PEM and ARI with PEM and negative association (statistically not significant) of hair zinc with PEM, ARI and ARI with PEM.

|              | <b>PEM (p=0.0001)</b>    | <b>ARI (p=0.0001)</b>    | <b>ARI + PEM (p=0.0010)</b> |
|--------------|--------------------------|--------------------------|-----------------------------|
| Serum Zinc   | PE-3.5411<br>(p=0.0020)* | PE-5.4503<br>(p=0.247)   | PE-4.6579<br>(p=0.0138)*    |
| Hair Zinc    | PE-0.0142<br>(p=0.08660) | PE-0.0423<br>(p=0.2741)  | PE-0.021<br>(p=0.3349)      |
| Age in month | PE-0.1109<br>(p=0.0085)* | PE-0.4240<br>(p=0.0482)* | PE-0.1654<br>(p=0.0346)*    |
| Sex          | PE-1.8008<br>(p=0.0782)  | PE-3.0854<br>(p=0.2173)  | PE-0.9346<br>(p=0.6045)     |

**Table – XVII**  
**Correlation of serum & hair zinc with various anthropometric measurement:**

|            | <b>Weight for age</b>             | <b>Height for age</b>           | <b>Weight for height</b>         |
|------------|-----------------------------------|---------------------------------|----------------------------------|
| Serum zinc | $r = .40006$<br>( $P < .001$ )*** | $r = .2991$<br>( $P = 0.043$ )* | $r = .0090$<br>( $P = .95$ ) NS  |
| Hair zinc  | $r = .2887$<br>( $P < .01$ )**    | $r = .314$<br>( $P < 0.01$ )**  | $r = -.1328$<br>( $P > 0.5$ ) NS |

\* Significant

NS = Not significant

**Table – XVIII**  
**Correlation of serum and hair zinc with age and with each other**

|                                               |                             |
|-----------------------------------------------|-----------------------------|
| Correlation of serum zinc with age in month r | = -0.0575 ( $P = .71$ ) NS  |
| Correlation of hair zinc with age in month r  | = 0.3821 ( $P < .001$ )*    |
| Correlation of serum zinc with hair zinc r    | = -0.0925 ( $P > 0.05$ ) NS |

\* Significant

NS = Not significant

**Table – XIX****Zinc status in various types of PEM (combined PEM and ARI with PEM)**

|                         | <b>N<br/>(n = 79)</b> | <b>Hair Zinc in ppm<br/>(174±25)</b> | <b>S. Zinc in ppm<br/>1.18±.62)</b> |
|-------------------------|-----------------------|--------------------------------------|-------------------------------------|
| Marasmus                | 50                    | 168±25                               | 1.16±.50                            |
| Kwashiorkor             | 9                     | 184±32                               | 1.64±.72                            |
| Marasmic<br>kwashiorkor | 20                    | 172±13                               | 0.87±.04                            |

**Table XX****Serum proteins and haemoglobin (Hb) in two malnourished (PEM & PEM + ARI) groups**

| <b>Serum Protein and Hb<br/>concentration</b> | <b>PEM</b> | <b>PEM + ARI</b> |
|-----------------------------------------------|------------|------------------|
| Serum total protein (gm/dl)                   | 4.41±0.64  | 4.28±0.53        |
| Serum total albumin                           | 1.90±0.37  | 1.78±0.42        |
| Serum total globulin                          | 2.47±0.56  | 2.50±0.28        |
| Haemoglobin (gm/dl)                           | 6.02±1.15  | 6.78±1.20        |



**Table XXI**  
**Correlation of Serum and Hair Zinc with Serum Proteins and Haemoglobin**

|            | <b>STP</b>                  | <b>S.Albumin</b>             | <b>S.Globulin</b>           | <b>Hb</b>                   |
|------------|-----------------------------|------------------------------|-----------------------------|-----------------------------|
| Serum Zinc | $r=-.0248$<br>( $p=0.876$ ) | $r=-0.209$<br>( $p=0.889$ )  | $r=-.1092$<br>( $p=0.465$ ) | $r=-.0266$<br>( $p=0.859$ ) |
| Hair Zinc  | $r=0.2769$<br>( $p=0.076$ ) | $r=0.2667$<br>( $p=0.008$ )* | $r=0.1353$<br>( $p=0.393$ ) | $r=0.0728$<br>( $p=0.647$ ) |

Correlation of Serum Total Protein with Hb :  $r = 0.4699$  ( $p=.001$ )\*

Correlation of Serum Albumin with Hb :  $r = 0.3336$  ( $p=.002$ )\*

Correlation of Serum Globulin with Hb :  $r = 0.2908$  ( $p=.056$ )

## **REFERENCES**

## References

1. Kabir M, Moslehuddin M. Mortality levels and pattern of oral therapy extension programme areas of Bangladesh Rural Advancement Committee (BRAC). Food and Nutr. Bull. 1986;8(3):008-11
2. UNICEF. The state of worlds children 1998. Oxford University Press. New York P-52-52.
3. Puffer RR, Serrand CU. Pattern of mortality on childhood. Pan American Sanitary Bureau. Pan American Health Organisation, Washington DC. 1973
4. WHO based on CJL, Murray and AD Lopez. The global burden of disease Harvard University Press, Cambridge (USA) 1996.
5. Tomkins A, Watson F. Malnutrition and infection A review ACC/SCN. State of the ARI series United Nations 1989;1-41.
6. Frank S. Protein energy malnutrition. In: Anncil GC, Metioff J eds. Paediatric Nutrition, Butter Woth 1985.
7. Kenneth H, Robert H, Gaffar A, Alamgir S et al. Infection associated with severe protein calorie malnutrition in hospitalized infants and children. Nutr Res 1981;1:33-46.
8. Shakur S, Bano N, Ehsan MA. Biochemical and Socio-economic factors associated with severe degree malnutrition in children admitted in Dhaka Shishu (children) Hospital - Dhaka Shishu (Children) Hospital Journal 1991;7:5-12.



9. World Health Organisation (WHO). Technical basis to WHO recommendation on the management of Pneumonia in children of first level health facilities programme for control of Acute Respiratory Tract Infections. ARI 20. WHO 1991.
10. Leoski J. Mortality from acute respiratory infections in children under 5 years of age: Global estimation. *World Health State Q.* 1986; 138-144.
11. Denny FW, Luda FA. Acute respiratory infections are the leading cause of death in children in developing countries. *Am J Trop Med Hyg* 1986; 35:1-7.
12. Garenne M, Ronsmans C, Cambell H. The magnitude of mortality from acute respiratory infections in children under 5 years in developing countries. *World Health State Q.* 1992;45:180-191.
13. Baqui AH, Black R, Arefin SE et al. 1998. Causes of childhood deaths in Bangladesh: Results of a nationwide verbal autopsy study. *Bulletin of World Health Organization* 76: 161-171.
14. James JW. Longitudinal study of the morbidity of diarrhoeal and respiratory infections in malnourished children. *Am J Clin Nutr* 1972;25: 690-694.
15. Zaman K, Baqui AH, Younus MD et al. Association between nutritional status, cell mediated immune status and acute lower respiratory infections in Bangladeshi children. *Eur J Clin Nutr* 1996; 50: 309-314.
16. de Francisco A, Morris J, Hall AJ. Risk factors for mortality from acute lower respiratory tract infections in young children. *Int J Epidemiol* 1993;22:1174-1182.

17. World Health Organisation (WHO). The treatment and management of severe protein-energy malnutrition. Geneva 1981;15-18.
18. Mohammed MA. Evaluation of current strategies in the management of protein energy malnutrition among children under five years old, Belbela Hospital (Djibouti). M.Sc report, London School of Hygiene and Tropical Medicine, University of London 1993.
19. UNICEF. Child malnutrition: Progress towards the world summit for children goal, UNICEF, New York, 1993.
20. Schofield C, Ashworth A. Why have mortality rates for severe malnutrition remained so high? Bull WHO 1996;74:223-29.
21. WHO management of severe malnutrition: a manual for physicians and other senior health workers. Geneva: WHO, 1999.
22. Scherbaum V. Management and rehabilitation of protein energy malnutrition in Ethiopia M.Sc dissertation, Institute of Child Health, University of London, 1992.
23. Waterlow JC. Protein energy malnutrition. Edward Arnold, London, 1992.
24. Suskind D, Murthy KK, Suskind RM. The malnourished child: an overview. In: Suskind RM and Suskind LL eds. The malnourished child: New York: Vavey/Raven press, 1990,1-22.
25. Chandra R.K. Am J Clin Nutr 1983;33:736-38.

26. Good RA, Fenandez G, West A. Nutrition. Immunity and cancer. A review part I: Influence of protein caloric malnutrition and zinc deficiency on immunity. Clin Bull 1979;9:3-12.
27. Khanom S. Alam AN, Anwar I et al. Effect of zinc supplementation on the dietary intake and weight gain of Bangladeshi children recovering from protein energy malnutrition. Eur J Clin Nutr 1988; 42: 709-14.
28. Smith Z.M. Pritirions P.J. J. Trop. Paedat, 1964;9:105-112.
29. Hansen JDL and Lehman BH, S Afr Med J 1969, 43: 1248-1250.
30. Kumar S, Rao R.S., J. Nutr. Metobolism 1977;15:364-371.
31. Cheek DB. Hill DE. Cordano A. Graham GG. Malnutrition in infancy:change in muscle and adipose tissue before and after rehabilitation. Pediatr Res 1970,4:135-44.
32. Golden BE., Golden MHN. Plasma zinc and the clinical features of malnutrition. Am J Clin Nutr 1979;32:2490-9.
33. Warren PJ. Hansen JBL. Lehman BN. The concentration of copper, zinc and manganese in the liver of African children with marasmus and kwashiokor. Proc Nut Soc 1969;26 6A-7A.
34. Malek MA., Chowdhury MHW. Trace element concentration of Bangladeshi malnourished children. Nutrition News 3, Jan 87.
35. Hambidge KM. Silver A. Arch Dis Child 1973; 48: 567-568.



36. Hambidge K.M., Hambidge C, Jacob M and Baum J.D. Low levels of zinc in hair, anorexia, poor growth and hypogeusia in children. *Pediatr Res* 1972;6:868-874.
37. Golden M.H.N. Trace elements in human nutrition. *Human Nutrition: Clinical Nutrition* 1982;36c:185-202.
38. Kratzer FH. Allred JB. Davis PN et al. *J Nutr* 1959; 68: 313-322.
39. Golden M.H.N, Golden B.E., Benette FI. Relationship of Trace Element Deficiency to Malnutrition. In R.K. Chandra Editor. *Trace Elements in Nutrition of children*. Nestle Nutrition. Vevey/Ravon Press, New York 1988, PP 183-193.
40. Golden MHN, Golden BE, Benette FI. Relationship of trace element deficiencies to malnutrition. Relationship of trace element deficiencies to malnutrition. RK Chandra Editor. *Nestle Nutrition*. Vevey/Ravan Press New York 1985; 185-189.
41. Golden BE & Golden MHN. Plasma zinc, rate of weight gain and energy cost of tissue deposition in children recovering from severe malnutrition on cow's milk or soya protein based diet. *Am J Clin Nutr*, 1981;34: 892-899.
42. Jackson MJ. Metabolic studies of zinc nutrition in the third world. In: Momeilovic B editor. *Trace elements in man and animals*. Institute for medical research and occupational health university of Zagreb, Zagreb, Yougoslavia. 1991; pp 147-148.
43. Mocl W. Solomons MD. Assessment of zinc and copper nutrition in man. *Am J Clin Nutr*, 1979;32:856-871.

44. Prasad AS, Schoomakers J, Ortega G et al. Zinc deficiency in sickle cell disease. Clin Chem 1975;21:582-587.
45. Prasad AS, J Ortega GJ. Brewer D et al. Trace elements in sickle cell disease. J Am Med Assoc 1976;235:2396.
46. Mcbean LD, Mahloudji M, Rejnhold JG et al. Correlation of zinc concentration in human plasma and hair. Am J Clin Nutr 1971; 29:506-509.
47. Aggett P.J. Zinc. Annals Nestle 1994;52:94-106.
48. Application of hair as an indication for trace element exposure in man. A review. NAHRES 22, IAEA, Vienna (1994).
49. Pallauf J, Kirchgessner M. Zinkkonzentration des Ratten haarus bei zink-Depletion und Repletion. Zbl. Vet Med A20 1973; 100-109.
50. Cavan KR, Gibson RS, Graziose C.F, Isalgue A.M, Ruz M, Solomons N.W. Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc interventional trial. Am J Clin Nutr 1993;57:344-52.
51. Gibson RS, Vander Kooy PD, McDonald AC Goldman A. Ryan BA, Berry M. A growth limiting mild zinc deficiency syndrome in some southern Ontario boys with low height percentiles. Am J Clin Nutr 1989;49:1266-73.
52. Sandstead HH, Lanier VC, Shephard GH and Gillespie DD: Zinc and wound healing: effects of zinc deficiency and zinc supplementation. Amer J Clin Nutr 1970; 23:514.

53. Hallbook T, Lanner E. Serum zinc and healing of venous leg ulcers. *Lancet* 1972; 2: 780-782.
54. Raulin, J.: Etudes cliniques sur la vegetation. *Ann. Sci. Nat. Bot. Biol. Vegetale*, 1869;11:93.
55. Sommer, A. I., and Lipman, C. B.: Evidence of the indispensable nature of zinc and boron for higher green plants. *Plants Physiol.*1926; 1:231.
56. Todd, W.R., Elevehjem, C.A., and Hart, E.B.: Zinc in the nutrition of the rat. *Amer. J. Physiol.*1934;107:146.
57. Parisi, A.F., and Vallee, B. L.: Zinc metalloenzymes: characteristics and significance in biology and medicine. *Amer. J. Clin. Nutr.*1969; 22:1222.
58. Holt, A. B., Mellits, E.D., and Cheek, D. B.: Comparisons between nucleic acids, protein, zinc, and manganese in rat liver: a relation between zinc and ribonucleic acid. *Pediat. Res.*1970; 4:157.
59. Mills. C. F., Quarterman, J., Chesters, J. K., Williams, R. B., and Dalgarno, A. C.: Metabolic role of zinc. *Amer. J. Clin. Nutr.*1969; 22:1240.
60. Prasad, A. S.: Nutritional metabolic role of zinc. *Fed. Proc.*, 1967;26:172-185.
61. Prasad, A. S.: A century of research on the metabolic role of zinc. *Am. J. Clin. Nutr.*1969; 22:1215.
62. Underwood, E. J.: Trace elements in human and animal Nutrition, Academic Press, New York, 1971.



63. Tucker, H. F., and Salmon, W. D.: Parakeratosis or zinc deficiency disease in the pig. *Proc. Soc. Exp. Biol. Med.* 1955; 33:613.
64. Barney, G. H., Macapinlac, M. P., Pearson, W. N., and Darby, W. J.: Parakeratosis of the tongue - a unique histopathologic lesion in the zinc deficient squirrel monkey. *J. Nutr.* 1967; 93:511.
65. Dynna, O., and Havre, G. N.: Interrelationship of zinc and copper in the nutrition of cattle: a complex zinc-copper deficiency. *Acta Vet. Scand.* 1963; 4:197.
66. Hurley, I. S., and Swenderton, H.: Congenital malformations resulting from zinc deficiency in rats. *Proc. Soc. Exp. Biol. Med.* 1966; 123:692.
67. Mills, C. F., Dalgarno, A. C., Williams, R. B., and Quarterman, J.: Zinc deficiency and the zinc requirements of calves and lambs. *Br. J. Nutr.* 1967; 21:751.
68. O'Dell, B. L., Savage, J. E., and Newberene, P.M.: significance of dietary zinc for the growing chicken. *J. Nutr.* 1959; 65:503.
69. Macapinlac, M. P., Pearson, W. N., and Darby, W. J.: In: A.S. Prasad: Zinc Metabolism, Thomas, Springfield, Ill., 1966;142.
70. Prasad, A.S., Halsted, J. A., and Nadimi, M.: Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. *Amer. J. Med.* 1961; 31:532-546.
71. Prasad, A. S., Miale, A., Farid, Z., Sandstead, H. H., Schulert, A. R., and Darby, W. J.: Biochemical studies on dwarfism, hypogonadism and anemia. *Arch. Intern. Med.* 1963;111:407.

72. Halsted, J. A.: Human zinc deficiency. *Trans. Amer. Clin. Clim. Ass.* 1970;82:170.
73. Caggiano, V., Schnitzler, R., Strauss, W., Baker, R. K., Carter, A. C., Josephson, A. S., and Wallack, S.: Zinc deficiency in a patient with retarded growth, hypogonadism, hypogammaglobulinemia and chronic infection. *Amer. J. Med. Sci.* 1969;257:305.
74. Mac Mahon, R. A., Parker, L. M., and McKinnon, M. C.: Zinc treatment in malabsorption. *Med. J. Aust.* 1968; 210.
75. Pories, W. J., Henzel, J. H., Rob, C. G., Strain, W. H. Acceleration of healing with zinc sulfate. *Ann. Surg.* 1967;165:432-436.
76. Henkin, R. I., Schechiter, P. J., Hoyer, R., and Mattern, C. F. T.: Idiopathic hypogeusia with dysgeusia, hyposmia, and dysosmia: a new syndrome. *J. A M A*, 1971; 217:434.
77. Henkin, R. I., and Bradley, D. F.: Hypogeusia corrected by  $Ni^{++}$  and  $Zn^{++}$ . *Life Sci.* 1970;9:701.
78. Halsted, J. A., Smith, J.C., Jr. and Irwin, M. I., *J. Nutr.* 1974;104, 345-378.
79. Cheek, D.B., Spango, P.M. and Holt, A. B. *Med. J. Aus.* 1981;1, suppl. No. 2, 4-5.
80. Coble, Y.D., VanReen, R., Schulert, A. R., et al. *Am. J. Clin. Nutr.* 1966;19: 415-421.
81. Solomons, N.W., *Am. J. Clin. Nutr.* 1979;32: 856-861.

82. Prasad, A. S., *Ann. Rev. Phar. Toxicol.* 1979;20:393-407.
83. Smith, S. E., and Larsen, E.T., *J. Biol. Chem.* 1946; 146: 29-32.
84. VanReen, R. *Arch. Biochem. Biophys.* 1953;46:337-342.
85. Golden, M.H.N., Golden, B.E., Harland, P.S.E.C. & Jackson, A.A. Zinc and immunocompetence in protein-energy malnutrition. *Lancet* 1,1978; 1226-1227.
86. Brooke, O.G., Wheeler, E.F. *Arch. Dis. Child.* 1976;51: 968-971.
87. Hansen-Smith FM, Picou D, Golden MHN. Growth of muscle fibres during recovery from severe malnutrition in Jamaican infants. *Br J Nutr* 1979; 41:275-82.
88. Belton, H., Thirumalai, C., Golden, M.H.N., Harland, P.S. and Jackson, A.A. In: *Proc. of 25th Common. Carrib. Med. Res. Counl.* 1980; 33-34.
89. Hambidge, K. M. *Pediat. Clin. North. Am* 1977;24: 95-99.
90. Golden MHN, Golden BE. Effect of zinc supplementation on the dietary intake rate of weight gain and energy cost of tissue deposition in children recovering from severe malnutrition. *Am J Clin Nutr* 1981;34:900-8.
91. Failla, M. I., Zinc: Functions and transport in microorganisms. In E.D. Weinberg (ed.). *Microorganisms and minerals.* Marcel Dekker, Inc., New York, 1977, p. 151-214.
92. Pories, W. J., Henzel, J. H., Rob, C. G., and Strain, W. H.: Acceleration of wound healing in man with zinc sulphate given by mouth. *Lancet* 1967; 1:121.



93. Cross, R.F., Parker, C. F. Oral administration of zinc sulfate for control of ovine foot rot. *J. Am. Vet. Med. Assoc.* 1981;178:704-705.
94. Verma, K.C., Saini, A. S., Dhamija, S. K. Oral zinc sulfate therapy in acne vulgaris: a double-blind trial. *Acta Derm. Venereol. (Stockholm)* 1980;60:337-340.
95. Landman J, Jackson AA. The role of protein deficiency in the aetiology of kwashiorkor. *West Indian Md J* 1980; 29:229-38.
96. Jackson AA, Golden MHN. Protein-energy malnutrition. In: Weatherall DJ, Ledingman JGG, Warrell DA, eds. *The oxford textbook of medicine*. Oxford: Oxford University Press, 1983; 812-21.
97. Golden MHN, Golden BE, Jackson AA. Albumin and nutritional oedema. *Lancet* 1980;1:114-6.
98. Golden MHN. Protein deficiency, energy deficiency and the oedema of malnutrition. *Lancet* 1982; 1:1261-5.
99. Golden, M.H.N., & Golden, B.E. & Jackson, A.A. (1980): skin breakdown in kwashiorkor responds to zinc. *Lancet* 1, 1980; 1256.
100. Golden, M. H.N., Golden, B. E., Harland, P.S.E.G., Jackson, A.A. Zinc and immunocompetence in protein-energy malnutrition. *Lancet* 1:1978; 1226-1227.
101. Walraven PA, Krebs NF, Hambidge KM. Linear growth of low income preschool children receiving a zinc supplement. *Am J Clin Nutr* 1983;38:195-201.

102. Buzinia R, Justice M, Sapaner J et al. Zinc nutrition and taste acuity in school children with impaired growth. *Am J Clin Nutr* 1980;33:2262-7.
103. Walraven PA, Hambidge KM, Neldner KH et al. Zinc metabolism in acrodermatitis enteropathica. *J Pediatr* 1978;93:71-3.
104. Jackson AA, Picou D, Reeds PJ. The energy cost of repleting tissue deficits during recovery from protein-energy malnutrition. *Am J Clin Nutr* 1977;30:1514-7.
105. Golden, M.H.N., Jackson, A.A., Golden, B.E. Effect of zinc on thymus of recently malnourished children. *Lancet* 2: 1977;1057-1059.
106. Patric J, Golden BE, Golden MHN. Leucocyte sodium transport and dietary zinc in protein energy malnutrition. *Am J Clin Nutr* 1980;33:619-620.
107. Golden, M.H.N. & Golden, B.E. Trace elements : potential importance in human nutrition with particular reference to zinc and vanadium. *Br. Med. Bull.* 1981;37: 31-36.
108. Chesters JK, Quarterman J. Effects of zinc deficiency on food intake and feeding patterns of rats. *Br J Nutr* 1970;24:1061-9.
109. United States Food & Nutrition Board: Recommended dietary allowances, Washington: National Academy of Science. 1974;92-96.
110. Hurley, L.S. & Sharder, R.E. abdominal development of preimplantation rat eggs after three days of maternal dietary zinc deficiency. *Nature* 1975; 254: 427-429.

111. Moynahan, E.J. Acrodermatitis enteropathica: a lethal inherited human zinc deficiency disorder. *Lancet* 2, 1974; 399-400.
112. Wolman, S.L., Anderson, G.H., Marliss, E.B. & Jeejeebhoy, K.N. Zinc in total parenteral nutrition: requirements and metabolic effects. *Gastroenterology* 1979; 76: 458-467.
113. Schlappner, O.L.A., Shelly, W.B., Ruberg, R.C. & Dudrick, S.J. Acute papulopustular acne associated with prolonged intravenous hyperalimentation. *J. Am. Med. Ass.* 1972;219: 877-880.
114. Van Vloten, W.A. & Bos, L.P. Skin lesions in acquired zinc deficiency due to parenteral nutrition. *Dermatologica* 1978;156: 175-183.
115. Kay, R.G., Tasman-Jones, C., Pybus, J., Whiting, R. & Black, H. A syndrome of acute zinc deficiency during total parenteral nutrition in man. *Ann. Surg.* 1976;183: 331-340.
116. Singh KP, Zaidi SIA, Raisuddin S et al. Effect of Zinc on immune functions and host resistance against infection and tumor challenge. *Immunopharmacology and immunotoxicology* 1992;14:813-840.
117. Lehman BH, Hansen JDL, Warren PJ. The distribution of copper, zinc and manganese in various regions of the brain and other tissues of children with protein calorie malnutrition. *Br J Nutr* 1971;26:197-202.
118. Landman J, Jackson AA. The role of protein deficiency in the aetiology of kwashiorkor. *West Indian Med J* 1980;29:229-35.



119. Fujioka M and Lieberman A.  $Zn^{++}$  requirement for synthesis of deoxyribonucleic acid by rat liver. *J Biol Chem* 1964;239:1164.
120. O'Rians, Copenhagen HS, Colman JS. The effect of zinc sulphate on the healing of incised wounds in rats. *British J Plastic Surg* 1968;21:240.
121. Sazawal S, Jalla S, Majumder S et al. Effect of zinc supplementation on cell mediated immunity and lymphocyte subsets in preschool children. *Indian Pediatrics* 1997;34:589-597.
122. Walravens, P.A. & Hambidge, K.M. Growth of infants fed a zinc supplemented formula. *Am. J. Clin. Nutr.* 1976;29: 1114-1121.
123. Chvapil, M. New aspects in the biological role of zinc: a stabilizer of macromolecules and biological membranes. *Life Sci.* 1973;13:1041-1049.
124. Chvapil, M., Ryan, J. N., Zukiski, C.F. Effect of zinc on lipid peroxidation in liver microsomes and mitochondria. *Proc. Soc. Exp. Biol. Med.* 1972;141:150-153.
125. Chvapil, M. Effect of zinc on cells and biomembranes. *Med. Clin. North Am.* 1976;60:799-812.
126. Hsu, J. M. Biochemistry and metabolism of zinc. In Z.A. Karcioğlu, C.C. Thomas, Springfield, 1980;66-93.
127. Burch, R.E., Sullivan, J.F. Clinical and nutritional aspects of zinc deficiency and excess. *Med. Clin. North Am.* 1976;60:675-685.

128. Prasad, A.S. Zinc deficiency in man. In K.M. Hambidge and B.L. Nichols (ed.). Zinc and copper in clinical medicine. Spectrum, New York, 1978;1-14.
129. Hambidge, K.M., Neldner, K.H., Walravens, P. A., Weston, W. L., Silverman, A., Sabol, J.L., Brown, R.M. Zinc and acrodermatitis enteropathica. In K.M. Hambidge and B.L. Nichols (ed.). Zinc and copper in clinical medicine. Spectrum, New York, 1978;81-98.
130. Williams, R.B., Russell, R.M., Dutta, S.K., Giovetti, A.C. Alcoholic pancreatitis: patients at high risk of acute zinc deficiency. *Am. J. Med.* 1979;66: 889-893.
131. Rodin, A.E., Goldman, A.S. Autopsy findings in acrodermatitis enteropathica. *Am. J. Clin. Pathol.* 1969;51: 315-322.
132. Julius, R., Schulkind, M., Sprinkle, T., Tennert, O. Acrodermatitis enteropathica with immune deficiency. *J. Pediatr.* 1973;83:1007-1011.
133. Anderson, E., Basse, A., Brummerstedt, E., Flagstsd, T. Zinc and the immune system in cattle. *Lancet* 1:1973;839-840.
134. Businco, L., Menghi, A., Rossi, P., D'Amelio, R., Galli, E. Zinc-dependent chemotactic effect in an infant with acrodermatitis. *Arch. Dis. Child.* 1980;55:966-968.
135. Fernandes, G., Nair, M., Onoe, K., Tanaka, T., Floyd, R., Good, R. A. Impairment of cell-mediated immunity functions by dietary zinc deficiency in mice. *Proc. Natl. Acad. Sci.* 1979;76: 457-461.

136. Luecke, R. W., Simonel, C. E., Fraker, P. J. the effect of restricted dietary intake on the antibody-mediated response of the zinc deficient A/J mouse. *J. Nutr.* 1978;108: 881-887.
137. Phillips, J. L. Specific binding of zinc transferrin to human lymphocytes. *Biochem. Biophys. Res. Commun.* 1976;72:634-639.
138. Williams, R. O., Loeb, L.A. Zinc requirement for DNA replication in stimulated human lymphocytes. *J. Cell. Biol.* 1973;58:594-601.
139. Duchateau, J., Delespesse, G., Vereecke, P. Influence of oral zinc supplementation on the lymphocyte response to mitogens of normal subjects. *Am. J. Clin. Nutr.* 1981; 34:88-93.
140. Duchateau, J., Delepesse, G., Vrijens, R., Collet, H. Benefician effects of oral zinc supplementation on the immune response of old people. *Am. J. Med.* 1981;70:1001-1004.
141. Kirchner, H., Ruhl, H. Stimulation of human peripheral lymphocytes by  $Zn^{2+}$  in vitro. *Exp. Cell Res.* 1970;61:229-230.
142. Ruhl, H., Kirchner, H., Bochert, G. Kinetis of the  $Zn^{2+}$  stimulation of humans peripheral lymphocytes in vitro. *Proc. Soc. Exp. Biol. Med.* 1971;137:1089-1092.
143. Allen, J. I., Kay, N. E., McClain, C. J. Severe zinc deficiency in humans: association with a reversible T-lymphocytes dysfunction. *Ann. Intern. Med.* 1981;95:154-157.



144. Pekarek, R.S., Sandstead, H. H., Jacob, R.A., Barcome, D.F. Abdominal cellular immune responses during acquired zinc deficiency. *Am. J. Clin. Nutr.* 1979;32:1466-1471.
145. Beach, R.S., Gershwin, M. E., Hurley, L.S. Nutritional factors and autoimmunity. I. Immunopathology of zinc deprivation in New Zealand mice. *J. Immunol.* 1981;126: 1999-2006.
146. Zanzonico, P., Fernandes, G., Good, R.A. the differential sensitivity of T-cell and B-cell mitogenesis to in vitro zinc deficiency. *Cell. Immunol.* 1981;60:203-211.
147. Chandra, R.K., Au, B. single nutrient deficiency and cell-mediated immune response. I. Zinc. *Am. J. Clin. Nutr.* 1980;33:736-738.
148. Fraker, P. J., DePasquale-Jardieu, P., Zwickl, C.M., Luecke, R. W. Regeneration of T=cell helper function in zinc-deficient adult mice. *Proc. Natl. Acad. Sci.* 1978;75:5660-5664.
149. Fraker, P. J., Haas, S.M., Luecke, R.W. Effect of zinc deficiency on the immune response of the young adult A/J mouse. *J. Nutr.* 1977;107:1889-1895.
150. Good, R.A., West, A., Fernandes, G. Nutritional modulation of immune responses. *Fed. Proc.* 1980;39:3098-3104.
151. Rabinovitch, M., Destefano, M.J. Macrophase spreading in vitro. II. Manganese and other metals as inducers or as Co-factors for induced spreading. *Exp. Cell Res.* 1973;79:423-430.
152. Snyder, S.L., Walker, R.I. Inhibition of lethality in endotoxin-challenged mice treated with zinc chloride. *Infect. Immun.* 1976;13:998-1000.

153. Avigad, L.S., Bernheimer, A. W. Inhibition by zinc of hemolysis induced by bacterial and other cytolytic agents. *Infect. Immun.* 1976;13:1378-1381.
154. Jackson, MJ. Physiology of zinc: General Aspects. In: *Zinc in human biology*. London: C.F. Mills ed. Springer-Verlag, 1989: 1-14.
155. Lowe NN, Bremner I, Jackson NJ. Plasma  $^{65}\text{Zn}$  kinetics in the rat. *Br. Nutr* 1991; 65: 445-55.
156. Torre M, Rodriguez AR, Saura Calixto F. Effects of dietary fiber and phytic acid on mineral availability. *Crit Rev Food Sci Nutr* 1991; 30: 1-22.
157. Lee HH, Prasad AS, Brewer GJ, Owyang C. Zinc absorption in human small intestine. *Am J Physiol* 1989; 256: G87-G91.
158. Matseshe JW, Phillips SF, Malagelada JR, McCall JT. Recovery of dietary iron and zinc from proximal intestine of healthy man studies of different meals and supplements. *Am J Clin Nutr* 1980; 33: 1946-53.
159. Cousins RJ. Absorption, transport and hepatic metabolism of copper and zinc : special reference to metallothionein and ceruloplasmin. *Physiol Rev* 1985; 65: 238-309.
160. Conrad ME, Umbreit JN, Moore EG. A role for mucin in the absorption of inorganic iron and other metal cations. A study in rats. *Gastroenterol* 1991;100: 129-36.
161. Aggett PJ. Acrodermatitis enteropathica. *J Inher Metab Dis* 1983; 6(suppl 1): 39-43.

162. Hempe JM, Cousins RJ. Cysteine rich intestinal protein binds zinc during transmucosal transport. *Proc Natl Acad Sci USA* 1991; 88:9671-4.
163. Crofton RW, Gvozdanovic D, Gvozdanovic S et al. Inorganic zinc and the intestinal absorption of ferrous iron. *Am J Clin Nutr* 1989; 50: 141-4.
164. Fuller NJ, Bates CJ, Evans PH, Lucas A. High folate intakes related to zinc status in preterm infants. *Eur J Pediatr* 1992; 151: 51-3.
165. Coleman JE. Zinc proteins: enzymes, storage proteins. Transcription Factors, and Replication Proteins. *Annu Rev Biochem* 1992; 61:897-946.
166. Cousins RJ, Leinart AS. Tissue specific regulation of zinc metabolism and metallothionein genes by interleukin 1. *FASEB J* 1988; 2: 2884-90.
167. Taylor CM, Bacon JR, Aggett PJ, Bremner I. Homeostatic regulation of zinc absorption and endogenous losses in zinc-deprived men. *Am J Clin Nutr* 1991; 53: 755-63.
168. Hempe JM, Carlson JM, Cousins RJ. Intestinal metallothionein gene expression and zinc absorption in rats are zinc responsive but refractory to dexamethasone and interleukin 1 alpha. *J Nutr* 1991; 121: 1389-96.
169. Hambidge KM. Mild zinc deficiency in human subjects. In: Mills CF, ed. *Zinc in human biology*. New York: Springer Verlag, 1989:281-96.
170. Aggett PJ. Severe zinc deficiency. In: Mills CF, ed. *Zinc in human biology*. London: Springer-Verlag, 1989:259-80.



171. Crofton RW, Aggett PJ, Gvozdanovic S, Gvozdanovic D, Mowat NA, Brunt PW. Zinc metabolism in celiac disease. *Am J Clin Nutr* 1990;52:379-82.
172. Aggett PJ, Favier A. The assessment of zinc status. *Int J Vit Nutr Res*. 1993;63:301-7.
173. Golden MHN. The diagnosis of zinc deficiency. In: *Zinc in human biology*. Mills CF, ed. London: Springer-Verlag, 1989:323-33.
174. Altigani M, Murphy JF, Gray OP. Plasma zinc concentration and catchup growth in preterm infants. *Acta Paediatr Scand* 1989; suppl 357:20-23.
175. Butrimovitz GP, Purdy WC. Resolution of age dependent reference intervals: polynomial regression methodology with applicability to plasma zinc levels in childhood population. *Clinical Biochemistry* 1979;12:33-6.
176. Gibson RS, Dewolfe MS. Changes in serum zinc concentrations of some Canadian term and low birth weight infants from birth to six months. *Acta Paediatr Scand* 1981;70:497-500.
177. Simmer K, Khanum S, Carlsson L, thompson RP. Nutritional rehabilitation in Bangladesh- the importance of zinc. *Am J Clin Nutr* 1988;47:1036-40.
178. Meftah S, Prasad AS, Lee DY, Brewer GJ. Ecto 5' nucleotidase (5'NT) as a sensitive indicator of human zinc deficiency. *J Lab Clin Med* 1991;118:309-16.
179. Prasad A, Meftah S, Abdullah J, et al. Serum thymulin in human zinc deficiency. *J Clin Invest* 1988; 82:1202-10.

180. Grider A, Bailey L, Cousins R. (1990) Erythrocyte metaallothionein as an index of zinc status in humans. *Proc Natl Acad Sci* 1990;87:1269-12.
181. Aggett PJ, Atherton DJ, More J, et al. Symptomatic zinc deficiency in a breast fed preterm infant. *Arch Dis Child* 1980;58:547-50.
182. Schlesinger L, Arevalo M, Arredondo S, Diaz M, Lonnerdal B, Stekel A. Effect of a zinc-fortified formula on immunocompetence and growth of malnourished infants. *Am J Clin Nutr* 1992;56:491-8.
183. Walravens PA, Chakar A, Mokni R, Denise J, Lemonnier D. zinc supplements in breastfed infants. *Lancet* 1992;340: 683-5.
184. Walravens PA, Hambidge KM, Koeplet DM. Zinc supplementation in infants with a nutritional pattern of failure to thrive a double blind controlled study. *Pediatrics* 1989; 83:532-8.
185. Golden BE, Golden MH. Effect of zinc on lean tissue synthesis during recovery from malnutrition. *Eur J Clin Nutr* 1992;46:697-706.
186. Bates CJ, Evans PH, Dardenne M, et al. A trial of zinc supplementation in young rural Gambian children. *Br J Nutr* 1993;69:243-55.
187. Cagnetti, P., Cigna-Rossi, L., Clemente, G., Santaroni, G., "Mercury pathways to man in-vivo content of the population of the Mt. Amiata area", *Proceeding of the Effects of Environmental Pollution, Commission of the European Communities Reports EUR 5360*, 1975; 1451-1460.
188. Wiadrowskia, B., Syrowatka, T., *Rocz. Panstw. Hig.* 1983;34: 87-94.

189. Greenwood MR, Dhahir P, Clarkson TW. *J Anal Toxicol* 1977; 265-269.
190. Kalman, S.M., *Anal. Toxicol.* 1977;1: 277-281.
191. Campbell, B.C., Baird, A.W., *Brit. J. Intern. Med.* 1977;34:298-304.
192. Kopito, L., Briley, A.M., Shwachman, H., *J. Anrer. Med. Assoc.* 1969;209:243-248.
193. Kopito, L., Byers, R.K., Shwachman, H., *J. New England J. Med.* 1967;276: 949-953.
194. Versieck J, Cornelis R. *Anal Chem Acta* 1980;116:217-254.
195. Marzulli, F.N., Watlington, P.M., Maibach, H.I., *Current problems dermatol.* 1978;7: 196-204.
196. Niculescu, T., Dumitru, R., Botha, V., Aalexandrescu, R., Malolescu, N., *Brit. J. Inter. Med.* 1983;40: 67-70.
197. Clayton, E., Wooller, K.K., *Ieee Trans. Nucl. Sci* 1983; NS-30:1326-1328.
198. Fergusson, J.E., Hibbard, K.A., Ting, R.L.H., *Environ. Pollut.* 1981;2:235-248.
199. Benguera, J.L., Burguera, M., Rondon, C.E., Rivas, C., Burguera, J.A., Alarcon, O.M., *J. Trace Elem. Electrolytes Health Dis.* 1987;1: 21-26.
200. Weber, C.W., Nelson, G.W., De Vaquera, M.V., Pearson, P.B., *Nutr. Rep. Internat.* 1984;30:1009-1018.
201. Paciga, J.J., Chattopadhyay, A., Jervis, R.E., *Proc. 2nd Int. Conf. on Nuclear Methods in Environmental Research* 1974; 286-300.



202. Jervis, R.E., Tiefenbach, B., Chattopadhyay, A., *Radioanal. Chem.* 1977;37:751-760.
203. Chattopadhyay, A., Roberts, T.M., Jervis, R.E., *Arch. Environ. Health* 1977;30:226-236.
204. Wibowo, A.A.E., Brunekreef, B., Lebret, E., Pieters, H., *Intern. Arch. Occup. Environ. Med.* 1980;46: 275-280.
205. Schroeder, H.A., Frost, D.V., Balassa, J.J., *L. Chron. Dis.* 1970;23:227-243.
206. Iyenger, G.V., Gopal-Ayengar, A.R., *Ambio* 1988;17:31-35.
207. Thimaya, S., Ganapathy, S.N., *Sci. Total Environ.* 1982;24:41-49.
208. Salbe, A.D., Levander, O.A., *J. Nutr.* 1990;120:200-206.
209. Limic N, Vazkovic V. *Bull Environ Centam Toxicol.* 1986;37: 925-930.
210. Limic, N., Valkovic, V., *Nucl. Instrum. Methods Phys. Res.* 1987;B22:163-165.
211. Salem, H., Katz, S.A., *Sci. total Environ.* 1989;86:59-64.
212. Strain WH, Steadman LT, Lankau CA et al. Analysis of zinc levels in hair for the diagnosis of zinc deficiency in man. *J Lab Clin Med* 1966;68:244-249.
213. Moynahan EJ. Zinc deficiency and disturbances of mood and behaviour. *Lancet*;9:1976.
214. Tupasi TE, Mangubat NV, Sunico Ma ES et al. Malnutrition in acute respiratory tract infection in Filipins children. *Rev Infect Dis* 1990;12:1047-1054.

215. Graham GG, Crud HM, MacLean WC et al. Determinants of growth among poor children: nutrient intake-achived growth relationship. *Am J Clin Nutr* 1981;34:539-54.
216. Halstead JA, Ronaghy P, Abadi M et al. Zinc deficiency in man; the Shiraj experiment. *Am J Med* 1972;53: 277.
217. Sandstead HHKP, Vokhactu and N Solomons. Conditional zinc deficiency In: Trace elements in human health and disease. Vol 1. Zinc and copper edited by A.S.Prasad. New York Academic Press; 1976, p33.
218. World Bank. Enriching lives. Overcoming vitamin and mineral malnutrition in developing countries. World Bank. Total GDP table 1995. World development indicators 1997 (CD-Rom). International Bank for Reconstruction and development/ World bank, Washington DC, 1997.
219. Child Nutrition Survey of Bangladesh 1995-96. Bangladesh Bureau of Statistics, Statistics division, Ministry of Planning, 1997.
220. WHO Programme for control of Acute respiratory infections: ARI 20, Geneva 1991.
221. Gomez F, Ramos Galvan, Frank S et al. *J Trop Pediat* 1956; 2: p77.
222. Management of severe malnutrition: a mannual for physicians and senior health workers. WHO Geneva 1999.
223. Lukasaki HC, Johnson PE, Bolon cheek WW et al. Assessment of fat free mass using bioelectric impedance measurement of human body. *Am J Clin Nutr* 1985; 41: 810-817.

224. Fjeld CR, Freundt- Thurne J and Shoeller DA. Total body water measured by  $^{18}\text{O}$  dilution and bioelectric impedance in well and malnourished children. *Pediatr Res* 1990;27(1): 98-102.
225. Fomon SJ, Haschke F, Ziegler et al. Body composition of reference children from birth to age 10 year. *Am J Clin Nutr* 1982;35:1169-75.
226. Report IAEA, Vienna 1978
227. Analytic method for atomic absorption spectrophotometry- Perkin Elemer revised edition 1982. Nor Walk. USA, P-5-15.
228. Hilder brand DC. White DH. *Clin Chem* 1974; 20: 148-151.
229. Hambidge DM. *Amer J Clin Nutr*- 1982; 36:943-949.
230. Rivlin RS. *Amer J Med* 1983; 75: 489-493.
231. Barette SJ. *Amer J Med Assoc* 1985;254: 1041-2045.
232. Katz SA. Wood JD. *Chem. Internate* 1980;6:12-15.
233. Chatt A. Saddey M. De Silva KN. Et. al. Human scalp hair as an epidemiological monitor of environmental exposure to elemental pollutants, Report TECDOC 330. Interventional Atomic Energy Agency, Vienna (1983).
234. Katz SA, Chatt A. Hair analysis: Application in the Biomedical and Environmental Senners, VCH publishers New York 1988.
235. Dorea JG, Paine PA. Hair zinc in children. Its uses, limitation and relationship to plasma zinc and anthropometry. *Clinical Nutrition* 1985;39:389-398.



236. Iyengar V, Woltiez J. Clin Chem 1988;34:474-481.
237. Roy SK, Tomkin AM, Akhramuzzaman SM et al. Randomized control trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhoea. Arch Dis Child 1997;77:196-200.
238. Islam S, Malek MH, Akbar MS et al. Classification of malnutrition according to fat content of body of children measured by bioelectrode impedance analyzer, Bangladesh Journal Nutrition 1991;4:73-82.
239. Halsted JA and Smith JC Jr. Plasma zinc in health and disease. Lancet 1:1970, 322:
240. Katz SA, Chatt A. Application of hair as an indicator for trace element exposure in man. A review. NAH RES-22, IAEA, Vienna 1994, pp 1-13.
241. Jackson MJ. Physiology of zinc: General aspects. In: CF Mills editor. Zinc in human biology. Springer verlog London 1989;1-14.
242. Conrad ME, Umbruit JN, Moore EG. A role for mucin in the absorption of inorganic iron and other metal cations. A study in rats. Gastroenterol 1991;100:129-36.
243. Foley BS, Johnson A, Hackley B et al. Zinc contents of human platelets. Biol Med 1968;128:265.
244. Kollmer WE, Berg D. The influence of zinc deficiency on the resorption, kinetics and amount of zinc in hair of the rat. In: Anke M, Baumann W, Braunlich H, Bruckner C, Groppel B (eds) Proceedings of the Fifth Symposium on Trace

Elements, Jena 14-17 July 1986. WEB Kongreß und Werbedruck, Oberlungwitz pp 502-507.

245. Hurry VJ, Gibson RS. The zinc, copper, and manganese status of children with malabsorption syndromes and inborn errors of metabolism. *Biol Trace Elem Res* 1982;4:157-173.
246. Bradfield RB, Yee T, Baertl JM. Hair zinc levels of Andean Indian children during protein-calorie malnutrition. *Am J Clin Nutr* 1969;22:1349-1353.
247. Erten J, Arcasoy A, Cavdar AO, Cin S. Hair zinc levels in healthy and malnourished children. *Am J Clin Nutr* 1978;31:1172-1174.
248. Hambidge KM, Walravens PA, Kumar V, Tuchinda C. Chromium, zinc, manganese, copper, nickel, iron and cadmium concentrations in the hair of residents of Chandigarh, India and Bangkok, Thailand. In: Hemphill DD (ed) *Trace substances in environmental health. VIII. University of Missouri, Columbia* 1974;39-44.
249. Bradfield RB, Poresky B, Cordano A. Hair diameter changes during protein-calorie malnutrition. In: Masta J (ed) *Proceeding of the 2nd Pacific Science Congress, Tokyo, Japan* 1966;319-320.
250. Hsu JM, Anthony WL. Zinc – an essential element for hair growth. In: Kirchgessner M (ed) *Trace element metabolism in man and animals. 3. Proceedings of the 3rd International Symposium, July 1977. Institut für Ernährungsphysiologie, Technische Universität, Munich, Freising-Weihenstephan* 1978;222-225.

251. Lombeck I, Wilhelm M, Hafner D, et al. Hair zinc of young children from rural and urban areas in North Rhine-Westphalia, Federal Republic of Germany. *Eur J Pediatr* 1988;147:179-183.
252. Van Wouwe JP, de Wolff FA, van Gelderen HH. Zinc in hair and urine of paediatric patients. *Clin Chim Acta* 1986;155:77-82.
253. Bondestam M, Foucard T, Gebri-Mednedin M Subclinical trace elements deficiency in children with undue susceptibility to infections. *Acta Paediatr Scand* 1985;74:515-520.
254. Taneja DP. Observations of serum zinc in patients of pulmonary tuberculosis. *J Indian Med Assoc* 1990;88(10):280-281.
255. Castillo-Duran C, Heresi G, Fisberg M et al. Controlled trial of zinc supplementation during recovery from malnutrition: effects on growth and immune function. *Am J Clin Nutr* 1987;45:602-8.
256. Gibson RS, DeWolfe MS. Copper, zinc manganese, vanadium and iodine content of hair from 38 Canadian neonates. *Ped Res* 1979;13:959.
257. Bergmann KE, Bergmann RI. The role of zinc in paediatric nutrition. In Fiorella Balli ed. *Nutrition problems in childhood*. Padeva Piccini Med Books. 1979.
258. Gentle P, Trentalange MJ, Coleman M. The relationship of hair zinc concentration to height, weight, age and size in normal population. *Ped Res* 1981;15:123.



259. Petering HG, Yeagger DW and Witherup SO. Trace metal content of hair zinc and copper; content of human hair in relation to age and sex. *Arch Environ. Health* 1971;23:202.
260. Moser PB, Kreb NK, Blyller F. Zinc concentrations and estimated zinc intakes of functionally delayed normal sized and small for age children. *Nutr Res* 1982;2:585.
261. Hambidge KM, Chavez MM, Brown RM et al. Zinc nutritional status of young middle income children and effects of consuming zinc fortified breakfast cereals. *Am J Clin Nutr* 1979;32:2532.
262. Erten J, Arcasoy A, Cavdar AD. Hair zinc levels in healthy and malnourished children. *Am J Clin Nutr* 1978;31:1172.
263. Klevay LM. Hairs as a biopsy material. Assessment of zinc nutriture. *Am J Clin Nutr* 1970;23:284-289.
264. Chase HP, Hambdridge KM, Barnette ST et al. Low vitamin A and zinc concentrations in Maxican-American migrant children with growth retardation. *Am J Clin Nutr* 1980;33:2346.
265. Eminians JG, Reinhold GA, Kfoury GH et al. Zinc nutrition in children in Fars Province of Iran. *Am J Clin Nutr* 1967;20:934.
266. Buzina R, Jusie M, Sapuner J et al. Zinc nutrition and taste acquity in school children with impaired growth. *Am J Clin Nutr* 1980;33:2262.
267. Sandstead HH. Zinc in human nutrition. In: Branner F, Coburn JW eds. *Disorders of meneral metabolism. Trace minerals*. New York NY. Academic press 1981.

268. O'Leary MJ, Mata IJ, Hegarty PVG. Hair zinc level in rural Costarican infants and preschool children. *Am J Clin Nutr* 1980;33:2194.
269. Griger JL, Higgins MM, Abbernathy RP et al. Nutritional status of adolescent girls in regard to zinc, copper and iron. *Am J Clin Nutr* 1978;31:269-306.
270. Bai KI, Sabbiha DV, Rao SK. Assessment of nutritional status of preschool children: a comparison of methods. *J Trop pediatr*. 1977;23(5): 224-5.
271. Gumeri M, Jutsum P. The Gomez classification. Time for a change ? *Bull WHO* 1980; 50(5):773-77.
272. Ramchandran K, Parmar BS, Jain JK et al. Limitations of film strip and Bangle test for identification of malnourished children. *The Am J Clin Nutr* 1978;31(8):1469-72.
273. Agarwal CM, Gupta SD, Jain JP, Joshi S. Age independent indices of malnutrition. *Indian Pediatr* 1980;17(8):683-7.
274. McMurry DN, Loomis SA, Casazza LJ et al. Development of impaired cell-mediated immunity in mild and moderate malnutrition. *Am J Clin Nutr* 1981;34:68-77.
275. Chandra RK. Nutrition and immunity: lesions from the past and new insights into the future. *Am J Clin Nutr* 1991;53:1087-1101.
276. Fraker PJ, Jardieu P and Cook J. Zinc deficiency and immunological function. *Arch Dermatol* 1987;123:1699-1701.

277. Roy SK, Tomkins AM, Haider R et al. Impact of zinc supplimentation in Bangladeshi children with acut3 diarrhoea. *Eur J Clin Nutr* 1999;53:529-534.
278. Neumann CG, Lawlor GJ, Stiehlm ER et al. Immunologic responses in malnourished children. *Am J Clin Nutr* 1975;28:89-104.
279. Mims CA. The pathogenesis of infectious disease. Host and microbial factors influencing susceptibility. Academic Press, London 1991;pp 270-295.
280. Sempertegui F, Estrella B, Correa E et al. Effects of short-term zinc supplementation on cellular immunity respiratory symptoms and growth of malnourished Equadorian children. *Eur J Clin Nutr* 1996;50:42-46.
281. Chandra RK. Immunology of nutritional disorders. London: Edward Arnold, 1980.
282. Gross RL, Osdin N, Fong L et al. In vitro restoration by levamisole of mitogen responsiveness in zinc-deprived rats. *Am J Clin Nutr* 1979;32:1267-71.
283. Alford RH. Metal cation requirements for phytohemagglutinin-induced transformation of human peripheral blood lymphocytes. *J Immunol* 1970;104:698-703.
284. Chvapil M, Zukoski CF, Hattler BG et al. In: Brewer J, Prasad AS, eds. Zinc metabolism; Current aspects in health and diseases. New York: Academic Press 1977;269-81.
285. Kielmann AA, Uberoi IS, Chandra RK et al. The effect of nutritional status on immune capacity and immune responses in preschool children in a rural community in India. *Bull World Health Organ* 1976;54:477-483.



286. Beisel WR. Single nutrients and immunity. *Am J Clin Nutr* 1982;35:417-468.
287. Sazawal S, Black RE, Bhan MK et al. Effect of zinc supplementation during acute diarrhea on duration and severity of the episode-A community based, double-blind controlled trial. *N Eng J Med* 1995;333:839-844.
288. Fraker PJ, De Pasquale-Jardieu P, Zwick CM et al. Regeneration of T cell helper function in zinc deficient adult mice. *Proc Natl Acad Sci USA* 1979;76:457-461.
289. Fraker PJ, Zwicki CM, Luecke RW et al. Delayed type hypersensitivity in zinc-deficient adult mice: Impairment and restoration of responsivity to dinitrofluorobenzene. *J Nutr* 1982;112:309-313.
290. Baqui AH, Black RE, Sack RB et al. Malnutrition, cell-mediated immune deficiency, and diarrhea: A community based longitudinal study in rural Bangladeshi children. *Am J Epidemiol* 1993;137:355-365.
291. Black RE, Lanata CF, Lazo F. Delayed cutaneous hypersensitivity: Epidemiologic factors affecting and usefulness in predicting diarrheal incidence in young Peruvian Children. *Pediatr Infect Dis J* 1989;8:210-215.
292. Chandra RK, Au B. Single nutrient deficiency and cell-mediated immune responses. Zinc. *Am J Clin Nutr* 1980;33:735-738.
293. Kay RG, Tasman-Jones C. Zinc deficiency and intravenous feeding. *Lancet* 1975;2:605-606.
294. Coello-Ramirez P, Diaz-Bensussen S. Zinc deficiency in malnutrition. In: *Pediatric Nutrition*. Ed. Lifshitz F. New York, Dekker 1982; pp 197-207.

295. Luecke RM, Simonel CE, Fraker PJ. The effect of restricted dietary intake on the antibody mediated response of the zinc deficient A/J mouse. *J Nutr* 1978;108:881-887.
296. Frost P, Chen JC, Rabbani I et al. The effect of immune deficiency on the immune response. In: *Zinc metabolism: Current Aspects in Health and Disease*. Eds. New York, Brewer GJ, Prasad AS, Alan R, Liss 1977;143-150.
297. Wazewska-Czyzewska M, Wezierska-Gadek J, Legutko L. Immunostimulatory effect of zinc in patients with acute lymphoblastic leukemia. *Folia Hematol* 1978;105:727-732.
298. Rao KMK, Schwartz SA, Good RA. Age dependant effects of zinc on the transformation response of human lymphocytes to mitogen. *Cell Immunol* 1979;42:270-278.
299. Muger JW. The lymphorecicular system in kwashiorkor. *J Pathol* 1971;105:105-108.
300. Schloen LH, Fernandez G, Garafalo JA et al. Nutrition immunity and cancer-A review: Part II: Zinc, immune function and cancer. *Clin Bull* 1979;9:63-75.
301. Hadden JW. The treatment of zinc deficiency is an immunotherapy. *Int J Immunopharmac* 1995;17:697-701.
302. Martin AA, Moor J, Collin C et al. Infectious disease Surveillance during emergency relief of Bhutanese refugees in Nepal. *JAMA* 1994;272(5):377-81.

303. Ferguson EL, Gibson RS, Obisaw CO et al. The zinc nutriture of preschool children living in two African Countries, Community and International Nutrition American Institute of Nutrition. *J Nutr* 1993;1239:1487-1496.
304. Michaelsson C, Vahiquist A, Juhlin B et al. *Scand J Clin Lab Invest* 1976;36:827-832.
305. Bhaskaram P. Zinc deficiency. *Indian Paediatrics* 1995;32:1153-1156.
306. Sundaresan PR, Cope EC, Smith JC. *J Nutr* 1977;107:2189-2197.
307. Malek MA, Khan A. Kinetics and mechanism of enzyme catalysed reactions. In: A Mannan, A Rahman Eds. *Zinc in Nutrition*. Bangladesh Agriculture Research Council. Eden Press. Dhaka 1988;209-226.
308. Cohen JJ, Schwartz WB. Evaluation of acid based equilibrium in pulmonary insufficiency. An approach to and diagnosis dilemma. *Am J Med* 1966;41:162.
309. Prasad AS, Oberleas D, Miller ER et al. Biochemical effects of zinc deficiency: changes in activities of zinc dependant enzymes and ribonucleic acid deoxyribonucleic acid content of tissues. *J Lab Clin Med* 1971;77:144.
310. Richard E, Behrman Victor C, Vaughan Waldo E Nelson eds. *Nelson Text book of Paediatrics*. Thirteen edition. WB Saunders company, Philadelphia 1987; 185-189.
311. Kawakami Y, Kondo T, Kishi F et al. Faculty of Medicine, University of Hokkaido, *Medical Bulletin* 1978;52(6):327-35.



312. Kirchgessner M, Stadler AE, Roth HP. Carbonic anhydrase activity and erythrocytic count in the blood of zinc deficient rats. *Bioinorg Chem* 1975; 5(1):33-8.
313. Mustafa MG. Effects of divalent metal ions on alveolar macrophage membrane ATP activity. *J Lab Clin Med* 1971;77:563-71.
314. Stankova L. Regulations of some functions of granulocytes by zinc of the prostatic fluid and prostate tissues. *J Lab Clin Med* 1997;88:640-8.
315. Chvapil M. Inhibition of some functions of PMN's by in vitro zinc. *J Lab Clin Med* 1977;89:135-46.
316. Chandra RK. Excessive intake of zinc impairs immune responses *JAMA* 1984;252:1443-6.
317. Schlesinger L, Arebalo M, Arredondo S et al. Zinc supplementation impairs monocyte function. *Acta Paediatr* 1993;82:734-8.
318. Sugarman B. Zinc and infection. *Rev Infect Dis* 1983;5:137-47.
319. Powanda MC, Cockerell GL, Pekarek RS. Amino acid and zinc movement in relation to protein synthesis early in inflammation. *Am J Physiol* 1973;225:399-401.
320. Pekarek RS, Wannemacher RW Jr, Beisel WR. The effect of leukocytic endogenous mediator (LEM) on the tissue distribution of zinc and iron. *Proc Soc Exp Biol Med* 1972;140:685-8.

321. Coyle P, Philcox JC, Rofe AM. Corticosterone enhances the zinc and interleukin-6-mediated induction of metallothionein in cultured rat hepatocytes. *J Nutr* 1993;123:1464-70.
322. Klasing KC. Effect of inflammatory agents and interleukin 1 on iron and zinc metabolism. *Am J Physiol* 1984;247:R901-4.
323. Morimoto A, Sakara Y, Watanabe T et al. Characteristics of fever and acute-phase response induced in rabbits by IL-1 and TNF. *Am J Physiol* 1989;256:R35-41.
324. Murata R, Soda S, Yamamoto A et al. The effect of zinc on the production of various toxins of *Clostridium perfringens*. *Japan J Med Sci Biol* 1969;22:133-48.
325. Jeasen SE, Fecycz IT, Campbell JN. Nutritional factors controlling exocellular protease production by *pseudomonas aeruginosa*. *J Bacteriol* 1980;144:844-7.
326. Sugarman B. Effect of heavy metals on bacterial adherence. *J Med Microbiol* 1980;13:351-4.
327. Biesel WR. Trace elements in infectious processes. *Med Clin North Am* 1976;60:831-49.
328. Smith IF, Taiwo O, Golden MHN. Plant protein rehabilitation and iron supplementation of the protein-energy malnourished child. *Eur J Clin Nutr* 1989;11:763-8.
329. Cordano A, Baertl JM, Graham GG. Copper deficiency in infancy. *Pediatrics* 1964;34:324-36.

330. Hemalatha P, Bhaskaram P, Khan MM. Role of zinc supplementation in the rehabilitation of severely malnourished children. *Eur J Clin Nutr* 1993;47:395-9.
331. Vyas D. Thymic factor activity, lymphocyte stimulation response and antibody forming cells in copper deficiency. *Nutr Res* 1983;3:343-50.
332. Babu U, Failla ML. Superoxide dismutase activity and blastogenic response of lymphocytes from copper-deficient rats fed diets containing fructose or cornstarch. *Nutr Res* 1989;9:272-82.
333. Botash AS, Nasca J, Dubowy R et al. Zinc induced copper deficiency in an infant. *Am J Dis Child* 1992;146:709-11.
334. Sandstrom B, Cederblad A, Lindblad BS et al. Acrodermatitis enteropathica, zinc metabolism, copper status, and immune function. *Arch Pediatr Adolesc Med* 1994;148:980-5.
335. Honkanen V, Lamberg-Allardt CH, Vesterinen MK et al. Plasma zinc and copper concentrations in rheumatoid arthritis: influence of dietary factors and disease activity. *Am J Clin Nutr* 1991;54:1082-6.
336. Porter KG, McMaster D, Elmes ME et al. Anaemia and low serum-copper during zinc therapy. *Lancet* 1977;2:774.
337. Prasad AS, Brewer GJ, Schoomarker EB et al. Hypocupremia induced by zinc therapy in adults. *JAMA* 1978;240:2166-8.
338. Festa MD, Anderson HL, Dowdy RP et al. Effect of zinc intake on copper excretion and retention in men. *Am J Clin Nutr* 1985;41:285-92.



339. Ronaghy HA, Reinhold JG, Mahloudji M et al. Zinc supplementation of malnourished schoolboys in Iran: increased growth and other effects. *Am J Clin Nutr* 1974;27:112-21.
340. Sachdev HPS, Mittal NK, Mittal SK et al. A controlled trial on utility of oral zinc supplementation in acute dehydrating diarrhoea in infants. *J Pediatr Gastroenterol Nutr* 1988;7:877-81.
341. Nakamura T, Nishiyama S, Futagoishi Suginoara Y et al. Mild to moderate zinc deficiency in short children: effect of zinc supplementation on linear growth velocity. *J Pediatr* 1993;123:65-9.
342. Shrivastava SP, Roy AK, Jana UK. Zinc supplementation in protein energy malnutrition. *Indian Pediatr* 1993;30:779-82.
343. Doherty CP, Sarkar MAK, Shakur MS et al. Zinc and rehabilitation from severe protein-energy malnutrition: higher-dose regimens are associated with increased mortality. *Am J Clin Nutr* 1998;68:742-8.
344. Riordan JF. Biochemistry of zinc. *Med Clin North Am* 1976;60:661-674.
345. Vallee BL. Biochemistry, physiology and pathology of zinc. *Physiol Rev* 1959;39:443-490.
346. Falchuk KH, Fawcett, Vallee BL. Role of zinc in cell division of *euglena gracilis*. *J Cell Sci* 1975;17:57-78.
347. Torriani A. Alkaline phosphatase subunits and their dimerization in vivo. *J Bacteriol* 1968;96:1200-1207.

348. Feeney RE, Lightbody HD, Garibaldi JA. Zinc as an essential element for growth and subtilin formation by bacillus subtilis. Arch Biochem 1947;15:13-17.
349. Collier HB. Binding of  $Zn^{2+}$  by buffers. Clin Chem 1979;25:495-496.
350. Chvapil M, Stankova L, Zukoski C et al. Inhibition of some functions of polymorphonuclear leukocytes by in vitro zinc. J Lab Clin Med 1977;89:135-146.
351. Sato H, Yamakawa Y, Ito A et al. Effect of zinc and calcium ions on the production of alpha-toxin and proteases by clostridium perfringens. Infect Immun 1978;20:325-333.
352. Soda S, Sato H, Murata R. The effect of calcium on the production of various toxins of clostridium perfringens. Japan J Med Sci Biol 1969;22:175-179.
353. Sazawal S, Black RE, Jalla S et al. Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children: A double-blind, controlled trial. Pediatrics 1998;102:1-4.
354. Mora JO, Herrera MG, Sucscun J et al. The effect of nutritional requirement on physical growth on children at risk of malnutrition. Am J Clin Nutr 1981;34:1885-1891.
355. Hussain MA, Karyudi L, Husaini YK et al. Developmental effect of short term supplementary feeding in nutritionally at risk. In Indonesian infants. Am J Clin Nutr 1991;54:799-804.