



Original Article



Evaluation of Zinc and Iron deficiency in Children up to 12 years

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Abstract

This cross-sectional observational study assessed the frequency of iron and zinc deficits in 100 children at the Hayatabad Medical Complex in Peshawar, Pakistan, who were between the ages of three and twelve. Serum iron, ferritin, total iron-binding capacity (TIBC), hemoglobin, hematocrit, serum zinc, plasma zinc, and zinc protoporphyrin (ZPP) were among the anthropometric and biochemical characteristics measured. Significant micronutrient deficits were found in the children: 25% had hemoglobin levels suggestive of iron-deficiency anemia, 16% had low ferritin, and 20% had low serum iron. Additionally, common was zinc deficiency, which affected 30% of people based on serum zinc levels and 35% based on plasma zinc levels. A dietary analysis revealed inadequate intake of protein sources, fruits, and vegetables. The results show that this pediatric group has a significant burden of zinc and iron deficiencies, highlighting the need for focused nutritional treatments, public health initiatives, and increased dietary diversity to address these deficiencies and enhance health outcomes.

Keywords: Zinc deficiency, Iron deficiency, Children, Malnutrition, Pakistan, Micronutrients, Anaemia, Dietary assessment

Introduction

Food and nutrition are considered vital for promoting optimal growth and development. Individuals from different age groups, categories, and backgrounds require essential nutrients to boost their energy levels. A proper diet plays a crucial role in promoting optimal development, supporting healthy ageing, and enhancing the body's ability to fight off diseases. Conversely, a deficiency in vital nutrients within an individual's diet can result in various health complications and ailments. There is a vast opportunity to create innovative products and implement more precise dietary guidelines, which will ultimately enhance health conditions through nutrition. Once individuals grasp the significance of this potential, they will realise the criticality of meeting their dietary needs and requirements in a suitable manner. Developing food processing techniques is crucial for improving the nutritional value of diets (Ferriday et al., 2018).

Food and nutrition play a vital role in providing individuals with the necessary fuel to carry out their tasks and activities. Food is essential for providing nourishment to the body, while nutrition encompasses all the processes that occur from the

moment food is consumed until it is utilised for various bodily functions. Food contains seven major nutrients, including carbohydrates, proteins, vitamins, minerals, dietary fibre, fats, and water. These nutrients can be categorised into two groups: macronutrients and micronutrients. Macronutrients are acquired in significant quantities, while micronutrients are acquired in smaller quantities. It is important for individuals to carefully regulate their nutrient intake. Staying healthy is a fundamental goal for everyone. The nutritional status of an individual is determined by the types of food they consume and how it affects their body. The nutritional status can vary between different levels, ranging from good to fair or poor [27].

There are instances where individuals struggle to grasp the meaning and importance of food and nutrition. Consuming nutrient-rich food items is essential for promoting healthy growth and development. Food and nutrition play a vital role in providing individuals with the energy they need. When diet and nutrition are combined with physical activity, they can make a significant impact on maintaining a healthy weight,

reducing the risk of chronic diseases, and promoting overall health conditions [69].

Various types of food are consumed by individuals on a daily basis, such as rice, cereals, bread, vegetables, fruits, milk, eggs, fish, meat, sugar, butter, oils, and more. These various food items consist of a variety of chemical components known as nutrients. These are categorised based on their chemical composition. Every nutrient class serves a distinct purpose. Food contains a variety of essential nutrients, such as carbohydrates, proteins, vitamins, minerals, dietary fibre, fats, and water. The vital role of nutrients is to support overall health and prevent the onset of health issues and diseases. The functions of nutrients have been described in the following manner [60].

Carbohydrates in foods include starch found in cereals and sugar found in sugarcane and fruits. Carbohydrates play a crucial role in supplying the body with the energy it requires. Carbohydrates that are not immediately stored for this purpose are either stored as glycogen or converted into fat for future energy needs.

Proteins found in various foods include casein from milk, albumin in eggs, globulins in legumes, and gluten in wheat. Proteins play a crucial role in constructing fresh tissues and preserving and mending those that are already present. Regulatory and protective substances, such as enzymes, hormones, and antibodies, also serve as food proteins. Proteins in the diet contribute approximately 10 percent of the total energy intake. When protein is consumed in excess of the body's needs, it is converted into carbohydrates and fats, which are then stored in the body.

Vitamins in foods can be classified as either fat soluble or water soluble. Vitamins A, D, E, and K are considered fat-soluble, while vitamins C and B are water-soluble. Vitamins play a crucial role in promoting optimal growth and supporting the proper functioning of the body and its processes. When people don't get enough vitamins from their food, they turn to vitamin tablets as a supplement. Vitamins are crucial for the proper functioning of the body.

Minerals like calcium, phosphorous, iron, iodine, sodium, potassium, and others can be found in various foods, along with organic and inorganic components. Minerals play crucial roles in the development and maintenance of the body, contributing to the formation of bones, teeth, and the structural components of soft tissues. Minerals are crucial for regulating various bodily processes, such as muscle contraction, blood clotting, and nerve stimuli.

Dietary fibre is a type of carbohydrate that the body's enzymes are unable to digest. It can be found in a variety of edible plants, including cereals, fruits, dry fruits, vegetables, dried peas, nuts, lentils, and grains. Fibre is classified based on its physical properties into three categories: soluble, insoluble, and resistant starch. All three types of fibre have a crucial role to fulfil. Dietary fibre plays a crucial role in maintaining gut health and significantly lowers the risk of diseases like diabetes and heart disease. Soluble fibre and resistant starch

also serve as prebiotics and provide support for prebiotics (Dietary Fibre, n.d.).

Some common examples of fats found in foods include oils, butter, ghee, and other similar substances. Fats play a crucial role as concentrated sources of energy, as well as carriers of fat-soluble vitamins and essential fatty acids. When an individual consumes an excessive amount of fats in their diet, these fats are stored as reserves in the body. Put simply, when an excess of energy is consumed, it is stored as fat within the body, leading to obesity. Thus, to regulate body weight, individuals are cautious about their fat intake.

Water is acquired through the consumption of food, with a significant portion being obtained from the consumption of beverages and drinking water. It is advised to consume water in significant amounts. Water is a crucial component of the body's structure, constituting a significant portion of its weight, approximately 60 percent. Water plays a crucial role in the body's ability to process and eliminate food materials. It is also considered the controller of the body's processes, such as maintaining body temperature.

Malnutrition refers to an imbalance at the cellular level, where the body's demand for nutrients and energy for growth, maintenance, and specific functions is not adequately met. It focusses mainly on the role that nutrients play in the body's growth, development, and maintenance. Malnutrition occurs when the body lacks the necessary vitamins, minerals, and nutrients to sustain healthy tissues and organ functions (Elia, 2000).

Malnutrition in children is a significant issue that has far-reaching consequences in the field of public health worldwide. Children who are malnourished face a higher likelihood of succumbing to infectious diseases. Shockingly, research suggests that malnutrition is responsible for nearly half of all deaths in children under the age of 5 worldwide [19,84]. The link between malnutrition and infections may be influenced by poverty, which is a common factor in both. Additionally, there is a potential two-way causal relationship: malnutrition can make individuals more susceptible to infections, while infections can worsen malnutrition by reducing appetite, promoting catabolism, and increasing the body's need for nutrients [103]. There has been ongoing discussion regarding the relationship between malnutrition and infections. However, reliable evidence suggests that children who are malnourished face a greater risk of mortality when they become infected. Impaired immune function due to malnutrition may contribute to the increased susceptibility to infections [28].

Given the prevalence of infections and deaths in malnourished children in low-income settings, it is often challenging to identify the specific organisms responsible for causing these diseases. Consequently, there is limited knowledge regarding the potential distinctions between these pathogens and those that infect children who are well-nourished. Additionally, it remains uncertain whether malnourished children are more vulnerable to opportunistic infections. While studying

malnourished children, researchers have reported cases of opportunistic infections such as *Pneumocystis jirovecii* and severe varicella. However, it is important to note that these studies were conducted before the discovery of HIV, so they may actually represent cases of undiagnosed paediatric AIDS. Recent studies have indicated that *Pneumocystis jirovecii* pneumonia is not commonly observed in malnourished children who are not infected with HIV (Ikeogu et al., 1997). Nevertheless, certain pathogens such as cryptosporidium and yeast are commonly responsible for causing diarrhoea in children who are malnourished. Additionally, malnourished children face an increased susceptibility to invasive bacterial infections, leading to conditions like bacterial pneumonia, bacterial diarrhoea, and bacteraemia, with a prevalence of gramme negative bacteria. Given the widespread occurrence of invasive bacterial infections, it is advised by current guidelines to administer antibiotic treatment to all children suffering from severe acute malnutrition, despite the limited strength of the supporting evidence [88].

Other factors besides the immune system can also play a role in the higher mortality rates observed in malnourished children. For example, their reduced muscle mass can make it harder for them to breathe during lung infections. Additionally, their ability to absorb electrolytes from the gut may be compromised, and their kidneys may struggle to concentrate urine, making them more prone to dehydration from diarrhoea. Furthermore, their cardiac function may be diminished, putting them at a higher risk of experiencing heart failure. Therefore, immune function could potentially be a crucial factor connecting malnutrition, infections, and higher mortality rates [97].

There are two types of severe acute malnutrition in children. The first is non-edematous malnutrition, also known as marasmus, which is characterized by severe wasting. It is currently defined by a weight-for-length z-score of less than -3 of the WHO growth standards, or a mid-upper arm circumference (MUAC) of less than 11.5 cm. The second type is oedematous malnutrition, which is defined by bilateral pitting oedema [88]. Kwashiorkor is a type of malnutrition that causes swelling, particularly in the abdomen. It is a severe condition that can lead to a range of symptoms, including an enlarged liver, changes in mental function, and alterations in the skin and hair. Children who exhibit both wasting and oedema have been referred to as having "marasmic kwashiorkor" [23,109].

Income affects children's health and nutrition, as is well known. A US study examined how race and ethnicity affects nutrition. Overweight people were used to measure malnutrition in this study. African youngsters are more likely to be overweight, according to studies. African girls are slightly more likely to be overweight than Mexican American boys. Wealthier families in emerging countries are more likely to be overweight than poor households. Their predilection for unhealthy, expensive fast food is to blame. In industrialized

countries, low-income migrants eat traditional, unhealthy diet, whereas wealthier people eat healthier [39].

A 12-country study found that household or national income directly reduces malnutrition. Alderman et al (2006). examined a four-round panel data set from North-western Tanzania to assess child nutrition determinants. The data show that nutritional interventions can boost income. Policy models show that decreasing hunger by 2015 will require more than income growth. Complementary attempts will require large-scale program interventions.

Research shows that economic and social inequality contribute to malnutrition. Hackett et al (2008). investigated how small Colombian communities affect children's physical parameters. The study focused on low-income kids. They explored how household consumption and public infrastructure affect childhood malnutrition. Household assets and municipality average wage were used to account for household consumption endogeneity. Both factors affect a child's nutrition, it was found. A well-developed piped water network improves a child's health, as long as the parents are educated, according to the study [3,49].

According to Brekke et al. Nordic nations have a stronger income-health link than other countries. According to the authors, when more income-influencing factors are considered, the income-health link will weaken. If the Nordic countries can minimise other income-influencing variables like social class, the income-health association may strengthen [22].

A 2011 Bangladeshi survey found that age, gender, mother's body mass index, mother's education level, father's education level, place of residence, socioeconomic status, community status, religion, region of residence, and food security all affect childhood malnutrition. Children from underprivileged homes and food-insecure families are more likely to be malnourished. Research shows that malnutrition rates vary by community and income.

Urban youngsters are taller than their peers. City and town healthcare are usually superior to rural healthcare. Bharati et al (2008). observed that spatial difference, notably rural-urban difference, affected health status along with other socio-economic aspects. The spatial effect diminished when age and socioeconomic characteristics were excluded. According to Ghosh, urban children, regardless of gender, are more fat than rural children due to urban lifestyles and lack of physical activity [16,25,42].

Bassolé et al (2007) 25 found that in India, access to safe drinking water improves the HAZ—in other words, reduces the stunting of the lowest (10th) income quintile—and that developed health facilities are also improving the health status of the 10th, 25th, and 50th percentiles of income groups at the national level. However, in rural areas, only health facilities have a positive and significant effect on a child's health.

Increased latrine coverage is effective for reducing exposure to faecal pathogens and preventing disease, and in the long run, reducing malnutrition. This has been proven through a study in Orissa [13,29].

Child nutrition is mostly affected by intra-household factors. One indicator is childbirth order. Birth sequence is birth order. First-born children in a household are born one. Behrman et al. estimated essential parental preferences for nutrient distribution among children using a model. Latent variable estimation in rural south India shows that parents choose older children and trade productivity for equity. When parents cannot afford food, they feed their older children first because they think they can start earning sooner. This puts their younger children at danger of malnutrition. Ranger et al. suggested that lack of competing younger siblings promotes feeding. Birth order strongly correlates with childhood malnutrition, according to Zakaria et al.²⁸. Children with higher birth orders are more likely to be malnourished [25,11].

Child gender is another intra-household factor. Due to cultural preference mapping, women prefer to feed boys better, according to several research. Sen and Sengupta²⁹ found a similar result in Santiniketan, West Bengal. The authors evaluated the nutritional condition of under-5s in Sahajapur and Kuchli villages. Girls had a much higher rate of undernourishment and deprivation, even 'severe' and 'disastrous' categories, than boys. Interestingly, the hamlet with the best nutrition discriminated against girls more. Land reform in Kuchli appears to have benefited boys more than girls economically. The two villages had similar girl nutrition. Overall, Kuchli is healthier than Sahajapur. Boys have greater nutrition, proving a sex bias [91].

Girls in Pakistan are malnourished more than boys, highlighting a child-level gender imbalance. This gender bias is linked to family income and breastfeeding, according to the study. Bharati et al. used Z-scores of weight-for-ages, HAZ, and WAZ to investigate the regional distribution of nutritional status for children under three years old in India's National Family Health Survey-2 data. Regression results demonstrate that the gender difference is small and nearly zero when age and socio-demographic variables are excluded [8].

Parents, particularly mothers, are committed to giving their children every conceivable benefit of excellent health care and improving their health to meet their needs. In addition, mothers are considered health care workers for their children. Thus, women's education, employment level, and health care behaviour should have a significant impact on their child's health [64].

Many studies have examined the association between a mother's education and employment and her child's nutrition. However, researchers on maternal features and childhood malnutrition are divided among groups. Several studies found a positive linear relationship between maternal education and child nutrition. A mother's education improves knowledge, feeding, and hygiene, as is increasingly accepted. Children of educated mothers are better fed than those of illiterates. A

study from Indonesia found that a mother's education strongly predicts her child's nutritional improvement.³⁶ Sahn and Stifel³⁷ found that in South Africa, a mother's education improves her daughter's nutrition more than her son's, while a father's education improves his son's. A Nigerian study suggests combining public health and women's education to prevent child malnutrition. Lindelow observed that in Mozambique, effective education, including home education, determines health service usage [2,65,71,106].

Barrera showed that mother education improves child health, as evaluated by HAZ. Its effects are strongest on pre-schoolers. According to research, maternal education enhances children's health by efficiently using public health resources. This improves childhood nutrition at a cheaper cost of information [12]. Another Indian study found that grandmothers' education is more essential than mothers' in predicting a child's diet [98].

In the 1970s, the World Bank's first community nutrition loan to Indonesia improved the nutritional condition of 40% of target children just through nutrition education. Moestue et al. showed that mother education does not necessarily predict child malnutrition. Increased employment by educated women may harm children and modify nursing practices, outweighing the potential advantage of a mother's education. Community-level maternal literacy trumps individual schooling. If a mother lacks health information, her education has minimal effect on her child's nutrition, according to Glewwe. Thus, the mother's health awareness, not her education degree, affects her child's diet. While less important, a father's education affects his child's diet [44,72].

Not all employed moms have well-nourished children, therefore the influence of maternal employment on child malnutrition is debatable. On one side, more women working would empower mothers to make better feeding and other decisions for their children. A mother's employment status, seen to empower her, is thought to improve her child's nutrition by providing better food and medical care. However, other research has shown results that contradict these expectations. Mothers may have less time to care for their children due to work, which can lead to malnutrition. Rizzo et al.⁴⁵ found that rural Kenyan mothers forced into underemployment had lighter kids. León and Younger⁴⁶ showed that paying moms did not improve Ecuadorian children's nutrition. A US study found that maternal employment does not affect children's health. There may be a favourable influence due to financial resources and a working mother being a better role model [17].

Modernisation and urbanisation increase female work, which improves child health by sharing childcare costs, according to researchers. Berman et al. showed that employed mothers, especially from disadvantaged households, improve household income and have a positive impact on their children's health. Parents can use extra revenue from maternal employment to buy food, clothing, and medical treatment, which improves children's health [14]. A Dhaka case study found a high positive correlation between working mothers

and severe malnourishment in children. Full-time maternal employment dramatically reduces vitamin A levels in children under six, according to this study [58].

Tetanus toxoid injections, weight checks, and vitamin A and iron supplements throughout pregnancy may improve infant growth and ensure healthy delivery. Professional antenatal care may improve a child's health, but its use depends on a mother's education and media exposure. This shows that prenatal care usage is based on mother education. Known as the endogeneity dilemma. Given endogeneity, Halim et al. employed instrumental variables to examine the problem and discovered that children are healthier when their moms are healthy and seek antenatal care [50,77].

Tharakan et al. ordered logistic regression study of Botswana data demonstrated that other factors affect childhood malnutrition. The authors classify them as biological, cultural, economic, and morbidity. Age, birth weight, duration of breast-feeding, family head gender, mother and father education levels, and child caretaker presence have been discussed. Additional indicators include milk and dairy product consumption, staple meal, cereal, and beverage consumption, and cough and diarrhoea incidence. When designing interventions to prevent child malnutrition, these aspects should be considered [102]. Another Nigerian study suggested targeting mothers with educational initiatives, good water, and a healthier rural environment to prevent child malnutrition [31].

Bangladeshi researchers found that parental pregnancy aspirations are linked to stunting, wasting, and underweight children. If this link is causal, reducing unplanned births may reduce childhood malnutrition in Bangladesh. An intriguing South Indian study found that maternal mental distress during pregnancy or low maternal intellect are important risk factors for infant malnutrition [7,85].

Domestic violence has negative impacts on physical and mental health, but there is minimal evidence linking it to starvation. Ackerson et al examined 1998–1999 Indian National Family Health Survey data to examine this association. Women self-reported physical domestic violence in this poll. Anaemia and underweight were examined in this study. Haemoglobin tests measured Anaemia. A woman's BMI determined her underweight status. This survey also measured child stunting and wasting. The data imply a link between domestic violence in the last year, Anaemia, and underweight in women and child nutrition. Domestic violence may affect nutritional outcomes through stress-mediated effects and food withholding. These findings show that decreasing domestic violence is important morally and intrinsically and for its potential health advantages [1].

As childhood malnutrition persists in most nations, governments implement measures to eliminate it. Ethiopia is a low-income country with a long history of childhood malnutrition. The National Nutrition Strategy 2005–06 and

National Nutrition Policy 2008 aim to reach this goal, however there is still a long way to go. Semba said UN programs focus on protein malnutrition. These methods do not address micronutrient deficiencies, which is worsening. Goudet et al.⁶⁴ found in a systematic literature analysis that micronutrient supplementation does not benefit malnourished children in low- and middle-income countries. The New Partnership for Africa's Development (NEPAD) aims to reduce hunger and improve nutrition [41,45,90].

Bain et al. suggested boosting genetically modified food production and strengthening sociocultural conditions to reduce food insecurity, a major cause of childhood malnutrition. Khan and Raja⁶⁷ found that WASH improvements in Bangladesh can prevent child malnutrition. The government also wants to strengthen infrastructure. The Indonesian government launched various malnutrition-fighting programs. Health insurance, community capacity building, and microcredit are effective examples. India similarly seeks to end childhood malnutrition. ICDS, a nutrition program for children under five and pregnant and breastfeeding mothers, and the wheat-based Supplementary Nutrition Program through the Public Distribution System are key programs they have developed. In remote locations, higher-level malnutrition prevalence was estimated due to poorer socioeconomic position among the population (68 [6]). The government must improve economic possibilities, healthcare education, nutritional access, and personal hygiene through economic, political, and social policies. These initiatives will boost economic resources, nutritional safety, maternal schooling, and child health [11,84].

Early childhood malnourished schoolchildren have lower IQ, cognitive function, school achievement, and behavioural issues than matched controls and, to a lesser extent, siblings. Negative effects remain till adolescence. No cognitive deficit is consistently found [46].

Malnutrition can affect homeostasis, spreading oral illness, reducing biofilm resistance, and slowing tissue recovery. It may potentially impact oral cavity growth. Protein-energy malnutrition occurs when a body lacks an adequate amount of protein, energy, or both. Studies reveal that enamel hypoplasia, salivary gland hypofunction, and saliva compositional alterations may link malnutrition to caries, whereas changed eruption timing may complicate age-specific caries rates [92].

The results from 53 developing countries with nationally representative data on child weight-for-age indicate that 56% of child deaths were attributable to malnutrition's potentiating effects, and 83% of these were attributable to mild-to-moderate as opposed to severe malnutrition. For individual countries, malnutrition's total potentiating effects on mortality ranged from 13% to 66%, with at least three-quarters of this arising from mild-to-moderate malnutrition in each case. These results show that malnutrition has a far more

powerful impact on child mortality than is generally appreciated, and suggest that strategies involving only the screening and treatment of the severely malnourished will do little to address this impact [5].

Malnutrition reduces plasma complement, gut-barrier function, and exocrine protective chemical release. Delayed-type hypersensitivity reactions decrease when lymphatic tissue, especially the thymus, atrophy. Severely malnourished children develop less antibodies after vaccination than moderately malnourished children. Cytokine patterns favour Th2. leukocyte and lymphocyte counts are unaltered, and immunoglobulins, especially immunoglobulin A, are high. The acute phase response may be present without clinical infection. Multiple studies have linked malnutrition, cell-mediated immune deficits, and gastrointestinal or respiratory illnesses in children under 5 years old [88]. The WHO established the external Child Health Epidemiology Reference Group (CHERG) in 2001 to assess the proportion of children under 5 who died from pneumonia, diarrhoea, malaria, and measles. In 2008, infectious diseases killed 68% (5,970 million) of 8,795 million children under 5, with pneumonia (18%), diarrhoea (15%), and malaria (8%) [18]. There were greater links between nutritional status and death for gastrointestinal and acute respiratory illnesses that correlate with malnutrition in another study [110]. The WHO reported in 2009 that 27% of under-5s in poor nations are malnourished. About 178 million children (32% of developing world children) are chronically malnourished. Although childhood malnutrition is reducing in Asia, South Asia still has the highest rates and most malnourished children. Malnutrition is substantially higher in India, Bangladesh, Afghanistan, and Pakistan (38–51%) than sub-Saharan Africa (26%). The latest national nutrition study in Mexico suggested 1.8 million under-5s are malnourished [89].

Child malnutrition is common, and ultrasonography is used to evaluate kidney size. Kidney sizes were researched in healthy infants and paediatric patients, but not in impoverished children. The study group had 74 children with energy malnutrition (marasmus) and the control group 47 healthy youngsters. The same radiologist measured kidneys using ultrasonography. The average age of the marasmic group was 29.6 ± 14.0 months. Compared to controls, malnourished children exhibited reduced kidney length and volume but higher relative kidney volume ($\text{cm}^3/\text{body weight}$) ($P < 0.05$). Mean left kidney length and volume were higher than right kidney in both marasmic and control groups ($P < 0.05$). Marasmic group had the strongest positive relationships between body height and kidney length, depth, and volume. Height and age of marasmic children affected kidney volume, but only body height affected kidney length, according to regression analysis [34].

In 2018, nearly 149 million children under five are stunted, 49.5 million are wasted, and 40.1 million are overweight, indicating a slower decrease in stunting and wasting and an increase in overweight compared to 2000 (de la Santé, 2020; Organization & Fund, 2020). Asian region has higher stunting (81.7 million), wasting (33.8 million), and overweight (18.8 million) of the

world total. Southern Asian countries have the highest rates of stunting (57.9 million), wasting (25.3 million), and overweight (5.5 million) in the UN classification [77].

Around half of Pakistani children under five are stunted, and one in ten waste [4]. China, Bangladesh, Congo, Indonesia, India, and Ethiopia account for two-thirds of the world's undernourished (Vos, 2019). Pakistan is also one among them. Pakistan ranks 77 out of 113 nations in malnutrition, is the most stunted country in Southern Asian nations (SAC), and has a high wasting rate. Pakistan's performance is modest, and stunting 40.2%, wasting 17.7%, underweight 28.9%, and overweight 9.5% are higher than other developing countries [94]. Rural Pakistan has higher rates of stunting (43.2%), wasting (18.6%), and underweight (31.6%) than urban Pakistan does (34.8%), (16.2%), and (24%). Boys are more stunted, wasted, underweight, and overweight than girls. Baluchistan has more stunting and overweight than Punjab and Khyber Pakhtunkhwa, whereas Sindh has more wasting and underweight [101].

Micronutrient deficiencies occur when the body lacks essential vitamins and minerals, such as vitamin A, iron, and iodine. These deficiencies can cause a range of health problems, including:

- Anemia: A common micronutrient deficiency that can lead to fatigue, weakness, and impaired immune function.
- Can cause blindness and increased susceptibility to infections.
- Can lead to goiter (enlarged thyroid gland) and impaired cognitive function

Iron, iodine, vitamin A, and zinc deficiencies remain serious public health issues in developing nations, although vitamin C, D, and B deficiencies have diminished [7,77]. At least 2 billion people lack micronutrients.⁵ Since biochemical markers of marginal micronutrient status are often unreliable, randomised controlled supplementation trials are the best way to explore the relationship between micronutrient deficits and human health [101].

Protein–energy malnutrition and micronutrient deficits intersect, and a lack of one micronutrient usually leads to missing others. Iron is needed for haemoglobin, myoglobin, and enzymes. Thus, its shortage causes Anaemia and other side effects. Iodine deficiency lowers thyroid hormone production and boosts TSH. The thyroid gland becomes hyperplastic and goitrous, causing hypothyroidism. vitamin A deficiency reduces haemopoiesis and increases infection risk. Vitamin A supports the immune system and eyes. Vitamin A insufficiency is linked to diarrhoea and death, but acute lower respiratory tract infections and malaria are less so. Zinc is needed for several enzymes and metabolic activities, including RNA and DNA synthesis. Zinc deficiency affects gene expression, protein synthesis, skeletal growth, gonad development, hunger, and immunity. Zinc facilitates the activity of unspecific immune cells like neutrophils and natural killer cells and specific immunological processes like T

helper cell balance. Zinc deficiency causes diarrhoea and pneumonia; however, debate surrounds its function in malaria and growth retardation [75].

Iron is required for oxygen transport and cellular respiration and is found in haemoglobin, myoglobin, enzymes, and cytochromes. Growth and cognition require iron. Iron can be heme or nonheme. Fortification uses nonheme iron from plants, while heme iron comes from animals. While heme iron bioavailability is estimated at 12-25% and nonheme iron at <5%, iron *in vivo* is substantially preserved, save for menstruation and pregnant people [57].

Iron deficiency is the most frequent Motor neuron disease, affecting around 30% of the world's population, or 2 billion individuals. Iron deficiency causes Anaemia and impairs endocrine and immunological function. Due to increased foetal growth and development, iron insufficiency is frequent throughout pregnancy. Low birth weight, early delivery, and perinatal problems, including haemorrhage, are linked to maternal iron insufficiency. Approximately 20% of maternal fatalities are due to Anaemia. Children of iron-deficient mothers are more likely to have inadequate iron stores, poor physical and cognitive development, and weak immune systems. Early-life iron status greatly affects individual and national potential. Iron deficiency may increase cadmium absorption [55,95]. Pregnant women and infants under 6 months old are at highest risk for iron deficiency. Pregnant and infant iron supplementation is advised in malaria-free environments. Iron supplementation may worsen falciparum-related malaria complications and death [38].

Essential mineral zinc is involved in several cellular metabolism processes. Zinc is needed for over 200 enzymes, immune system function, cell division, and protein and DNA production. Zinc is needed for optimal development from foetal to puberty. Since the body has no long-term zinc storage system, constant dietary intake is needed to sustain these processes and preserve the limited exchangeable zinc pool. Plasma zinc concentrations have been utilised as biomarkers, although they are nonspecific. Zinc has many functions *in vivo*. Zinc is mostly in seafood and animal goods. Phytates, fibre, and lignins reduce zinc bioavailability from nonanimal sources, like iron. Calcium and casein may limit cow's milk zinc bioavailability. Human breast milk contains zinc [63].

Zinc status reduces diarrhoea, respiratory, and malarial infection incidence, severity, and mortality (Patel et al., 2010). Diarrhoea might reduce micronutrient absorption and meal intake due to infection. A recent Cochrane review of 80 randomized clinical trials involving 205,401 participants in children aged 6 months to 12 years found that zinc supplementation reduced all-cause and infectious disease mortality and slightly improved linear growth [70]. In a Peruvian clinical trial, zinc-supplemented mothers had greater weight gain, calf and chest circumference, and calf muscle area than mothers without zinc supplementation [53].

Zinc did not affect linear growth. Zinc supplementation during pregnancy significantly reduces preterm births without affecting birth weight [79]. 17.3% of the global population lacks zinc, with the highest estimates in Africa (23.9%) and Asia (19.4%). Zinc deficiency is more common in pregnant women and young children. The WHO and UNICEF recommend zinc supplements for 10-14 days and oral rehydration treatment for acute diarrhoea, but no routine zinc deficient supplementation. Zinc deficiency is likely a major cause of morbidity in developing nations, although little is known about the global situation. Due to biomarker difficulties, estimations of zinc deficiency are based on child stunting, nutritional consumption, and food zinc availability [32].

In iron insufficiency, brain, muscle, and blood tissues lack enough iron to function normally. Chronic iron deficiency causes it. Hemosiderin and ferritin, iron reserves, decline and cannot meet physiological needs. Several systemic signs of iron insufficiency appear. Iron deficiency symptoms are mild and non-specific, often appearing only in severe cases [30].

Iron status ranges from iron deficiency with Anaemia (IDA) to normal iron status to iron excess. Iron deficiency without Anaemia involves iron deficient erythropoiesis and depletion. Iron depletion reduces stored iron but does not influence needed iron, therefore a person with iron depletion has no iron stocks to use if needed. In iron-deficient erythropoiesis, stored iron depletes and transport iron decreases, so absorbed iron is insufficient to restore lost iron or meet body function and development needs. Iron deficiency Anaemia, the most severe form of iron insufficiency, lowers iron storage and transport, causing underproduction of Hb and myoglobin [108].

Iron deficiency causes clinical symptoms by depleting iron reserves. All cells utilise iron for energy metabolism, gene control, cell growth and differentiation, oxygen binding and transport, muscle oxygen usage and storage, enzyme reactions, protein and neurotransmitter production, and enzyme reactions. Thus, iron deficiency is a multi-systemic illness, not just Anaemia. Children with iron deficiency have growth retardation, poor immunological function, impaired behavioural, mental, and psychomotor development, and diminished work capacity [66].

Iron is needed for cell differentiation and proliferation. Ribonucleotide reductase, an iron-containing enzyme, synthesizes DNA, which slows cell reproduction. Thus, iron shortage inhibits cell growth. the growth of 40 iron-deficient children aged 17.2 ± 12.4 months before and after supplementation. The authors found that iron-deficient children were substantially shorter and grew slower than controls. Additionally, iron-deficient children's growth indices improved following treatment [9].

Iron deficiency reduces work capacity. Multi-minute muscular action requires iron-containing cytochromes, iron-sulphur proteins, and electron transport proteins for mitochondrial oxidative energy generation [20].

Tay et al. examined exercise tolerance in 25 iron-deficient cyanotic congenital heart disease (CHD) patients over five months. Iron replacement therapy and cardiovascular exercise testing were given at beginning and 3 months following treatment. Researchers found that 3 months of iron replacement therapy in iron-deficient patients improved their quality of life and exercise tolerance. Fatigue, shortness of breath, irritability, weakness, and anorexia can result with severe iron deficiency [52].

The primary internal iron circuit arising from 30–40 mg Hb iron in senescent RBCs each day, which are eliminated and replenished, must be understood to comprehend laboratory iron status measurements. After bone marrow and spleen macrophages eat ancient red cells, iron is taken from Hb and returned to plasma, where it binds to transferrin, its extracellular carrier. Iron-containing transferrin binds to bone marrow red-cell precursor receptors after 1–2 hours. The iron cycle is completed when freshly produced erythrocytes re-enter to circulation after 7–10 days. Interpreting iron status measures by compartment is crucial. First, storage iron is depleted, next iron transport, finally erythroid compartments [30].

Ferritin, a high-molecular-weight protein, contains 20% iron. Iron reserves are found in reticuloendothelial cells, hepatocytes, and most tissues. It is also seen in tiny levels in the serum, reflecting normal iron storage. Ferritin helps absorb, store, and release iron. It stores iron in tissues until erythropoiesis. Iron molecules are liberated from the apo ferritin shell and attach to transferrin, a plasma protein that delivers iron to erythropoietic cells [24].

Khan (97) compared serum ferritin to various biochemical and haematological indicators of serum iron in iron-deficient children. Children 5 months–12 years old were researched. Red cell morphology, Hb, serum iron, and ferritin were assessed. TIBC was 95% sensitive, Tf saturation 82% sensitive, and serum ferritin 100% sensitive. The researchers found serum ferritin more sensitive than other measures. Guyatt et al. (95) examined iron deficiency laboratory test diagnostic results. Thus, ferritin values should be evaluated with acute infection biomarker CRP (98). Acute phase protein CRP is essential for pathogen killing, apoptosis elimination, and complement activation. Infections or inflammatory diseases raise CRP levels fast in the first 6–8 hours and peak at 350–400 mg/L after 48 hours. CRP binds to phosphocholine on injured cells and Pepto saccharides and polysaccharides on parasites, fungi, and bacteria (100). This binding triggers the immune system's classical complement cascade and modifies phagocytic cell activity, supporting CRP's role in opsonising infectious pathogens and dead or dying cells (100). Once inflammation subsides, CRP drops. CRP is a good disease activity marker (98,101). According to WHO, a blood CRP level of <5 mg/L is considered normal for fast tests and <3 to 10 mg/L for immunoassays like ELISA [47,62].

Iron deficiency Anaemia has poor red cell indices (MCHC, MCV, Red cell count, MCH, Hb concentration, and haematocrit). Red cell abnormalities occur late in the iron

storage depletion-to-absence process. Hb or haematocrit testing is commonly used to detect iron deficient Anaemia. Due to malnutrition, haemoglobinopathies, and persistent infection, its sensitivity and specificity are low. Standard indices MCV and MCH measure red cell quantity and Hb content. The time it takes for iron deficiency levels to become problematic limits these haematological parameters. Beutler et al. found that MCH and MCV did not exclude or confirm iron insufficiency. Researchers observed that controls had abnormal indices in 10% of cases and subjects had normal MCH and MCV in 20% and 50%. The incidence of aberrant indices rose with Anaemia severity, suggesting that they constitute a late sign of iron deficiency clinically [15,61].

ZEP testing is sensitive but limited in specificity since lead poisoning, inflammation, and haemoglobinopathies enhance ZEP. ZEP has a 42% sensitivity and 61% specificity for predicting iron insufficiency in infants and adolescents aged 6 months to 17 years [68].

TfRs bind transferrin-bound iron to cell membranes before absorption. Serum TfR may be a new and reliable indicator of cellular iron status. All cells include a transmembrane protein called TfR that transfers transferrin and iron inside the cell. Any iron shortage increases TfR synthesis. Cellular iron deficit increases serum TfR proportionately. Together with serum ferritin, serum TfR provides complete iron status information. Without chronic inflammatory disorders, blood ferritin and TfR reflect tissue iron reserves. Unlike plasma ferritin, infection and inflammation do not increase plasma TfR. Thus, plasma TfR levels may assist distinguish iron deficiency Anaemia from chronic inflammatory Anaemia [59].

Zinc deficiency is a common global issue affecting millions of people, particularly in developing countries. Zinc deficiency is prevalent among children in developing countries, with estimates ranging from 4% to 73% across subregions. In Pakistan, for example, major zinc deficiency levels of 43.8% were found in children, primarily due to low socio-economic status and decreased dietary intake of zinc it is often due to inadequate dietary intake, but can also result from malabsorption syndromes, chronic illnesses, or increased physiological needs such as during pregnancy and infancy.

Diagnosing zinc deficiency involves clinical assessment and laboratory tests. Clinically, characteristic skin lesions, along with other systemic symptoms, raise suspicion. Laboratory tests include measuring serum zinc levels, though these can be influenced by various factors and may not always reflect zinc status accurately. Therefore, a comprehensive approach considering dietary history, clinical signs, and biochemical tests is often necessary for accurate diagnosis. Zinc deficiency has significant dermatological and systemic consequences. Awareness and recognition of its signs, combined with appropriate diagnostic measures, are essential for timely management and prevention of associated complications [76].

Rationale

Health and growth are both adversely affected by zinc and iron deficiency in children under 12 years old. In addition to

supporting the immune system, both minerals are essential for cognitive function. The frequency and outcome of these inadequacies can help guide specific therapies, nutritional approaches, and public health policies to ensure the optimal health of children. This vulnerable group would benefit from better prevention strategies by studying the reasons causing these inadequacies.

Review of the Literature

Malnutrition is caused by consuming a diet with either too little and or too much of one or more nutrients, such that the body malfunctions. These nutrients can be the macronutrients, including proteins, carbohydrates, and fats that provide the body with its building blocks and energy, or the micronutrients including vitamins and minerals, that help the body to function. Infectious diseases, such as diarrhea, can also cause malnutrition through decreased nutrient absorption, decreased intake of food, increased metabolic requirements, and direct nutrient loss. A double burden of malnutrition (both overnutrition and undernutrition) often occurs across the life course of individuals and can also coexist in the same communities and even the same households. While about a quarter of the world's children are stunted, due to both maternal and young child undernutrition, overweight and obesity affects about one in three adults and one in ten children. Anemia, most commonly due to iron deficiency, is also affecting about a third of women of reproductive age and almost half of preschool children. Around 90% of nations have a serious burden of either two or three of these different forms of malnutrition [93].

WHO estimates that malnutrition (underweight) was associated with over half of all child deaths in developing countries in 1995. The prevalence of stunting in developing countries is expected to decline from 36% in 1995 to 32.5% in 2000; the numbers of children affected (excluding China) are expected to decrease from 196.59 millions to 181.92 millions. Stunting affects 48% of children in South Central Asia, 48% in Eastern Africa, 38% in South Eastern Asia, and 13–24% in Latin America. IDA affects about 43% of women and 34% of men in developing countries and usually is most serious in pregnant women and children, though non-pregnant women, the elderly, and men in hookworm-endemic areas also comprise groups at risk. Clinical VAD affects at least 2.80 million preschool children in over 60 countries, and subclinical VAD is considered a problem for at least 25 millions; school-age children and pregnant women are also affected. Globally about 740 million people are affected by goiter, and over two billion are considered at risk of IDD. However, mandatory salt iodization in the last decade in many regions has decreased dramatically the percentage of the population at risk. Two recent major advances in understanding the global importance of malnutrition are (1) the data of 53 countries that links protein-energy malnutrition (assessed by underweight) directly to increased child mortality rates, and (2) the outcome

in 6 of 8 large vitamin A supplementation trials showing decreases of 20–50% in child mortality [99].

Zinc deficiency (ZnD) has adverse health consequences such as stunted growth. Since young children have an increased risk of developing ZnD, it is important to determine its prevalence and associated factors in this population. However, only a few studies have reported on ZnD prevalence in young children from Western high-income countries. This study evaluated ZnD prevalence and associated factors, including dietary Zn intake, in healthy 1–3-year-old children from Western European, high-income countries. ZnD was defined as serum Zn concentration $<9.9 \mu\text{mol/L}$. A total of 278 children were included with a median age of 1.7 years (Q1–Q3: 1.2–2.3). The median Zn concentration was $11.0 \mu\text{mol/L}$ (Q1–Q3: 9.0–12.2), and ZnD prevalence was 31.3%. No significant differences were observed in the socio-economic characteristics between children with and without ZnD. Dietary Zn intake was not associated with ZnD. ZnD is common in healthy 1–3-year-old children from Western European countries. However, the use of currently available cut-off values defining ZnD in young children has its limitations since these are largely based on reference values in older children. [106].

A total of 238 patients at Pediatric Clinic of Kayseri Training and Research Hospital having Zn levels $<100 \mu\text{g/g}$, and 322 patients had Zn levels $>100 \mu\text{g/g}$. The median ferritin level was 16.2 (9.8–24.9) ng/mL in the Zn-deficient group and 18.7 (12–29.3) ng/mL in those without Zn deficiency group. The presence of Fe deficiency was higher in the Zn deficiency group (60.1%) than in the without Zn deficiency group (50%; $p<0.05$). The presence of Fe deficiency anemia was significantly higher in the Zn deficiency group (20.2%) than in the without Zn deficiency group (12.7%; $p<0.05$). There was very weak negative significant correlation between hair Zn and RDW level ($r=-0.24$; $p<0.001$) and weak positive correlation between hair Zn and MCV ($r=0.31$; $p<0.001$). Fe deficiency and Fe deficiency anemia increased in patients with zinc deficiency [36].

Atasoy and Bugdayci, (2018) studied, 349 of 483 children between 6.5 and 14.8 years old were included from primary schools in Bolu, Turkey. measured weight, length, body mass index, and complete blood count with serum zinc, ferritin, vitamin B12 and folate. Thirty-eight (10.9%) of 349 children had low serum zinc concentration, and 21 (6.0%) were anaemic. There were 12 anaemic children in the zinc-deficient group and nine in the zinc-sufficient control group (31.5% vs 2.9%) with similar ferritin levels. On regression analysis, zinc had the strongest association with haemoglobin. On receiver operating characteristic analysis, the cut-off for serum zinc for prediction of Anaemia was $71.5 \mu\text{g/dL}$. The strongest association of zinc with haemoglobin suggests that low zinc contributed the most to the observed Anaemia in children [10].

Motadi et al (2023) studied Iron and zinc status of children aged 3 to 5 years attending Early Childhood Development

centres in Venda, South Africa. This study assessed iron and zinc levels of preschoolers. 276 children were randomly chosen from 8 preschools. Weight and height were measured using standard techniques. After blood was obtained, serum zinc, iron, ferritin, transferrin saturation, and transferrin were assessed. Dietary intake was calculated using the Food Frequency Questionnaire. Prevalence of severe underweight, stunted, and acute malnutrition was 4.7%, 12.7% and 2.9%. Using transferrin saturation <5%, one-quarter were iron deficient. Using serum iron <40 µg/dl as indicative of depletion, 8% exhibited low serum iron while 18% were mildly deficient. Based on ferritin <12 µg/L, 99% had iron deficiency [74].

Zinc deficiency is an important cause of morbidity due to infectious diseases and growth faltering among young children. Prevalence of iron-deficiency anemia among children is much higher than among adult women and may be partly attributable to the high prevalence of hookworm infestation among children: Sobeih, A. A., (2023) was investigate the Zinc level correlated positively with BMI, Height, times of eating meat per week, diversity of food and iron level. And iron level correlated positively with age, height, times of eating meat per week, diversity of food, and correlated negatively with age of introduction to complementary foods. In our community, 27.9% of children had zinc deficiency and 37% of children had iron deficiency. Zinc and iron deficiency were associated with male sex, illiterate mothers, lower diversity of food, lower weight, height and BMI. Zinc level correlated positively iron level, and both were correlated positively with times of eating meat per week and diversity of food [96].

Consuming a diverse diet is essential to ensure an adequate intake of micronutrients. The aim of this study was to assess the nutritional status and dietary diversity of women of reproductive age (WRA) living in a marginalized community in rural Pakistan. Forty-seven WRA (35 ± 7 years old) who were not pregnant or lactating at enrollment, were recruited to participate in the study. Twenty-four-hour dietary recall interviews were conducted by the study nutritionist, and the data collected were used to create a minimum dietary diversity for women score (MDD-W) on five occasions during the monsoon and winter seasons (October to February). Nutritional status was assessed using anthropometry and biochemical markers of micronutrient status. Height and weight were used to determine body mass index (BMI), and mid-upper-arm circumference was measured. Plasma zinc, iron, and selenium concentrations were measured using inductively coupled mass spectrometry, and iron status was assessed using serum ferritin and blood hemoglobin concentrations. The mean (±SD) food group diversity score was 4 ± 1 with between 26% and 41% of participants achieving an MDD-W of 5. BMI was 27.2 ± 5.5 kg/m² with 28% obese, 34% overweight, and 6% underweight. The prevalence of zinc deficiency, based on plasma zinc concentration, was 29.8%; 17% of the participants had low plasma selenium levels; 8.5% were iron deficient; and 2% were suffering from iron deficiency anemia. The findings indicate that the women living in this

community consume a diet that has a low diversity, consistent with a diet low in micronutrients, and that zinc deficiency is prevalent. Public health interventions aimed at increasing the dietary diversity of WRA are needed to improve the micronutrient intake, particularly of zinc, in this population [20].

Palacios, et al (2020) investigated and find out that one in four children younger than age five in Guatemala experiences Anaemia (haemoglobin <11.0 g/dl). This study characterized the factors and micronutrient deficiencies associated with Anaemia in a baseline cross-sectional sample of 182 Guatemalan infants/toddlers and 207 preschoolers, using generalized linear mixed models. Associations between Anaemia and maternal, child and household variables, and biomarkers (soluble transferrin receptor, ferritin, zinc, folate, vitamin B12, C-reactive protein, and α-acid glycoprotein) were explored. Rates of Anaemia were 56% among infants/toddlers and 12.1% among preschoolers. In children with Anaemia, rates of iron deficiency (low ferritin based on inflammation status, and/or high soluble transferrin receptor, ≥1.97 mg/L) and zinc deficiency (serum zinc <65 µg/dl) were 81.1% and 53.7%, respectively. Folate deficiency (either plasma folate <3 ng/ml or erythrocyte folate <100 ng/ml) was 3.3%. Vitamin B12 deficiency (plasma vitamin B12 <148 pmol/L) was 7.5%. For infants and toddlers (<24 months), the odds ratio of Anaemia was lower when higher number of adults lived in the household (OR = 0.69; 95% CI [0.53, 0.90]), and higher when children were zinc deficient (OR = 3.40; 95% CI [1.54, 7.47]). For preschoolers (36–60 months), the odds ratio of Anaemia was lower for every additional month of age (OR = 0.90; 95% CI [0.81, 1.00]). Findings suggest that micronutrient deficiencies coexist in Guatemalan rural children, and zinc deficiency is associated with Anaemia in children <24 months, highlighting the need of continued multidisciplinary interventions with multiple micronutrients. Further research examining how household composition, feeding practices, and accessibility to micronutrient supplements and to animal source foods is needed to incorporate strategies to improve the nutritional status of Guatemalan children [80].

Anemia affects >2 billion people worldwide and is negatively associated with child development and academic performance. The purpose of this dissertation was to identify variables associated with anemia in children from Guatemala and Haiti. Generalized linear mixed models were used to identify significant associations with anemia (dependent variable) and maternal, child and household variables utilizing a cross-sectional design. For the Guatemala study, serum biomarkers (soluble transferrin receptor, ferritin, zinc, folate, vitamin B12, C-reactive protein, and α-acid glycoprotein) were explored. For the Haiti study, a mediation analysis was constructed to understand the relationship between rural or urban residence and anemia. In Guatemala, 56% of children ages 6–24 months had anemia. For this age group, anemia was inversely associated with higher numbers of adults living in the household [OR=0.69; 95% CI (0.53-0.90)], and zinc deficiency increased the odds of anemia [OR=3.40; 95% CI (1.54-7.47)]. Conversely, only 12.1% of children ages 36–60

months had anemia, which was inversely associated with age [OR=0.90; 95%CI (0.81-1.00)]. In Haiti, 52.3% of children from rural areas had anemia, and stunting increased the odds of anemia [OR=3.42; 95%CI (1.47-7.97)], while children in households with more adults had lower odds, [OR=0.74; 95%CI (0.62-0.89)]. Among urban children, anemia prevalence was 75.4% and helminth morbidities increased the odds of anemia [OR=1.74; 95%CI (1.13-2.68)]. Age was inversely associated in both rural and urban children, [OR=0.88; 95%CI (0.81-0.96)] and [OR=0.93; 95%CI (0.88-1.00)], respectively. Only helminth morbidities partially mediated the relationship between anemia and place of residence ($b=0.10$, $SE=0.05$, $P=0.037$). In conclusion, zinc deficiency was associated with anemia in young Guatemalan children, highlighting the need of continued multidisciplinary interventions with multiple micronutrients. In Haitian school-aged children, anemia continues to be a severe public health problem. Key differences between urban and rural children were identified that should be considered when developing cost-effective interventions to improve nutritional and health status of this population, especially deworming measures in children residing in urban areas. Further research examining how household composition, feeding practices, accessibility to micronutrient supplements, and decreasing intestinal morbidity is needed to develop effective interventions seeking to improve the nutritional status of children in Guatemala and Haiti [80].

Rouhani et al. (2022) conducted a systematic review and meta-analysis of randomized clinical trials assessed the impact of zinc supplementation on mortality in children under five. Combining data from 28 trials involving 237,068 participants revealed a significant 16% reduction in all-cause mortality risk among children receiving zinc supplementation. Subgroup analyses indicated notable reductions in mortality risk with doses of ≥ 10 mg/d zinc, interventions lasting less than 11 months, and in low birth weight infants. Additionally, zinc supplementation was associated with decreased mortality from pneumonia and infection, with potential benefits on mortality from diarrhea and sepsis. These findings highlight the potential of zinc supplementation as a strategy to reduce mortality in this vulnerable population [87].

Aabdien et al. (2022) aimed to determine the prevalence and the determinants of iron deficiency among 10-19-year adolescents in Qatar. The cross-sectional study was done for 450 adolescents from different nationalities. The iron deficiency was defined based on the serum ferritin level of <15 $\mu\text{g/L}$ in the absence of an elevated C-reactive protein level. The present study revealed that 26.4% of adolescents were iron-deficient. These percentages were significantly associated with the gender, nationality, menarche status, and the consumption of iron absorption enhancers from citrus fruits and juice. According to the WHO classification, these results reveal that there is a moderate public health concern. The

prevalence is similar to other LMIC, but it was less common than in high-income countries.

The study by Ismail et al. (2020) reveals a concerning double burden of malnutrition among infants and toddlers in the UAE, with both under-nutrition and overweight/obesity prevalent in the population. While breastfeeding initiation rates were high, exclusive breastfeeding at 6 months was low, and there were inconsistencies in the timing of introducing complementary foods. Macronutrient intake exceeded recommended levels for some toddlers, but there were notable deficiencies in iron and vitamin D intake, particularly among specific age groups. Excessive consumption of free sugars was also observed, especially in older toddlers. Additionally, many toddlers did not meet recommended intake levels for essential food groups such as vegetables, fruits, and dairy [26].

Bevis et al. (2023) investigated how soil zinc deficiency in Nepal is linked to child stunting. The Tarai region studied in this paper is critical to the country's food production. As a result, Bevis et al. aimed to study the link between soil zinc deficiency and child health. Soil zinc deficiency is known to reduce crop yields and reduce the zinc content of food consumed from crops, which may in turn lead to zinc deficiency and ultimately, child stunting. The evidence found supported the hypothesis. Bevis et al. found that increasing the amount of available zinc in the soil, perhaps through increasingly used zinc-enriched fertilizer, is expected to reduce child stunting from 1 to 7.5% per 1 ppm of plant-available zinc [73].

Lu et al. (2023) carried out an extensive assessment of zinc nutritional status and zinc deficiency's prevalence among Chinese children aged 6 to 18 years. The study used cross-sectional data from monitoring surveys held on the China National Nutrition and Health Survey of Children and Lactating Mothers in 2016-2017 which covered 64 850 cases from 150 monitoring sites from 31 provinces. The median serum zinc was 88.39 $\mu\text{g/dL}$, with a zinc deficiency prevalence of 9.62%. Shi et al. L. found several risk factors, which were male sex, anemia, nutrition status, and city type and income. The subgroup showed that males, anemia, stunting, low income, and those in a rural area are at risk of zinc deficiency [67].

Sunuwar et al. (2020) studied the prevalence and factors associated with anemia among women of reproductive age in over seven South/East Asian countries. Sunuwar et al. utilized recent demographic and on-the-ground data between 2011 and 2016 from all DHS available. A total of 726,164 women among seven countries were included in the analysis. The overall prevalence of anemia was 52.5%, with the lowest in Timor-Leste, 22.7%, and the highest in the Maldives, which was 63.0%. Some of the factors associated with anemia included age group 15-24, no education, poorest, poor, no toilet and no clean water source, underweight, and multiple children birth in the last five years. These findings call for urgent evidence-

based policies and programs targeting maternal health and nutrition as well as those that address women's education and socioeconomic status to lower the high burden of anemia among women of reproductive age in these two regions.

Gautam et al. (2019) investigated the determinants of anemia prevalence among women of reproductive age in Nepal using recent national survey data. They aimed to identify factors associated with anemia risk and its implications for maternal health. Utilizing data from a national survey, the study employed logistic regression analysis to assess associations between various factors and anemia prevalence. Results revealed a high prevalence of anemia among women of reproductive age in Nepal, with certain factors such as the source of drinking water and contraceptive use significantly influencing anemia risk. Interestingly, the study found no significant association between women's decision-making autonomy regarding healthcare and experiences of intimate partner violence with anemia after adjusting for background characteristics [40].

Ghosh et al. (2021), drawing upon data from the National Family Health Survey-IV (2015–16), this study delves into the spatial patterns and determinants of anemia prevalence among children aged 6–59 months. By employing spatial analysis tools and logistic regression techniques, the research identifies Dadra & Nagar Haveli as having the highest prevalence of anemia, with 321 districts surpassing the national average. Moreover, 110 hot-spot areas emerge across central, western, south-western, northern, and eastern India. The analysis reveals that factors such as maternal age, education level, receipt of full antenatal care, and socio-economic status are associated with lower anemia risk, while maternal underweight status, children aged 12–23 months, underweight children, and higher birth order pose higher risks [43].

Epidemiological studies have suggested an association between diet and mental health. Hajianfar et al. (2021) conducted a study aimed to explore the relationship between dietary zinc intake and the risk of depression, anxiety, and sleep quality among female students of Iran. 142 female students were randomly selected. Food frequency questionnaires assessed dietary intake, while depression, anxiety, and sleep quality were evaluated using standardized tools. Results indicated a significant association between dietary zinc intake and depression, anxiety, and sleep disorders, suggesting a potential link between zinc intake and mood disorders among Iranian female students [6].

Habib et al., 2023 identified the prevalence of iron deficiency anemia in Pakistan among children under five and women of reproductive age: Furthermore, a significant proportion of the risk of IDA among CU5 is associated with the child's age, signs of diarrhea or fever, place of residence, household size, wealth status, and access to improved sanitation. For WRA, factors such as the level of education, body mass index, vitamin A and zinc status, household food security in the last 24 hours and in the past one-week wealth status, and access to improved sanitation facilities, and existence of a toilet at home are significantly associated with IDA. The findings underscore the

urgent need for large-scale, government-financed programs for micronutrient supplementation, food fortification, dietary diversification, and prevention and appropriate treatment of infectious and parasitic diseases to address IDA and anemia burden in children and women [48].

Furthermore, Haq et al. (2021) examine the prevalence of major micronutrient deficiencies, such as calcium, zinc, iron, vitamin A, and iodine among children aged between 1 and 12 in the flood affected areas of Khyber Pakhtunkhwa, Pakistan. Following the distribution of a cross-sectional survey to 656 households, the study results indicate the presence of alarming deficiency levels that significantly varied with age groups and districts. In this case, children aged 10 to 12 showed low serum zinc and thus require immediate interventions, while children aged 1 to 3 also demonstrated low urinary iodine levels. The findings indicate an urgent need for intervention to help alleviate morbidity and other health complications associated with micronutrient deficiencies among children in the flood affected regions [104].

Similarly, Haq et al. (2021) studied the prevalence and determinants of stunting among preschool and school-aged children following floods in Pakistan. Through a cross-sectional survey to households, the researchers found a high prevalence of stunting, with the overall figure being 40.5%, where higher rates were prevalent among girls and children less than five years of age. Skills were majorly attributed to structural and programmatic interventions concerning restoration of normalcy post the disaster [51].

Materials and Methods

❖ Study Design

This study was designed as a cross-sectional observational study to evaluate the prevalence of zinc and iron deficiencies among children.

❖ Study Setting

The research was conducted at Hayatabad Medical Complex, Peshawar, Pakistan, a major healthcare facility providing services to a diverse paediatric population.

❖ Sample Size

The sample size was determined using the Rao soft calculator, ensuring adequate statistical power to detect significant differences and correlations.

❖ Sampling Technique

Convenient random sampling was employed to select participants. This method allowed for the inclusion of children who were readily available and met the study's inclusion criteria, thereby providing a representative sample of the target population.

❖ Inclusion Criteria

Children aged up to 12 years were included in the study. Participation required informed consent from the parents or

legal guardians. Only generally healthy children, without severe chronic illnesses that could independently affect their nutritional status, were considered eligible.

❖ Exclusion Criteria

Children with chronic illnesses that might independently influence zinc and iron status were excluded to minimize confounding factors. Additionally, children undergoing specific medical treatments or interventions that could impact nutritional measures were not included. Those without parental or guardian consent, as well as those outside the specified age range, were also excluded from the study.

❖ Sample Collection and Anthropometric Evaluations

Anthropometric measurements, including weight, height, and mid-upper arm circumference (MUAC), were conducted following World Health Organization (WHO) guidelines. These measurements were used to assess the nutritional status of the participants, identify malnutrition, and monitor growth trends over time. Body Mass Index (BMI) was calculated to categorize the nutritional status of the children, serving as a standardized tool to detect both undernutrition and overnutrition.

Blood samples were collected using sterilized tubes, and samples were transported to the laboratory within six hours for further processing. These samples were then analyzed to assess zinc and iron levels.

❖ Iron Blood Tests

Serum iron levels were measured to determine the amount of circulating iron in the blood, with normal ranges typically between 60 to 170 mcg/dL. Low serum iron levels can indicate iron deficiency, while high levels might suggest conditions such as hemochromatosis.

❖ Serum Ferritin

Serum ferritin levels were measured to assess the amount of stored iron in the body. Normal ranges vary by age and sex, with typical adult levels ranging from 12 to 300 ng/mL. Low ferritin levels are indicative of iron deficiency, often preceding decreases in serum iron.

❖ Transferrin and Total Iron-Binding Capacity (TIBC)

Transferrin, a protein that transports iron in the blood, and TIBC, which measures the maximum amount of iron that can bind to transferrin, were assessed. Normal TIBC levels range from 240 to 450 mcg/dL. Elevated TIBC may suggest iron deficiency, while lower levels might indicate iron overload.

❖ Hemoglobin and Hematocrit

Hemoglobin and hematocrit levels were measured to assess the oxygen-carrying capacity of the blood and the proportion

of blood volume occupied by red blood cells, respectively. Low levels of hemoglobin and hematocrit are indicators of iron-deficiency anemia.

❖ Zinc Blood Tests

Serum zinc levels were measured to assess zinc status, with normal levels typically ranging from 70 to 120 mcg/dL. Low zinc levels can indicate a deficiency, while elevated levels may occur during acute inflammation. Plasma zinc levels, similar to serum zinc, were also measured.

Zinc protoporphyrin (ZPP) levels were assessed as an additional measure, with elevated levels potentially indicating iron deficiency.

❖ Considerations for Blood Tests

Some tests, such as those measuring serum iron and fasting serum zinc, required fasting for accurate results. The clinical context, including the patient's overall health, medical history, and symptoms, was considered when interpreting the results. Additional tests, such as complete blood count (CBC) or genetic testing, were conducted if warranted.

Results

This study examined the evaluation of zinc and iron deficiencies in 100 children between the ages of 3-12 years at Hayatabad Medical Complex in Peshawar, Pakistan. The participants were categorized into three separate age groups: 3-5 years, 6-8 years, and 9-12 years. In the entire samples, 45% were male, while 55% were female. The study sought to assess the nutritional status of these children by examining a range of biochemical markers, such as serum iron, ferritin, transferrin, total iron-binding capacity (TIBC), hemoglobin, hematocrit, serum zinc, plasma zinc, and zinc protoporphyrin (ZPP).

The participants were greatly concerned about iron deficiency. 20% of the children exhibited serum iron levels below the normal range (<60 mcg/dL), suggesting a susceptibility to iron insufficiency. In addition, it was found that 16% of the participants exhibited low serum ferritin levels, specifically below 12 ng/mL. This suggests a depletion of iron stores. Some of the children also had abnormal TIBC levels. About 18% of them had levels below the normal range (<240 mcg/dL), while 7% had elevated levels (>450 mcg/dL). This indicates a possible iron overload or deficiency.

There were some worrisome trends observed in the hematological parameters. It is quite common to observe hemoglobin levels below 11.5 g/dL and hematocrit levels falling below 35% in many children, which are indicative of iron-deficiency anemia. Based on the analysis of hemoglobin levels, it was observed that a significant portion of children, 58% to be precise, were found to have iron deficiency. Another 27% were categorized as borderline, while only 15% had normal iron levels.

There was also a high prevalence of zinc deficiency among the children. Approximately 30% of the participants displayed serum zinc levels below 70 mcg/dL, indicating a prevalent nutritional deficiency. In addition, it was found that 35% of the children had plasma zinc levels below the normal threshold, which suggests that their zinc intake is insufficient. Zinc and iron deficiencies were confirmed by the presence of elevated ZPP levels (≥ 70 mcg/dL) in 15% of the children.

When examining the distribution of zinc deficiency across different age groups, it becomes evident that the 5–8-year age group exhibited the highest prevalence of zinc deficiency at 50%. This was closely followed by the 3–5-year age group, which had a prevalence of 40%. Lastly, the 8–12-year age group showed a prevalence of 33.33%. The rates of borderline zinc deficiency were particularly high, especially among children aged 8–12, with 40% of them classified as borderline.

The dietary patterns of the participants, as observed in the questionnaire, revealed a restricted consumption of foods that are rich in nutrients. A notable proportion of the children had infrequent intake of fruits, vegetables, dairy, and protein sources, with a considerable number reporting sporadic consumption. As an expert in the field, it is fascinating to observe that a mere 15% of the children incorporate fruits such as apples and bananas into their daily diet, while a significant portion of 20–25% rarely or never consume these nutritious fruits. In the same vein, the regular intake of vegetables among children was quite low, as only 30–35% of them consumed vegetables such as carrots and broccoli daily.

The frequency of consuming protein sources like chicken, fish, and eggs was comparable. A mere 15% of the children included chicken in their daily diet, while a greater percentage consumed it 1–3 times per week. These dietary patterns indicate a possible connection between insufficient nutrient consumption and the observed deficiencies in micronutrients.

Table 1: Serum Iron Results Analysis

Serum Iron Level (mcg/dL)	Count	Percentage (%)
< 60	20	20%
60 – 170	78	78%
> 170	2	2%
Total	100	100%

Serum Iron Analysis: 20% of children had serum iron levels below the normal range (<60 mcg/dL), suggesting iron deficiency. Most children (78%) had levels within the normal range, but this still leaves a significant portion at risk.

Table 2: Serum Ferritin Results Analysis

Serum Ferritin Level (ng/mL)	Count	Percentage (%)
< 12	16	16%

12 – 150	83	83%
> 150	1	1%
Total	100	100%

Serum Ferritin Analysis: 16% of participants had ferritin levels below 12 ng/mL, a clear marker of iron deficiency. Low ferritin levels, even in the presence of normal serum iron, indicate depleted iron stores, which can be a precursor to iron deficiency anemia.

Table 3: Transferrin and TIBC Results Analysis

TIBC Level (mcg/dL)	Count	Percentage (%)
< 240	18	18%
240 – 450	75	75%
> 450	7	7%
Total	100	100%

Transferrin and TIBC Analysis: High TIBC levels were observed in 7% of participants, which is often associated with iron deficiency, as the body increases iron-binding capacity in response to low iron availability. Conversely, 18% of children had TIBC levels below normal, which can indicate inadequate protein intake, a component of malnutrition.

Table 4: Hemoglobin Results Analysis

Hemoglobin Level (g/dL)	Count	Percentage (%)
< 11.5	25	25%
11.5 – 13.5	70	70%
> 13.5	5	5%
Total	100	100%

Hemoglobin Results Analysis: This table presents the distribution of hemoglobin levels among the participants. The data shows that 25% of the children had hemoglobin levels below the normal range (<11.5 g/dL), indicating a significant prevalence of iron-deficiency anemia likely due to malnutrition. The majority (70%) fell within the normal range, while 5% had levels above normal.

Table 5: Hematocrit Results Analysis

Hematocrit Level (%)	Count	Percentage (%)
< 35	22	22%
35 – 45	73	73%
> 45	5	5%

Total	100	100%
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Hematocrit Results Analysis: This table shows the hematocrit levels among participants. The results indicate that 22% of the children had hematocrit levels below the normal range (<35%), further supporting the presence of iron deficiency. The majority (73%) had normal hematocrit levels, with 5% exceeding the upper limit, suggesting possible dehydration or other factors

Table 6: Serum Zinc Results Analysis

Serum Zinc Level (mcg/dL)	Count	Percentage (%)
< 70	30	30%
70 – 120	60	60%
> 120	10	10%
Total	100	100%

Serum Zinc Analysis: 30% of children had serum zinc levels below 70 mcg/dL, indicating zinc deficiency. Given zinc's crucial role in growth and immune function, this deficiency is particularly concerning in a pediatric population.

Table 7: Plasma Zinc Results Analysis

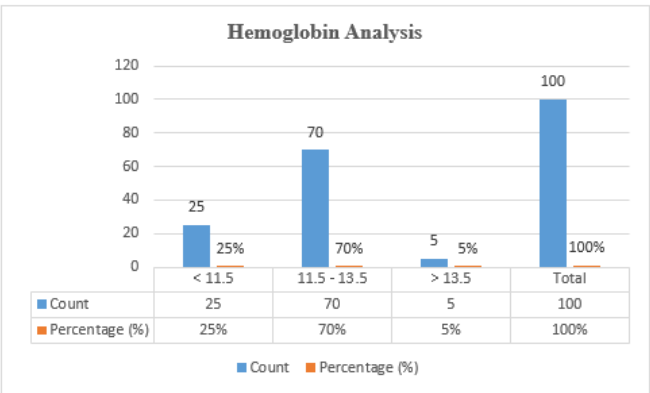
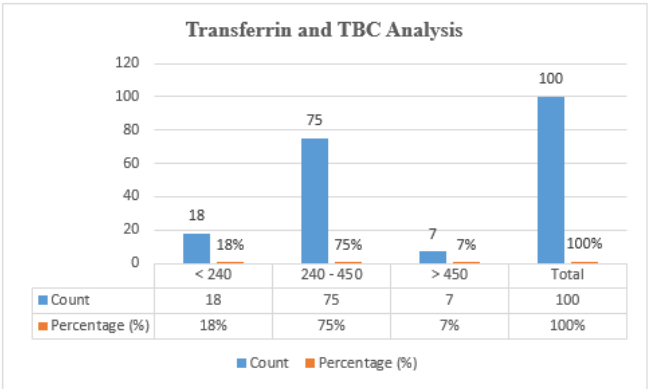
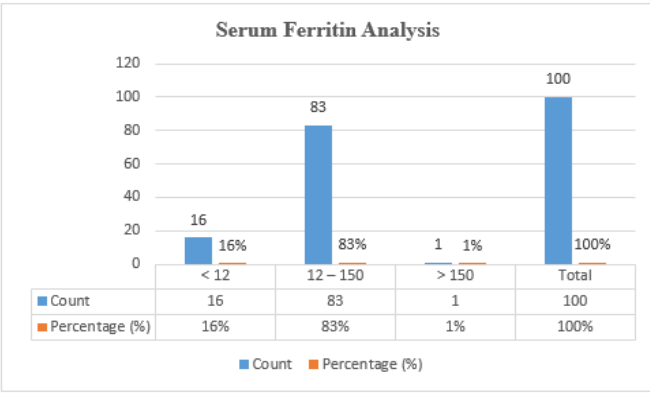
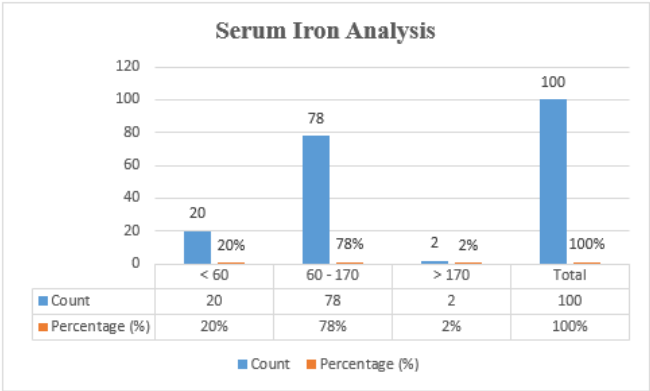
Plasma Zinc Level (mcg/dL)	Count	Percentage (%)
< 70	35	35%
70 – 120	58	58%
> 120	7	7%
Total	100	100%

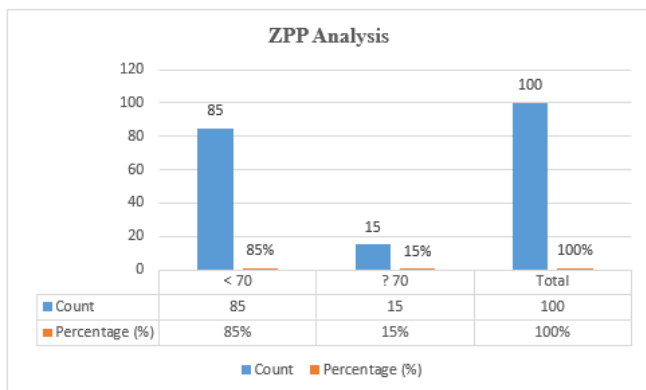
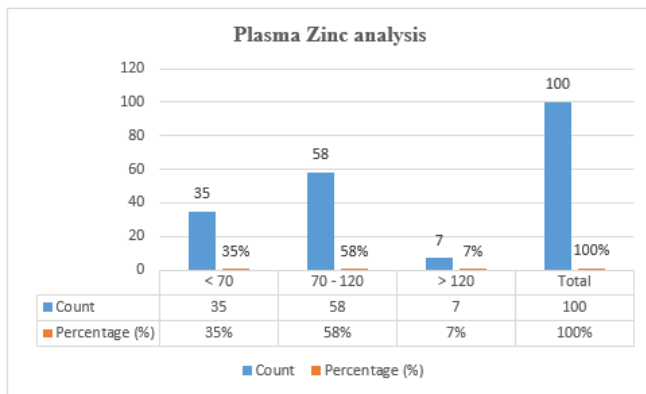
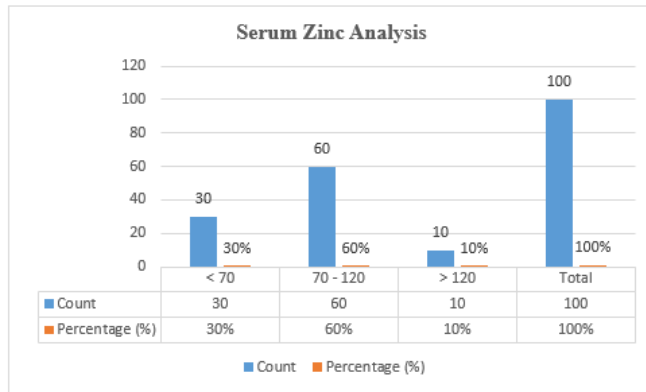
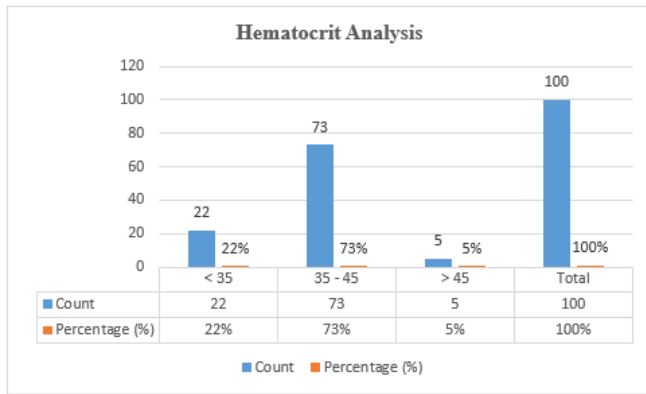
Plasma Zinc Analysis: Similarly, 35% of children had plasma zinc levels below the normal range, further supporting the finding of widespread zinc deficiency.

Table 8: Zinc Protoporphyrin (ZPP) Results Analysis

ZPP Level (mcg/dL)	Count	Percentage (%)
< 70	85	85%
≥ 70	15	15%
Total	100	100%

Zinc Protoporphyrin (ZPP) Analysis: Elevated ZPP levels (≥70 mcg/dL) were found in 15% of participants. Elevated ZPP is typically a sign of either iron deficiency or zinc deficiency, suggesting that a significant subset of the population is suffering from one or both of these deficiencies.





Discussion

The research conducted at Hayatabad Medical Complex in Peshawar, Pakistan, offers valuable insights into the nutritional status of children aged 3-12 years, with a specific emphasis on zinc and iron deficiencies. Based on the results of this study, it is evident that there is a significant occurrence of zinc and iron deficiencies among these children. This has

important implications for their overall health and development.

According to the findings of this study, it was observed that 25% of children had hemoglobin levels below the normal range. These results are consistent with global research, which highlights the persistent issue of anemia, primarily caused by iron deficiency. As an example, research conducted in Guatemala by Palacios et al. (2020) revealed that 56% of children under the age of five were found to be anemic. Interestingly, there was a significant correlation between anemia and zinc deficiency, as 53.7% of the anemic children also exhibited low levels of zinc. Atasoy and Bugdayci (2018) found a strong correlation between zinc deficiency and anemia in children. Their study revealed that 31.5% of zinc-deficient children were anemic, while only 2.9% of the zinc-sufficient group showed signs of anemia (96). The findings highlight the interconnectedness of zinc and iron deficiencies, as observed in the Peshawar study.

Based on the study conducted in Peshawar, it was observed that around 30% of children had serum zinc levels below normal, which aligns with similar findings in other regions. In a study conducted in South Africa by Motadi et al. (2023), it was discovered that 27.9% of children suffered from zinc deficiency. This finding highlights the widespread impact of this micronutrient deficiency on a global scale. On the other hand, the prevalence observed in Peshawar exceeds that reported in Western European countries. In a study conducted by von Salmuth and Vreugdenhil (2023), founded a zinc deficiency prevalence of 9.62% among children aged 1-3 years. These variations can be explained by disparities in eating habits, economic conditions, and public health measures in these areas.

On the other hand, the iron deficiency results from Peshawar, where 16% of children had low ferritin levels, which indicates depleted iron stores, are consistent with what is seen in high-risk populations around the world. The occurrence of iron deficiency aligns with the findings of Aabdien et al. (2022) in Qatar, where 26.4% of adolescents were identified as lacking iron. On the other hand, the Peshawar study reveals a lower occurrence of severe iron deficiency in comparison to regions with higher rates of malnutrition and infectious diseases. For instance, Sobeih's et al (2023) studied Africa found that up to 37% of children suffered from iron deficiency.

In addition, the study emphasizes the potential influence of a varied diet on the levels of essential nutrients in the body. The observed zinc deficiency prevalence in Peshawar aligns with findings from marginalized communities in rural Pakistan, where limited dietary diversity was associated with a high prevalence of zinc deficiency. Bevis et al. (2023) discovered a correlation between soil zinc deficiency in Nepal and child stunting, highlighting the importance of both environmental and dietary factors in determining zinc status.

The study's results in Peshawar are consistent with global and regional data on the occurrence of zinc and iron deficiencies, while also shedding light on the distinct obstacles

encountered by this population. Based on the data, it is evident that malnutrition, specifically caused by insufficient consumption of vital micronutrients such as zinc and iron, continues to be a noteworthy issue in public health. The parallels and differences with other studies emphasize the necessity of focused nutritional interventions and public health strategies to tackle these deficiencies and enhance the overall health outcomes of children in this region.

Conclusion

The study conducted at Hayatabad Medical Complex in Peshawar emphasizes the notable prevalence of zinc and iron deficiencies among children aged 3-12 years, which mirrors wider global and regional patterns. The findings highlight the interconnectedness of these deficiencies, especially their link to Anaemia, and emphasize the pressing requirement for focused nutritional interventions. Although there may be some differences in different areas, the fact that these deficiencies are consistently found in various settings highlights the importance of having a varied diet and implementing public health measures to tackle malnutrition and enhance the health of vulnerable populations.

Limitations

- As our research was limited to only one hospital in the region that's why we got the less amount of data.
- As we had done the random convenient sampling that's why the research can be affected by selection bias.
- Accurate diagnosis of deficiencies can be difficult due to overlapping symptoms with other conditions.
- Due to cross sectional study, we can continue this research further as we have done this research with a smaller number of parameters.
- We should had considered large number of parameters but the time was limited.
- Factors such as infections, chronic diseases and other nutritional deficiencies can complicate the analysis of zinc and iron deficiency specially.
- Small sample sizes are usually selected by most of the researchers which slightly affects the accuracy of the results.
- Socioeconomic status is very sensitive factor that affects the results of the research.

Recommendations

As we have done a descriptive study in Hayatabad Medical Complex Peshawar. We will suggest to future researchers that they should do the same study with the larger sample size and consider the other hospitals as well in the region.

More accurate results will be obtained.

On the other hand, dietary recommendations are as follow:

- Increase dietary intake of zinc and iron from different food groups.
- Enhance nutrients absorption.
- Monitor and diagnose.
- Educate and raise awareness.

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Declarations:

Authors' Contribution:

- a-b-c Conceptualization, data collection, interpretation, drafting of the manuscript
- d-e Data collection and intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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