Assessment of Trace Elements in Gastrointestinaltract and Skin Disorders

By

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Chapter 1

Introduction

I. INTRODUCTION

The essential trace elements can be defined simply as those elements necessary in the diet of humans or animals in trace amounts (less than 100 mg per day for human). Minerals are those elements that remain largely as ash when plant or animal tissues are burned. About 4% of the body weight consists of mineral matter (Briggs, 1979).

About 100 elements have been identified out of which 50 have been found to be present in human body. Those which have been proved to be essential constituents of living tissues are classified as major or minor elements on the basis of the amounts present in biological sample.

Those trace element (micronutrients) with no known function for either humans or laboratory animals are often referred to as trace contaminants (Clara Mixon Lewis, 1986).

Major elements are sodium, potassium, calcium, magnesium, silicon, chloride and phosphorus. These are required in the diet in levels of mg/day (Alfinslater and Mienda, 1980).

Minor elements are Iron, copper, cobalt, zinc, manganese, molybdenum, Iodine, Boron, Fluorine, selenium and Chromium. These are also required by the human body and these are required in µg quantities/day (Briggs and calloway, 1979).

Trace contaminants are cadmium, Lead, Arsenic, Barium, Strontium, Mercury, Boron, Aluminium, Lithium, Beryllium and others (Clara mixon Lewis, 1986).

The trace element field is one of the most active and interesting areas of nutrition today with important discoveries constantly being made. These serve as structural components of enzymes, vitamins, hormones and other protein containing tissues (Cameron, 1984).

Permeability of cell membrances, osmostic pressure and acid base balance are regulated in the presence of mineral elements in body fluids. Nerve response to stimuli and muscle contractility require the presence of mineral elements (Alfinslater et al., 1980).

The amount of inorganic material contained in foodstuffs varies with the material and in the case of plants with the type of soil, fertilizer etc. Approximately 4.4 per cent of the total weight of the body consists of inorganic compounds. The bones contain from 22 to 82 percent, whereas the muscles and the body fluids contain about 1 percent.

Cereals and vegetables are the chief sources of supply of the trace elements (Solomons, 1989).

In certain restricted areas in many parts of the world, animals and man have suffered from various debilitating diseases with well marked clinical and pathological manifestations that have been shown to be caused by naturally occurring trace element deficiencies, toxicities, and imbalances (Wenc, et al., 1983).

Gastroenteritis is a common disease causing morbidity and mortality amongst pediatric age groups(Kasliwal et al., 1982). Acute gastro-enteritis is a common clinical syndrome in our country. The etiological agents responsible for it are too many and still in a large number of cases, no causative agent could be identified (Vinod Kumar & Suman Kirti, 1981). Further, it has been found that common pathogens responsible for gastroenteritis vary in different parts of the country.

Toxicity due to metal compounds under acute and moderately severe chronic conditions can be distinguished by outward clinical symptoms. Toxicity due to chronic exposure to very low doses of matallic toxicants is difficult to diagnose, especially when clinical or outward symptoms are not well pronounced. Under these conditions blood, cerebrospinal fluids, and available excretory products of metabolism such as faces, urine, skin, nails and hair are analysed to identify and assess the dosage of the toxicant(Luckey and Venugopal, 1977).

The study of trace elements in the treatment of disease of the gastrointestinal tract will help to

1. Gain information about the special functions of the GITract **

2. Develop an understanding of the nature of some of the disease of the GITract

3. Develop a knowledge of relationship of nutrition and diet to the treatment of the different diseases (Martha Davis Dunn, 1983).

The GIT includes the esophagus, stomach, and intestines which perform critical functions in the digestion and

** GITract = Gastrointestinal tract

absorption of nutrients. The GITract might be compared to a most sensitive tube in the human body which responds to physical and psychological factors. The responses of the esophagus, stomach and intestines also reflect and are interrelated with the emotions and tensions of each individual (Martha Davis Dunn, 1983).

The drugs like Nalidixic acid are used for the treatment of GITract disorders. Gramoneg showed superiority in this trail over chloramphenicol, streptomycin, furazolidine and in some cases also over gentamycin (Kashiwal et al ., 1982).

The skin is the barrier as well as the principal organ of communication between the man and his environment (Montagna, 1982). It is a major organ of the body forming about 8% of the total mass. It has rich nerve supply which is concerned with sensory and thermo regulatory functions (William et al., 1989). The skin is divided sharply into 3 distinct layers.

i. a thin outer layer of epithelial tissue, the epidermis.

ii. a much thicker layer of connective and other tissues, the dermis, and

iii. Substaneous tissue consisting of adipose and areolar tissue ' the flesh'.

Starvation of patients with burns is a well - recognized cause of morbidity and mortality. Limited studies indicate that epithelialization of burns may be improved by treatment with zinc (Prasad, 1979).

The development of laser use in dermatology has been and continues to be, a mixture of serendipity and science shortly after the invention of lasers in 1960, the pioneoring studies of Leon Goldman established that the Ruby laser, a pulsed red laser, could be used for treatment of port-wine stains and benign pigmented lesions (R.Rox Anderson and Parrish, 1988).

The interesting and often close connection between the skin and the gut has been well reviewed by wormsley in a monograph. The association can arise in three ways:

a. There are diseases which primarily affect the alimentary tract with secondary or associated cutaneous manifestations.

b. There are generalised disease processes involving both the skin and the alimentary tract.

c. There is increasing evidence that some primary skin diseases affect the structure and function of the intestine (Jewell, 1988).

The crucial initial steps in trace-metal analysis, such as sample preparation and ashing and metal isolation and concentration, have been improved considerably. Instrumentation technology has developed atomic absorption, fluorimetry, emission spectroscopy, neutron activation, X-ray fluorescence, electron microscope, and spark source mass spectroscopy techniques into versatile and highly sensitive tools for the determination of metals in biologic sample (Luckey and Venugopal, 1977).

Hence an attempt has been made in this study to estimate the trace elements like zinc, iron, copper and manganese in the serum and urine of different types of GITract and skin disorders by Piper's method (1966) using atomic absorption spectrophotometer in order to see if they could serve as markers in the early diagnosis of GITract and skin disorders.

Chapter 2

Review of Literature

II REVIEW OF LITERATURE

The literature related to the investigation "Assessment of trace elements in Gastro intestinal tract and skin disorders" is presented under the following headings.

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1. Nutritional Importance of Minerals:

More than 60 elements have been discovered in bacteria, fungi, higher plants, animals and man, few of them have been studied intensively.

There are more than 25 minerals and trace minerals important to the body's nutrition and health. Nineteen of these are essential nutrients (Solomons and Young, 1988). Trace elements, micronutrient elements or minor elements are terms applied to the remaining elements or minor elements are terms applied to the remaining elements occurring constantly in biologic systems(Trowbridge, 1988).

Digestion and healthful assimilation of foods depend upon adequate "mineralisation" in the system (Gary and Steve Null, 1972). In the absence of a specific element, a deficiency state develops on diets otherise adequate and satisfactory, i.e., containing all other dietary essentials in adequate amounts and proportions and free from toxic properties. Dietary supplementation of this specific element alone reverses the deficiency state, resulting in repeated and significant responses in growth and health.

With some elements, the deficiency state has been correlated further with the finding of subnormal concentrations of the element in the blood or organs of the deficient animals and of altered metabolism (Alpers et al., 1987).

Excess amount of essential minerals can be toxic. Thus, it is important that we obtain minerals in our diets in properly balanced amount (Wenck et al., 1983).

In humans the elemental nutrients act in four ways:

1) as strctural components
2) as charged ions
3) as components of metalloenzymes, and
4) as miscellaneous effectors in small molecules (Paige 1988)

Substantial proportions of the body's magnesium and zinc are incorporated into bones, but their physiologic roles in this context are not well understood.

Certain trace elements (Mertz. W, 1981) are indispensable components of metalloproteins and metalloenzymes: hemoglobin (iron), alkaline phosphatase (zinc), ceruloplasimin (copper), glutathione peroxidase (selenium), pyruvate carboxylase (manganese), and xanthine oxidase (molybdenum). Other trace elements are found in various small molecules: Vitamin B12 (cobalt) and thyroxine / Triiodothyronine (iodine). Others functions as a soluble complex (chromium).

The zinc content of a mixed diet in the United States, for example, is about 3 mg/1000 kcal (Solomons, 1982). An adult would have to consume 5000 kcal to ingest 15 mg of zinc as specified by the RDA (NRC, 1980). Moreover, some dietary forms of nutrients are poorly absorbed and a nutrient's biologic availability varies according to its source. For example, muscle meats and blood are good sources of available iron, but iron from vegetable sources is less well absorbed.

Elemental nutrients are important in fulfilling the body's requirements for growth and maintenance. However, the dietary levels of certain elements may influence heath in other ways.

The dietary ratio of zinc to copper may influence cholesterol concentrations and atherogensis, with high zinc : copper ratios being detrimental (Klevay, 1989). seleniferous soils are associated with lower rates of certain malignancies in human (Schrauzer, 1977).

2. Trace elements and their role:

a. Iron:

i. Nutritional and Biochemical role of iron:

When dietary iron level or bioavailability becomes insufficient to meet the needs of a normal individual or one whose needs are elevated due to blood loss or other reasons, the body responds first by drawing upon iron stores. As stores become progressively depleted over time, the efficiency of dietary iron absorption increases (Crosby and O'Neil-Ca Hing, 1984). When this adjustment is not sufficient to remedy the situation, serum ferritin may drop below 12mg/L and essential iron in the tissues will decrease (Finch and Cook, 1984).

Dietary intake of iron for adult females is 18 mg and for adult males is 10 mg daily (Martha Davis Dunn, 1983). Asparagus, Beans, green, Broccoli, Lettuce, romaine, liver, calves, spinach (fresh, cooked) wheat bran (commercially

milled) are the rich sources of iron (2.0 mg iron / 100 kcal). Beef, Bread (White enriched) chicken, Eggs (whole), Raisins (uncooked, sugar (brown), wheat flour (white, enriched, whole grain) contain medium iron content (0.7-1.9 mg iron / 100 Kcal). Apples, raw, Bologna, frankfurters, boiled milk, potato chips, rice ,sugar (white) contain (< 0.7 mg iron/100 Kcal) (Thompson, 1988).

Enzymes such as the catalases, the cytochromes in hydrogen iron transport and xanthine oxidase contain iron as an integral part of the molecule. Iron is required as a co-factor for other enzymes (Robinson and Lawler, 1982). Enzymes (called cytochromes) in the electron transport system contain heme, without it they are unable to participate in the oxidation of carbohydrate, lipids, and protein for energy. Other enzymes involved in oxygen utilization also are heme containing (Wilson et al., 1971)

ii. **Absorbtion of Iron:**

Iron is absorbed primarily in the small intestine (Charlton and Bothwell, 1983). Iron in foods are much better absorbed than others. Heme iron is absorbec directly as the heme complex, and its iron is then released in the intestinal mucosal cells and subsequently be moved across the

cell, across the serosal membrane, and finally to the blood, where it is picked up by transferring and presumably becomes indistinguishable from previously absorbed iron. It is important to realize that absorption is the sum of uptake and transfer (Bjorn Rasmussen et al., 1974).

Due to the rapid turnover of the mucosal cells, uptake does not imply absorption. If the mucosal iron is not transferred before the cell is sloughed into the lumen, this iron is no longer part of the organism, rather it contributes to the lumenal contents (de Bruin, et al., 1970).

Most of the iron in food is also in the form of nonheme iron (S.R. Lynch, 1984). This form must be soluble and in ionic form to be absorbed. Stomach acid solubilizes both ferrous ((Fe^{2+}) and Ferric (Fe^{3+}) iron, but generally ferrous iron is better absorbed than ferric iron. Many food components from soluble chelates such as ascorbic acid with ferrous or ferric iron aiding in absorption (Gorman and Clydesdale, 1983)

The percentage of iron absorbed from either heme or nonheme iron is generally influenced by the extent of body iron stores. Men having markedly larger stores of iron than women, absorb lower percentages of both nonheme and heme iron than do women (Layrisse et al., 1974).

iii. **Excretion of iron:**

The daily excretion of body iron by adults is about 0.1 mg from the urine (Madhavan Nair, 1990) and 0.3 to 0.5mg in to the intestinal lumen. Small amounts of iron are also lost in the perspiration and by exfoliation of the skin. The iron losses through menstruation range from 0.3 to 1.0 mg on a daily basis, but about 5 per cent of women have losses in excess of 1.4 mg daily. Thus the total iron losses by women are 1 to 2 mg daily (Robinson and Lawler, 1982).

iv. **Levels in normal and changes in pathological conditions:**

Iron is an essential micromineral for the human body. The normal amount of iron present in blood of adult is 75-175 mg/dl) (Barland et al., 1980).

Beard and Finch (1985) have recently pointed out an important distinction between the cinical and the public health definition of the iron deficiency. Clinical iron deficiency will be manifested as the anaemia of iron deficiency (Narasinga Rao, 1990). The fnctional implication of iron deficiency have recetly been reviewed (Vyas and Chandra, 1984).

The " Bantu siderosis" appears to result from maize sorghum beverage, which can contain 40 mg iron/L. Moreover

this iron is present in a highly available form (Derman et al., 1980). Death mayoccur with 4-6 hours due to severe necrotizing gastroenteritis (Lynch, 1984) The daily intake levels of 25 to75 mg are unlikely to cause problems (Finch and Cook, 1984).

v. **Changes in GITract and Skin disorders:**

Nutritional siderosis :- Excessive ingestion of iron in elderly persons subsisting on marginal diets has resulted in so called nutritional siderosis. In patients with this condition, excessive iron has been found in the small intestine. The bone narrow generally shows an erythroblastic hypercellular state. Extensive liver diseases occur in this condition usually and there is extensive deposition of iron in the liver (Grace and Powell, 1974).

Idiopathic Hemochromatosis:

In idiopathic hemochromato sis, which is characterized by deposition of iron in the liver, pancreas, heart, and pituitary with resulting impaired function together with associated skin pigmentation (due to melanin) and hypogonadism (not due to iron depletion) there is a defect in absorption of iron by the small itestine (Alfinslater etal., 1979).

b. Zinc:

i. Nutritional and Biochemical role:

The 1980 RDA for zinc is 15 mg/day for people 11 years of age and older (NRC, 1980) but zinc available in the food supply amounts to only 12.3 mg per capita.

Normal, well balanced adult diets supply an average of 10-15 mg Zinc daily with the actual amount greatly influenced by protein content because of the association of zinc with the protein in foods and by refined cereal or white flour content because of the high concentration of this metal in the germ and "branny" layers of the grain and its removal therefore in the milling process (Underwood and Platell, 1979).

Zinc has been associated with several human disorders including beriberi, nephrosis, hepatic porphyria and post alcoholic cirrhosis of the liver.

Common foods vary greatly in zinc concentration. In general most (in particular organ meats) eggs, shellfish, and some vegetables are high in zinc. In contrast milk fruits, and other vegetables are reported to be lower in zinc content (Swanson and King, 1979).

Many dietary factors including other minerals, phytates, and dietary fiber, may adversely affect zinc absorption (Hambidge et al., 1986).

Zinc has been shown to have roles in enzyme functions (Vallee, 1983) structural integrity (Chvapil, 1976) and regulation of hormonal actions under normal conditions and during infectious episodes (Sugarman, 1983).

Zinc has well established roles in such basic enzymatic functions and DNA and RNA polymerases. Moreover it has been suggested that it may actually stabilize these nucleotides (DNA, RNA) as well (Solonmons, 1982).

Thymidine Kinase is yet another critical enzyme where zinc is involved biochemically and such basic involvement in nuclear, biochemical and regulatory events may partially explain the rapidly replicating tissues and cell types, i.e. intestinal and mucosal epithelia, may be particularly sensitive to zinc deficiency (Chvapil, 1976).

Zinc is an essential cofactor in the enzymatic profiles of over proteins from a wide variety of species. These enzymes have roles in the synthesis and degradation of all major cellular constituents. In fact, proteins, carbohydrates,

lipids and nucleic acids and have representative enzymatic functionalities which include zinc metalloeyzymes in their various metabolic pathways (Bettger, 1981). Some examples of these are collagenases, anhydrases, aldolases, ligases, carboxylases, creatinases, anhydrases, peptidase and dehydratases (Clara Mixon Lewis, 1986). Other enzymes activated by zinc include a variety of dipeptidases and aminopeptidases (Martin et al., 1981).

ii. Absorption of Zinc:

The prototypical "adult 70 kg man" contains an average of 1.5 - 2.5 gms of zinc. Zinc absorption is approximately 30% efficient, thus with an intake of 15mg/day (the current RDA), the average net absorption, barring other abnormalities, would be roughly 5 mg. Absorption for the most part, takes-place in the small intestine (Antonson et al., 1979).

Zinc absorption is homeostatically regulated. More recently, a second brush-border-membrane protein has been identified as being involved in the zinc absorption process ((Menard and Cousins, 1983). The actual process of zinc absorption appears to be regulated by a carrier mediated mechanism(Solomons and Cousins, 1984).

Zinc absorption is increased during pregnancy and lactation (Devies and Williams, 1977). Protein and amino acids have been shown to quantitatively increase zinc absorbed from the diet (Wapnier et al., 1983).

Perhaps one of the most controversial areas in the field of zinc absorption/nutrition has been the nature of the zinc binding ligands in human and bovine milk. The value of human milk zinc is useful for the treatment of acrodermatitis enteropathica (Moynahan, 1974).

iii. **Excretion of Zinc:**

Zinc is lost from the body predominately via the GITract with losses in the urine (Burch et al., 1975 and Sweat Bauer et al., 1984) as relative minor components. Zinc in the feces is a reflection of dietary zinc, pancreatic zinc, zinc from intestinal secretions, and sloughed intestinal mucosal cells. Hence one of the difficulties in the use of radio isotopes or stable isotopes of zinc as tracers in the study of absorption/distribution process is the fact that the specific activity of the isotope may infact be influenced by this endogenous secretory compartment. Estimates have been made that as much as 10-50% of the zinc in the feces may infact, be endogenous in origin (Smith, 1988).

iv. **Levels in normal and changes in Pathological conditions:**

Zinc is present in all living tissues. The normal concentration of zinc in the serum is 90-116 mg/dl (Tietz, 1986).

Common to the syndrome of zinc deficiency are a number of dominant features like retarded growth, loss of appetite, hypogonadism, dermatitis, alopecia, and resistance to infection (Sandstead et al., 1976). Not only is the sense of taste impaired, but smell and sight (night blindness) have also been identified as result of zinc deficiency (Morrison et al., 1978).

Similarly wound healing, Ulcerations (both external and internal) and parakeratosis have also been related to zinc deficiency (Larson, 1974).

In some cases, a rise in serum zinc values well above normal have been documented. In fact there is one extreme case of an individual who was exposed to dialysis fluid contaminated by stroage in a galvanized tank (Gallery et al., 1972). In this particular case, the serum zinc rose to over 400mg/dl and yet no toxic symptoms were obtained. In general most zinc salts are gastro intestinal irritants, and thus the immediate reflex response of nausea and vomiting may serve to self-limit exposure via the oral route. In a response known as metal fume fever, the inhalation of zinc metal (normally zinc oxide) results in a series of symptoms including weakness, fever, pain and hyperventilation (Moynahan and Barners, 1973).

Zinc salts in very large amounts, 60 to 120 times the recommended allowances will induce vomiting and cramps, but the sysmptoms subside shortly.

Changes in GITract and skin disorders:

Dietary zinc deficiency has been described which is characterized by iron deficiency anemia, hepatosplenomegaly, dwarfism, hypogonadism, low plasma zinc, increased plasma zinc turnover rate, decreased 24 hours exchangeable zinc pool, decreased zinc excretion in stool and urine, and decreased zinc in hair (Prasad, 1983).

The effect of zinc deficiency in GITract is due to small intestinal alkaline phosphatase (Alpers, 1988).

An acute zinc deficiency has been described in patients which developed 2-5 weeks after the start of a high caloric infusion. The patients becoame apathetic, depressed, and developed a facial rash, diarrhoea, and alopecia (Holman, 1971). In acrodermatitis enteropathica in which lesions occur on the hnds, feet, face, circumorally, and circumanally, there is an associated diarrhoea (Crofton et al., 1983, Chandra 1980) The disease is caused by defect in zinc abscrption and the condition is treated with pharmacological doses of

zinc (Golden et al., 1980). Steatorrhea may be the most common mechanism for zinc deficiency in patients with GITract disease.

Zinc also seems to be necessary for the release of retinol-binding protein (RBP) from the liver (Smith et al., 1974). In zinc deficiency a lowered RBP might result in inadquate treatment of vitamin A to peripheral tissues, with a resultant defect in the glycoproteins of cell membrances. Whether superoxide dismutase and/or vitamin A are involved in the pathogenesis of zinc deficiency (Robert H. Herman, 1979).

Problems in zinc nutrition have been identified in patients with malaborption, kidney disease, pancreatic insufficiency (Boosalis et al., 1983). Sickle cell anemia, and inflammatory bowel diseases (Prasad, 1983).

In psoriasis, the loss of large numbers of skin cells may results in zinc depletion. The skin contains approximately 20% of the body zinc(Prasad, 1979).

C. COPPER:

i. Nutritional and biochemical role of Copper:

Uncomplicated copper deficiency in man has never been demonstrated unequivocally, although hypocupremia, associated with iron deficiency anemia and hypoproteinemia, has been observed in infants (Joffe et al., 1981). Infants maintained

on nearly exclusive milk diets are liable to develop this multiple deficiency syndrome, which invariable responds to an improved diet. The hypocupremia, but not the anemia and hypoproteinemia, generally responds to copper therapy (Prasad et al., 1978).

The cause of the low blood copper level is obscure. It has been suggested that it may be due to a copper depletion in the infants or a failure to synthesize apoceruloplasmin or to an increased rate of destruction or loss of this protein from the body (Hillman et al. , 1981). Hypocupremia does not necessarily arise in infants on exclusive milk diets (Solomons, 1985).

The Board recommends an intake of 0.08 mg per kg of body weight for infants and children (Jean Bogert et al., 1973). The estimated safe and adequate intake proposed by the food and Nutrition Board is 2 to 3 mg/day for the adult, and that preadolescent girls maintain adequate balance with 1.3 mg per day.

Foods highest in copper include oysters liver, dried yeast, chocolate and lobster. Good sources are crabmeat, fresh vegetables and fruits, nuts, seeds, and legumes. Foods very low in copper are refined sugars, cereals and milk (Wilson et al., 1971).

Copper has been identified as a constituent of a number of enzymes: butyryl coenzyme a dehydrogenase required for the oxidation of fatty acids; tyrosinase required for melanin pigment formation; uricase in purine metabolism, and in the cytochrome oxidation system for energy production (Solomons 1985). Several copper containing proteins such as hepatocuprein and erythrocuprein help protect against the toxic effects of oxygen (Sandstead et al., 1979). Lysyloxidase is involved in the synthesis of elastin and collagen. Superoxide dismutase has an important role in protecting the cell against oxidative damage (Klevay and Forbush, 1976).

ii. **Absorption of Copper:**

Copper is absorbed chiefly from the stomach or duodenum. About 30 percent of dietary copper is absorbed by adults. Its absorption is inhibited by phytates. About 95 per cent of the copper in blood plasma is firmly bound to a protein complex ceruloplasmin and 5 per cent is loosely bound to albumin. Molybdenum, Zinc, and cadmium are antagonistic to copper, thus, an increased intake of these elements increases the requirement for copper. Vitamin C can also decrease the absorption of copper by decreasing its ability to bind one of the carrier proteins needed for absorption (Solomons, 1989).

iii. **Excretion of Copper:**

Almost all of the excretion of copper is in the feces, chiefly through the excretion of bile (Corinne H. Robinson, et al., 1986). Urinary loss is usually insignificant.

iv. **Levels in normal and changes in pathological conditions:**

Copper is an essential micromineral. The amount of copper present in serum is 75-140 µg/dl in normal adult (Devidson et al., 1979).

Copper deficiency can exist in special situations in milk fed infants (Graham et al., 1976). Such deficiencies on infants are most generally complicated with chronic diarrhoea or metabolic diseases, while deficiencies in adult man are unknown either naturally or experimentally (Jean Bogert et al., 1973). Long-term treatment of patients with sickle cell anemia with zinc supplements (150 mg, 6 x daily) resulted in the appearance of copper deficiency (Prasad et al., 1978).

High dietary copper produced increased pigmentation, increased hair growth, and increased thickness of the skin (Hurely and Bell, 1975). Elevated zinc intakes have been used in the treatment of the copper accumulation diseases such as Wilson's disease(Brewer et al., 1983) a rather rare chronic

metabolic disease in man in which the body has great difficulty in disposing the excess copper, the copper is stored in the liver and other tissues (such as the eyes), finally resulting in toxic concentrations. The level of serum ceruloplasmum is usually very low in this disease. The excess copper in these tissues leads to hepatitis, lenticular degeneration, renal malfunction, and neurologic disorders (Frieden,, 1970).

v. Changes in GITract and skin disorders:

Dietary copper Deficiency:

Dietary copper definciency rarely occurs, but a few cases have been described in infants (al Rashid and Spangler, 1971), and although hypocupremia, hypoferremia, neutropenia, hypoproteinemia, anemia, megaloblastic changes, and scorbutic bone changes occur, there is no known small intestinal abnormality (Danks, 1980). Copper deficiency is more likely to occur during total parenteral nutrition (Vilter et al.,1974).

Manke's Syndrome:

Manke's kinkyhair or steely-hair syndrome is caused by a defect in the GI absorption and transport of copper (Joffe et al., 1981). The overall syndrome consists of a progressive brain disease, culminating in death, pili torti (Kinky or steely hair), an abnormally of elastic fibers in arterial walls, scorbutic bone changes, and hypothermia (Menkes, 1972). The manifestations of the disease are the

result of systemic copper deficiency. The nature of the disease are the result of systemic copper deficiency (Darby, 1982). The nature of the transport defect in the gastrointestinal tract is unknown, but copper accumulates in the small intestinal epithelial cells. Pharmacological doses of copper can be absorbed however (Shike M. et.al., 1981).

The epidermis and increased mortality in early life are characteristics of copper deficiency (underwood, 1971). High dietary copper produced increased pigmentation, increased hair growth, and increased thickness of the skin in mutant offspring (Menkes, 1972).

The decrased copper level in serum might be due to defective from the intestine as a result of skin depigmentation (Bales, 1989). Chromic diarrhoea and short bowel syndrome (Trowbridge, 1988).

d. **Manganese:**

i. Nutritional and Bio Chemical role of manganese:

A dietary deficiency of manganese has never been recorded in man. Important but limited evidence that manganese depletion, that is an induced deficiency of this

element, may occur in hydraliazine or apresoline disease in lupus erythematosis disseminatus in man(WHO, 1981).

The recommended Dietary Allowances of the NAS/NRC (1980) state that safer and adequate intakes of Manganese in infants range from 0.5 to 1.0 mg/day and for adults from 2.5 to 5.0 mg/day.

Wheat bran, blue berries, whole wheat, split peas, beets and navy beans are the richest sources of the mineral in foods (FAO, 1974). The best sources of manganese are plant foods especially cereals, which contain between 10-100 mg/kg (Kanzantzis, 1981).

Manganese is an important catalyst and is a cofactor or component of many enzymes in the body (David Buss and Jean Robertson, 1982). Among them are glycosyl transferase (Leach, 1971), superoxide dismutase (McCord, 1976) and Pyruvate Carboxylase (Utter, 1976). On the basis of its relationship to these enzymes, it is needed for synthesis of complex carbohydrates in cells, for utilization of glucose for lipid synthesis and metabolism, cholesterol synthesis, normal pancreas development, muscle contraction, prevention of skeletal defects, prevention of sterility and other vital functions(Jean Bogert et al.,1973). Arginase is an enzyme which is required for the formation of urea, and a number of peptidases that bring

about the hydrolysis of proteins in the intestine. It is involved in the metabolism of biogenic amines (Hurley and Keen, 1987).

ii. Absorption of Manganese:

Manganese is rather poorly absorbed from the small intestine by a mechanism similar to that for the absorption of iron. It is loosely bound to a protein and transported as "Transmanganin" (Davidson et al., 1988). Tissues that are rich in mitrochondria take up manganese readily from the blood. A dynamic equilibrium exists between the intracellular and extracellular maganese (Robinson et al., 1985),

iii. Excretion of Manganese:

Manganese is poorly absorbed from the gut and is excreted very largely in the feces, most of the metabolic manganese is excreted into the intestine as a constituent of bile, but much of this is again reabsorbed, indicating an effective body conservation. Very little manganese is excreted in the Urine (Underwood, 1978).

iv. Levels in normal and changes in Pathological conditions:

The normal concentration of manganese in serum of adults were found to be 1.2 ± 0.4 µg/dl (Underwood, 1979).

Manganese deficiency of dietary origin occurs in the human. Invariably skeletal development is affected resulting in shortened and often deformed limbs (Pike and Brown, 1970).

Manganese toxicity has been reported in miners of manganic oxide who acquire toxic levels in the mineral through inhalation of ore dust (Ulrich et al., 1979). The toxic syndrome appears to be similar to viral encephalitis and in severe cases is characterised by tremor, a peculiar mask like facial expression, and incoordinated body movements. Dietary manganese appears to be non toxic.

v. **Changes in GITract and skin disorders:**

Manganese deficiency leads to hypocholesterolaemia retarded growth of hair and nails, mild dermatitis, and moderate weight lose (WHO, 1981).

However during the active phase of hepatitis serum concentrations of manganese were invariably elevated (Versieck et al., 1974). The highest concentration of manganese were found in the liver, pancreas, kidney and intestines, (Kitamura, 1974).

Chapter 3

Experimental Procedure

III. EXPERIMENTAL PROCEDURE

The experimental procedures followed in the study, "Assessment of trace elements in Gastrointestinal tract and skin disorders" are described in the following sequence.

1. Selection of subjects
2. Collection of blood and separation of serum
3. Collection of urine
4. Analysis of minerals in serum and urine
5. Statistical Analysis

1. Selection of Subjects:

Forty patients of both sexes suffering from different types of GITract and skin disorders undergoing treatment in various hospitals (G.H, K.G. Hospital, Kovai Medical Centre, Lalitha Hospital) at Coimbatore were selected for the study, of the 40 patients studied, half of them were found to be affected by GITract disorders and the remaining half of them were affected by skin disorders. The patients who had been diagnosed with a histologically confirmed GITract and skin disorders only were included for the investigation.

Those selected with GITract disorders belong to the age group of 35 to 65 years. Among the 20 patients selected with skin disorders one was a 5 year old child and the remaining 19 were between 35 to 55 years.

Twenty healthy persons of matching age and sex and free from diseases were selected as controls.

2. **Collection of blood and separation of serum:**

Venous blood samples from the patients of GITract and skin disorders as well as the normal subjects were collected according to the method of (Varley et al., 1980).

The arm was extended and a tourniquet was fastened firmly a few centimeters above the elbow without obliterating the arterial pulse at the wrist. The skin over the vein was sterilised. A disposable sterile needle fixed on to a disposable syringe of approximate capacity 5.0 ml was inserted. When the needle entered the plunges was withdrawn slightly. When the desired amount of blood had been drawn into the syringe the tourniquet was released and a small pad of cotton wool soaked with spirit was placed on the arm and the needle was withdrawn from the vein. The pad was placed in that place until the bleeding stopped. Then slowly transferred the blood from the syringe to an appropriate container using the minimum amount of pressure.

The blood was allowed to clot in a capped container to obtain the serum and it was then centrifuged. At room temperature clotting usually takes 15 to 30 minutes.

3. **Collection of Urine:**

Since the present study was undertaken to compare the trace element level in urine and serum, Urine samples were also collected at random from the patients of GITract and skin disorders and normal persons. 1 ml of xylene was added to the urine as a preservative.

4. **Analysis of minerals in serum and Urine:**

The levels of Zinc, Copper, Iron and Manganese were estimated in both serum and urine by pipers method (1966) using atomic absorption spectrophoto meter. The details of the method was given in Appendix. 1.

5. **Statistical Analysis:**

't' tests were conducted whenever necessary to check if the results were significant using formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S} \sqrt{\frac{n_1 \; n_2}{n_1 + n_2}}$$

$\bar{X}_1$ = mean of the first sample

$\bar{X}_2$ = mean of the second sample

S = Combined standard deviation

n_1 = Number of patients in the first sample

n_2 = Number of patients in the second sample

$$S = \sqrt{\frac{(n_1-1)S_1^2 + (n_2-1)\ S_2^2}{n_1 + n_2 - 2}}$$

Results and Discussion

IV. RESULTS AND DISCUSSION

The study "Assessment of trace elements in Gastrointestinal tract and skin disorders" was undertaken to evaluate the mineral metabolic status in human GI tract and skin disorders.

Forty patients of both sexes suffering from different types of GI .Tract and skin disorders undergoing treatment in various hospitals at Coimbatore were selected for the study. Of the forty patients studied, half of them were found to be affected by GITract disorders and the remaining half of them were affected by skin disorders. Those selected with GITract disorders belong to the age group of 25 to 65 years. Among the twenty patients selected with skin disorders, one was a five year old child and the remaining nineteen were between 35 to 55 years. Twenty healthy persons of matching age and sex and free from diseases were selected as controls.

The serum and Urine samples were collected from the patients and controls as discussed under chapter III. All the samples were collected before the patients underwent treatment. Further random Urinary excretions were used as indices of mineral status.

The serum and Urinary levels of Iron, zinc, Copper and Manganese were determined for both the patients and

controls. From the above parameters, the extent of damage to the GITract and skin disorders and impairment of mineral nutrititional status were assessed.

The results obtained in this study were discussed under the following headings.

1. Types of GITract and skin disorders among the selected patients.

2. Mean Serum levels of Iron in GITract and skin disorders.

3. Mean urinary levels of Iron in GITract and skin disorders.

4. Mean serum levels of Zinc in GITract and skin disorders.

5. Mean Urinary levels of Zinc in GITract and skin disorders.

6. Mean serum levels of Copper in GiTract and skin disorders.

7. Mean Urinary levels of copper in GITract and skin disorders

8. Mean serum levels of Manganese in GITract and skin disorders

9. Mean urinary levels of Manganese in GITract and skin disorders

10. Types of GITract and skin disorders among the selected patients.

Table 1 represents the various types of GITract and skin disorders among the selected patients.

TABLE I

TYPES OF GI TRACT& SKIN DISORDERS AMONG THE SELECTED PATIENTS

GI Tract Disorder	Number of Patients	Skin Disorder	Number of Patients
Epigastric Pain	2	Vitiligo	2
Abdominal pain with chronic diarrhoea	8	Hansen's	3
Bowel syndrome	1	Granuloma skin	2
Dysphagea	2	Urticaria	5
Carcinoma of Stomach	3	Teena Vesicular	2
Trans Urethero reseres prostrate (TURB)	1	Eczema	6
Gastrectomy and Carcinoma of Stomach	1		
Chronic Duodenal Ulcer	2		
Total	20		20

Among the selected patients suffering from GI Tract disorders many of them were affected by abdominal pain with chronic diarrhoea, Eczema Urticaria, Hansen's were found to be the major skin disorders seen among the selected patients

2. Mean Serum levels of Iron in GI Tract and Skin Disorders.

Table II shows the levels of Iron in serum of controls and GI Tract and skin Disorders.

TABLE II

MEAN LEVELS OF IRON IN SERUM OF CONTROLS AND GI TRACT AND SKIN DISORDERS

Group	Number selected	Iron in µg/dl Mean ± S.D	Groups Compared	't' value
Controls (1)	20	65.41 ± 4.45	1 Vs 2	23.19**
GI Tract Disorders (2)	20	30.55 ± 5.03	1 vs 3	23.55**
Skin Disorders (3)	20	18.6 ± 7.68	2 Vs 3	5.83**

** $P < 0.01$

The mean values of serum iron in controls were found to be 65.41 ± 4.45 µg per dl with a range of 60.95 to 69.86 µg per dl, where as in GI Tract disorder the mean value was found to be 30.55 ± 5.03 µg per dl with a range of 25.52

to 35.58 µg per dl and the mean value inskin disorder was found to be 18.6 ± 7.68 µg per dl with a range of 10.92 to 26.28 ug per dl.

The normal range of Iron is 80 - 165 µg per dl in men and 65-130 µg per dl in women (Robinson and Lawler, 1986).

In both the group of patients the serum levels of iron were decreased when compared to the control values and this decrease was found to be statistically significant at 1% levels. This is in accordance with the study by Schottenfeld and Fraumeni (1988) which says that the iron deficiency might be one of the factors that contribute to the occurrence of esophagus and stomach cancers.

Some other studies have also reported depletion of iron in serum during chronic diarrhoea and abdominal pain (Scully et al., 1989).

In this study, majority of the patients were suffered by abdominal pain and a few of them had diarrhoea.

Statistically significant differences ($P < 0.01$) in the levels of iron were found between the two groups of disorders. Fig. 1 indicates the mean serum levels of iron in controls and GITract and skin disorders.

MEAN SERUM LEVELS OF IRON IN CONTROLS AND G.I.TRACT AND SKIN DISORDERS

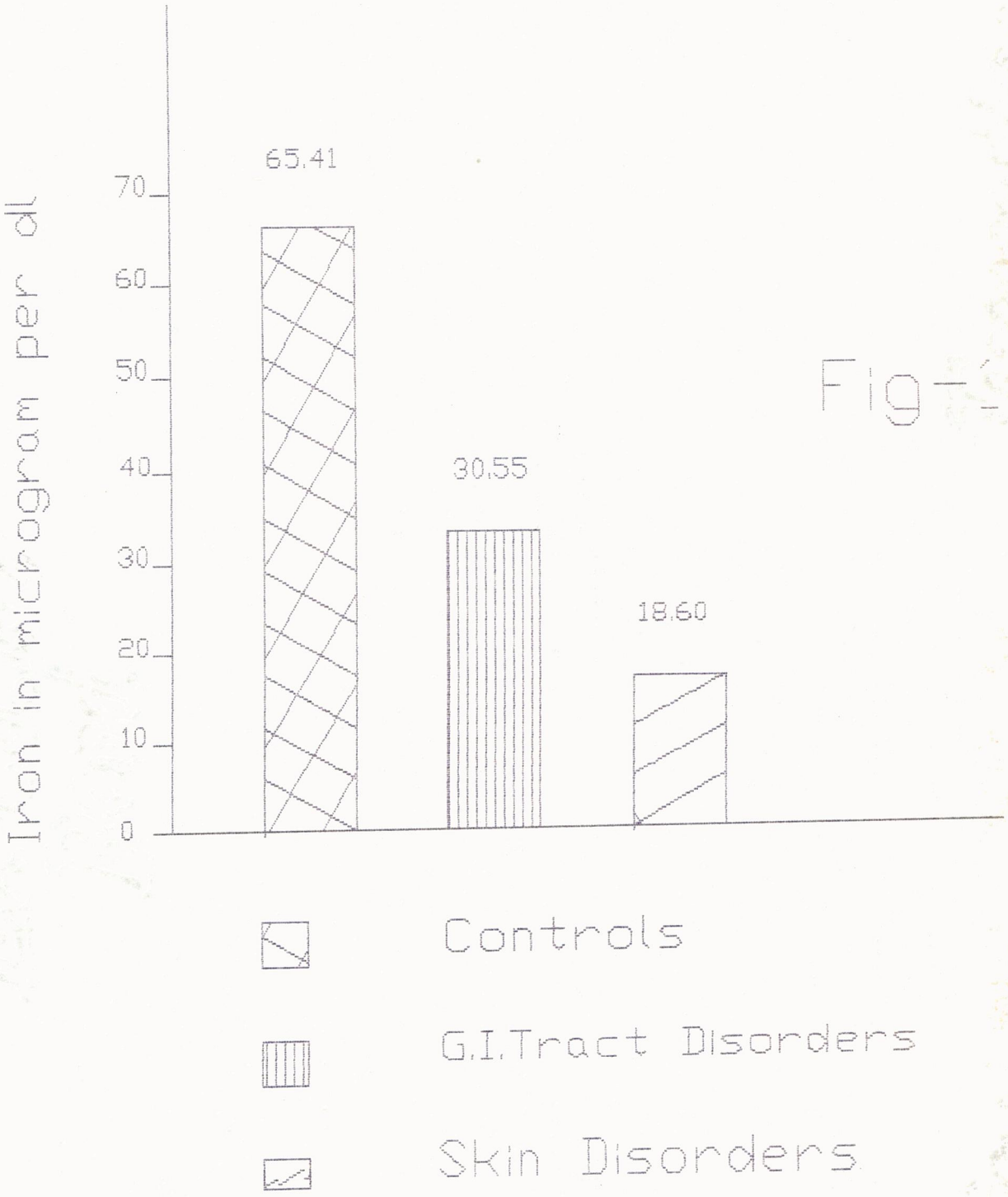

Fig-1

3. Mean Urinary levels of Iron in GI Tract and skin disorders.

Table III furnishes the details regarding the Urinary levels of iron in GI Tract and skin disorders.

TABLE III

MEAN URINARY LEVELS OF IRON IN CONTROLS AND GI TRACT AND SKIN DISORDERS

Group	Number selected	Iron in µg/dl mean ± S.D.	Groups compared	't' value
Control (1)	20	5.44 ± 1.05	1 Vs 2	12.15**
GI Tract Disorders	20	2.48 ± 0.30	1 Vs 3	7.28 **
Skin disorders	20	1.37 ± 0.25	2 Vs 3	12.53**

** $P < 0.01$

The mean levels of Urinary iron in controls were found to be 5.44 ± 1.05 µg per dl with a range of 4.39 to 6.49 µg per dl where as in GI Tract petients the same was found to be 2.48 ± 0.30 with a range of 2.18 to 2.78 µg per dl and in skin patients 1.37 ± 0.25 µg per dl with a range of 1.12 to 1.62 µg per dl.

The normal range of Urinary excretion of iron is 6.67 µg per dl (Robinson and Lawler, 1986).

It was evident from the table that the excretion of iron in skin disorders were half as that of GI Tract disorders and the difference in excretion was found to be statistically significant.

Fig. 2 indicates the mean urinary levels of iron in controls and GI Tract and skin disorders.

MEAN URINARY LEVELS OF IRON I
CONTROLS AND G.I.TRACT AND SKIN DISORDER

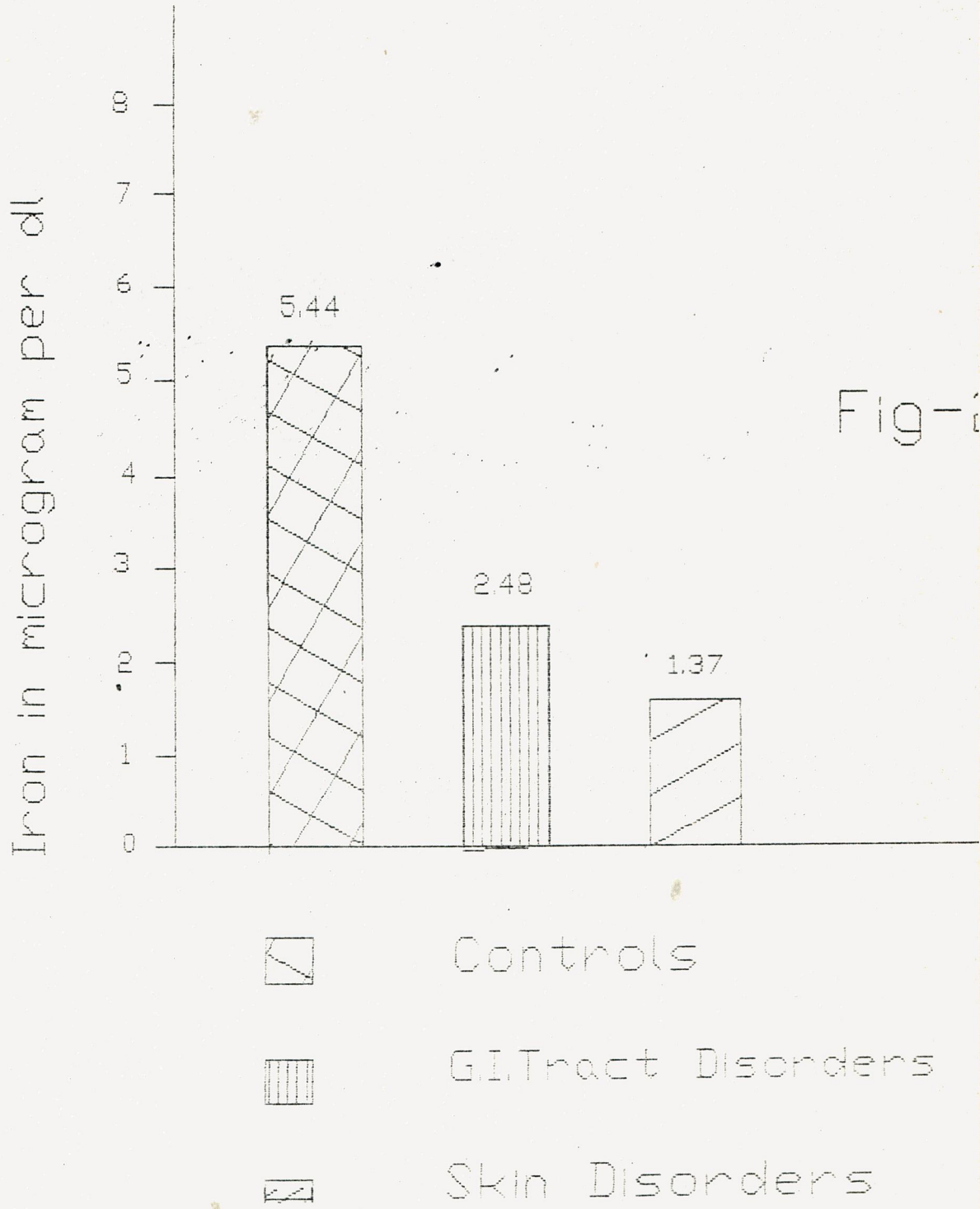

Fig-1

4. Mean Serum levels of Zinc in GITract and skin disorders.

Table IV shows the levels of Zinc in serum of controls and GI Tracts and skin disorders.

TABLE IV

MEAN LEVELS OF ZINC IN SERUM OF CONTROLS AND GI TRACT AND SKIN DISORDERS

Group	Number selected	Iron in µg/dl mean ± S.D.	Groups compared	't' value
Control (1)	20	71.36 ± 3.75	1 vs 2	14.61**
GI Tract disorders (2)	20	37.14 ± 9.77	1 vs 3	17.81**
Skin disorders (3)	20	34.1 ± 8.53	2 vs 3	1.05 NS

** : $P < 0.01$

NS : Not significant

The mean levels of Zinc in serum of controls, GI Tract and skin disorders were found to be 71.36 ± 3.75, 37.14 ± 9.77 and 34.1 ± 8.53 µg per dl respectively and the corresponding ranges were found to be 67.61 to 75.11, 27.37 to 46.91 and 25.57 to 42.63 µg per dl. The normal range of Zinc in serum is 50-150 µg per dl (Harper, 1988).

The levels of Zinc in serum of both the disorders were found to be decreased to one half when compared to the control and this decreases were statistically significant at 1% level.

The decreased serum levels of Zinc might be due to failure to reabsorb Zinc from the gut as a result of acrodermatitis and various GI Tract disorders (Trowbridge, 1988).

It was evident from the table that there was no significant difference in the levels of Zinc between the two groups of disorders.

Fig. 3 indicates the mean serum levels of zinc in controls and GITract and skin disorders.

MEAN SERUM LEVELS OF ZINC IN CONTROLS AND G.I.TRACT AND SKIN DISORDERS

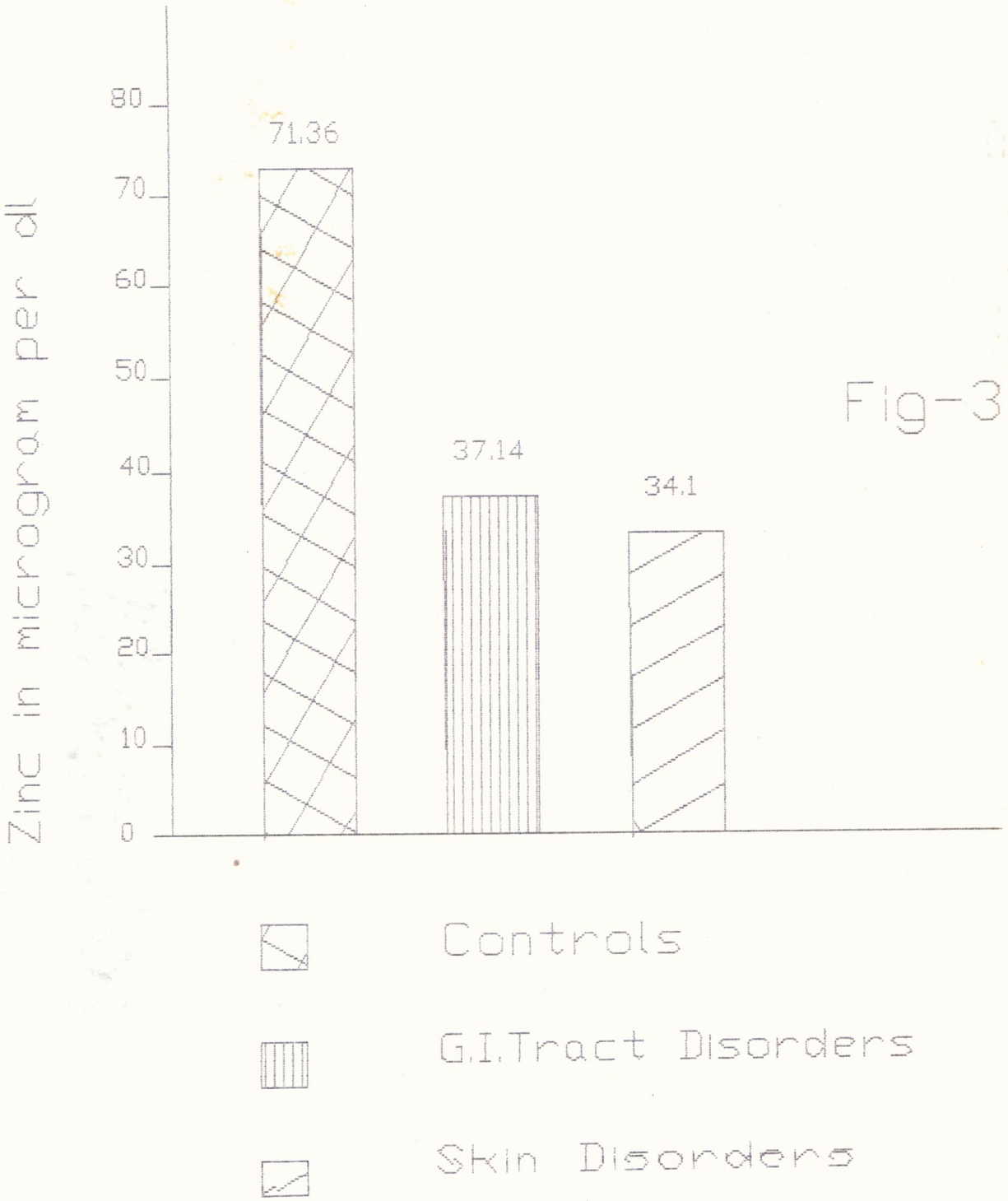

Fig-3

5. Mean Urinary levels of Zinc in GI Tract and skin disorders

Table V depicts the Urinary Zinc levels in controls and GI Tract and skin disorders.

TABLE V

MEAN URINARY LEVELS OF ZINC IN CONTROLS AND GI TRACT AND SKIN DISORDERS

Group	Number selected	Zinc in µg/dl Mean ± S.D.	Groups Compared	't' value
Control (1)	20	23.99 ± 6.24	1 vs 2	9.79**
GI Tract disorders (2)	20	8.32 ± 3.51	1 vs 3	12.60**
Skin disorders (3)	20	6.33 ± 0.63	2 vs 3	2.50*

** $P < 0.01$

* $P < 0.05$

The mean Urinary levels of Zinc in controls were found to be 23.99 ± 6.24 µg per dl with a range of 17.75 to 30.23 µg per dl, where as in the case of GI Tract patients the same ranged from 4.81 to 11.83 µg per dl with a mean value of 8.32 ± 3.51 µg per dl and in skin patients, the value ranged from 5.7 to 6.96 µg per dl with a mean value of6.33 ± 0.63 µg per dl.

The Urinary excretion of Zinc in normal adults is 10 - 18 µg per dl (Henry, 1986).

There was a decrease in the excretion of Zinc to one third in the case of GI Tract disorders, and the same was decreased to one-fourth in skin disorders and the decrease was statisfically significant at 1% level for both the disorders.

When the two groups were compared the results were found to be statistically significant at 5% level.

Fig 4 shows the mean Urinary levels of Zinc in controls and GI Tract and skin disorders.

MEAN URINARY LEVELS OF ZINC IN CONTROLS AND G.I.TRACT AND SKIN DISORDERS

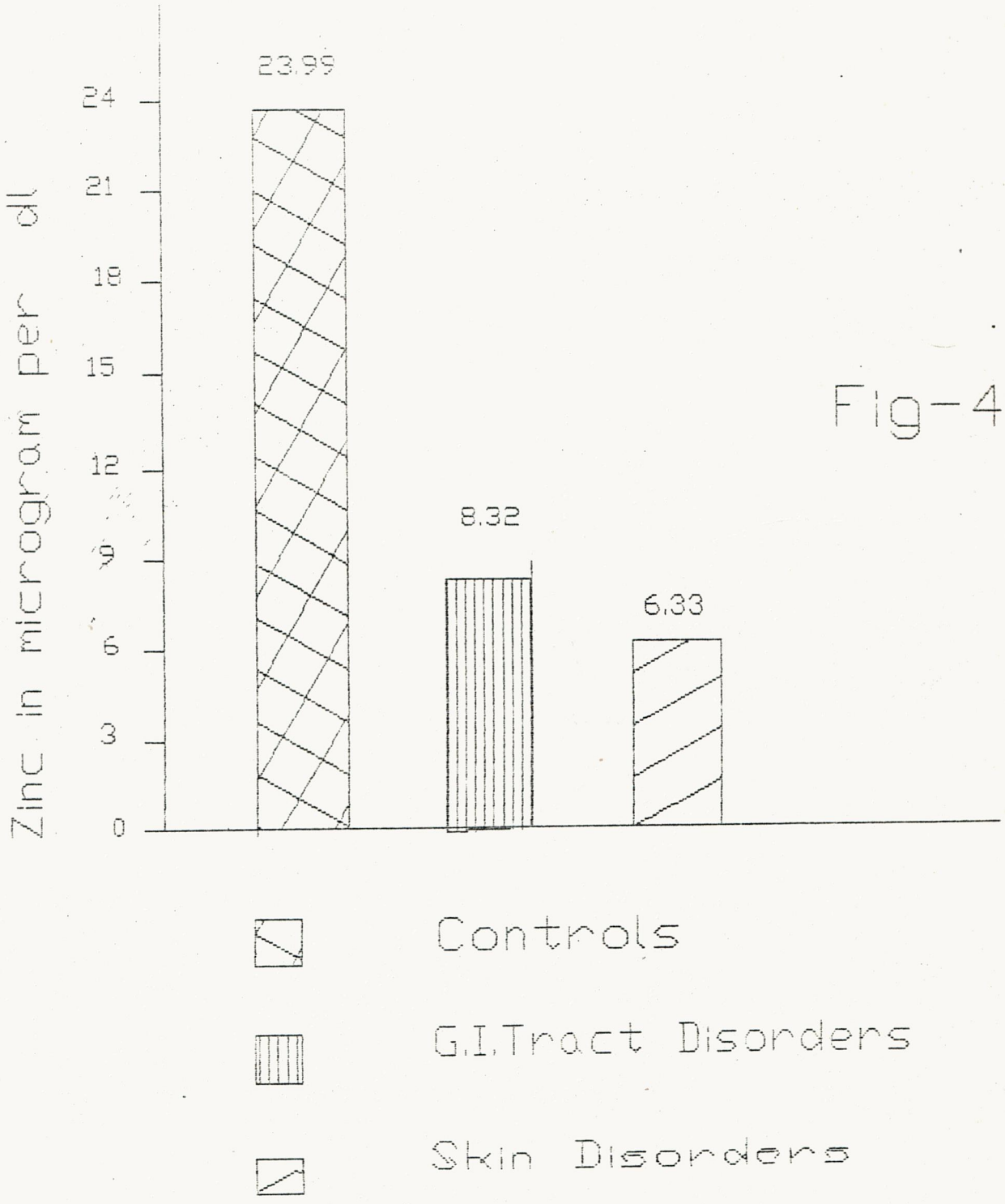

Fig-4

6 Mean serum levels of Copper in GI Tract and skin disorders

Table VI indicates the serum levels of Copper in controls and GI Tract and skin disorders.

TABLE VI

MEAN SERUM LEVELS OF COPPER IN CONTROLS AND GI TRACT AND SKIN DISORDERS

Group	Number selected	Copper in µg/dl Mean ± S.D.	Groups compared	't' value
Controls (1)	20	72.85 ± 3.34	1 vs 2	26.02**
GI Tract Disorders	20	40.00 ± 4.45	1 vs 3	25.64**
skin disorders	20	48.75 ± 2.25	2 vs 3	7.79**

** $P < 0.01$

The mean serum levels of Copper in control, GI Tract and skin disorders were found to be 72.85 ± 3.34, 40.00 ± 4.45, and 48.75 ± 2.55 µg per dl respectively and the corresponding ranges were found to be 69.51 to 76.19, 35.55 to 4.45 and 46.2 to 51.3 µg per dl.

The normal range of copper in serum is 70-155 µg per dl (Tietz, 1986).

The mean levels of copper were decreased in GI Tract and skin disorders when compared to the respective controls and the decrease was statistically significant at 1% level.

The decreased Copper level in serum might be due to defective absorption from the intestine as a result of skin depigmentation (Bales, 1989), Chronic diarrhoea and short bowl syndrome (Trowbridge, 1988).

The difference in mean values were also found to be significant at 1% level between the groups compared .

Fig 5 depicts the mean serum levels of controls and GI Tract and skin disorders.

MEAN SERUM LEVELS OF COPPER IN CONTROLS AND G.I.TRACT AND SKIN DISORDERS

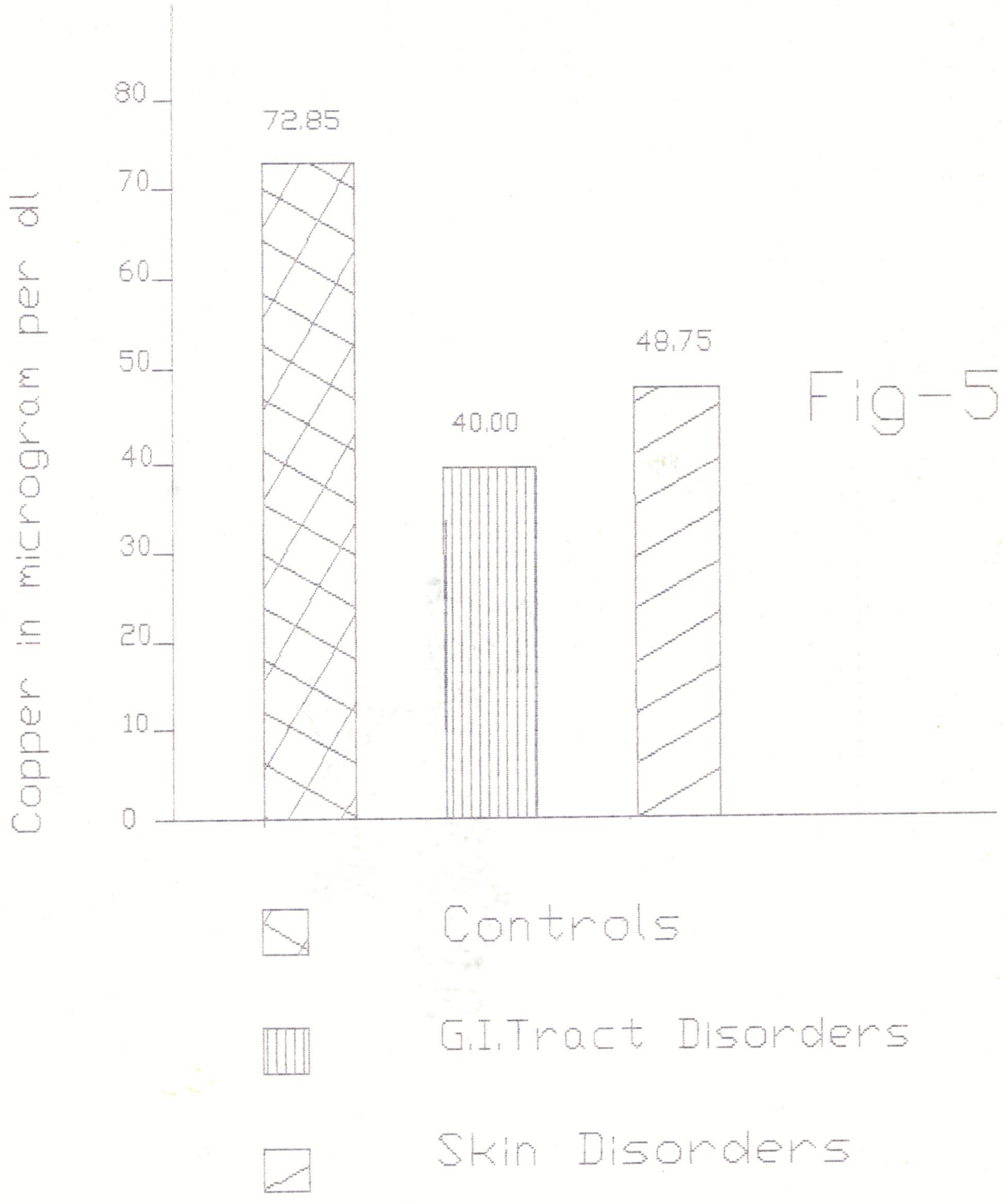

Fig-5

7. Mean Urinary levels of Copper in GI Tract and skin disorders.

Table VII reveals the Urinary levels of Copper in controls, GI Tract and skin disorders.

TABLE VII

MEAN URINARY LEVELS OF COPPER IN CONTROLS AND GI TRACT AND SKIN DISORDERS

Group	Number selected	Copper in µg/dl Mean ± S.D.	Groups Compared	't' value
Controls (1)	20	20.65 ± 1.42	1 vs 2	16.36**
GI Tract Disorders (2)	20	9.0 ± 2.85	1 vs 3	12.51**
Skin disorders (3)	20	10.2 ± 3.46	2 vs 3	1.69 Ns

** $P < 0.01$

Ns Not significant

The mean levels of Urinary Copper in Controls, GI Tract and skin disorders were found to be 20.65 ± 1.42, 9.0 ± 2.85 and 10.2 ± 3.46 µg per dl respectively and the corresponding ranges were found to be 19.23 to 22.07, 6.15 to 11.85 and 6.74 to 13.66 µg per dl.

The normal excretion of Copper in Urine is 15-30 µg/dl (Tietz, 1986).

There was a decrease in the excretion of copper to one-half in both GI Tract and skin disorders and this decrease was found to be significant at 1% level. The decreased excretion of Copper might be due to low levels of the same in serum of both the group of disorders.

There was no significant difference in the excretion of Copper between the two groups of disorders.

Fig 6 depicts the mean urinary levels of copper in controls and GI Tract and skin disorders.

MEAN URINARY LEVELS OF COPPER IN CONTROLS AND G.I.TRACT AND SKIN DISORDERS

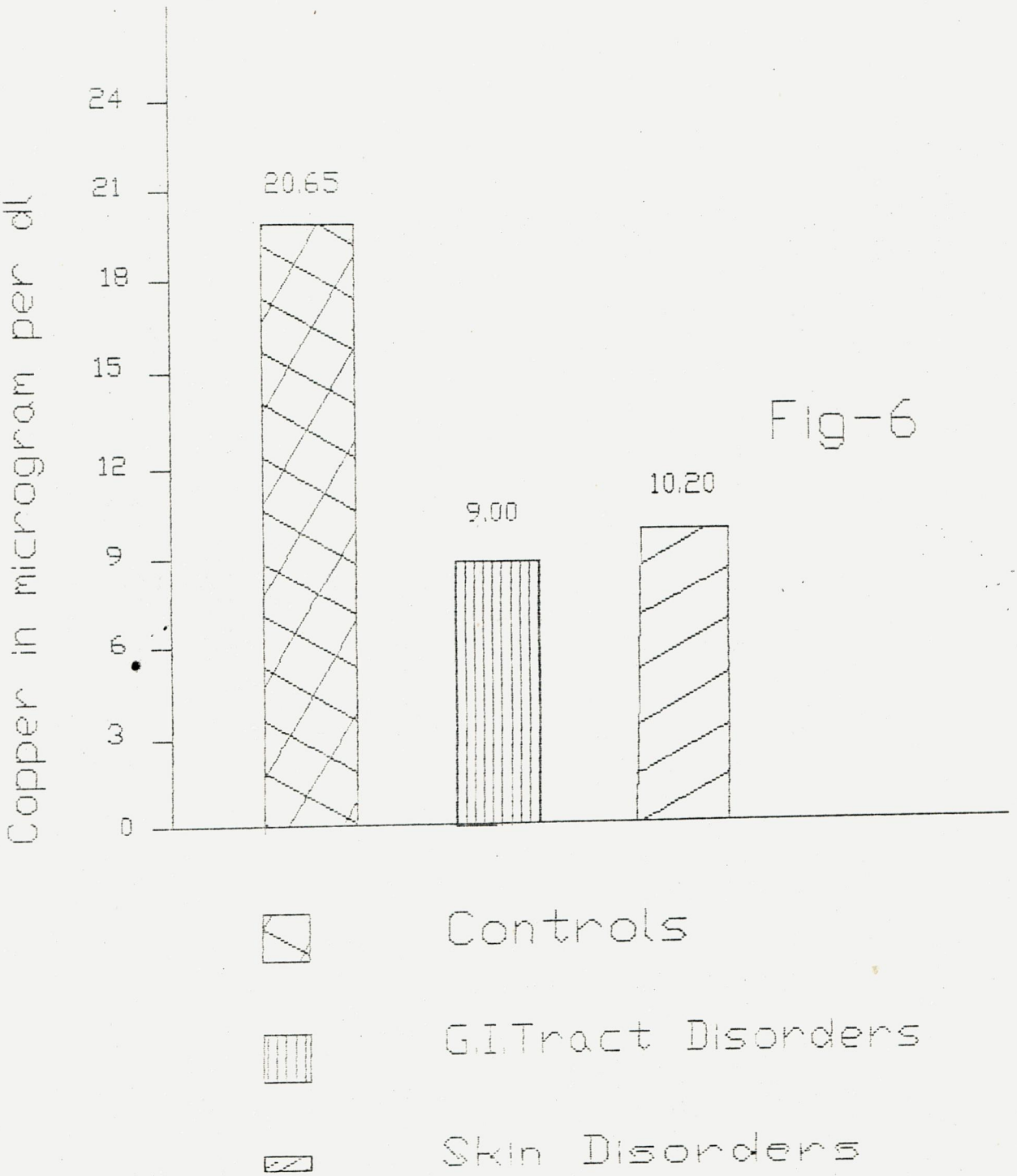

Fig-6

8. Mean serum levels of manganese in GI Tract and skin disorders

Table VIII projected the mean serum levels of manganese in controls and GI Tract and skin disorders.

TABLE VIII

MEAN SERUM LEVELS OF MANGANESE IN CONTROLS AND GI TRACT AND SKIN DISORDERS

Group	Number selected	Manganese in µg/dl Mean ± S.D.	Groups compared	't' value
Controls (1)	20	1.90 ± 0.05	1 vs 2	15.89**
GI Tract disorders (2)	20	1.06 ± 0.23	1 vs 3	16.11**
Skin disorders (3)	20	1.12 ± 0.21	2 vs 3	0.86 NS

** < P 0.01

NS Not significant

The serum levels of manganese in controls ranged between 1.85 to 1.95 µg per dl with a mean value of 1.90 ± 0.05 µg per dl, where as in the case of GITract and skin patients the same ranged between 0.83 to 1.29 and 0.91 to 1.33 µg per dl respectively with a corresponding mean values of 1.06 ± 0.23 and 1.12 ± 0.21 µg per dl.

The normal range of manganese in serum is 1.2 - 3.8 µg per dl (WHO, 1981).

The mean levels of manganese in serum were found to be approximately one half as that of controls and the differences were significant at 1% level in both the groups of disorders.

This was in agreement with the study by Kuttel (1984) that the manganese depletion might have occurred due to dermatitis and GITract disorders, in which there was a deposition of manganese in liver, pancreas, kidney and intestine (Kitamurra, 1974).

The difference in manganese levels of serum between the two groups were found to be insignificant.

Fig. 7 represents the mean serum levels of manganese in controls and GITract and skin disorders.

MEAN SERUM LEVELS OF MANGANESE IN CONTROLS AND G.I.TRACT AND SKIN DISORDERS

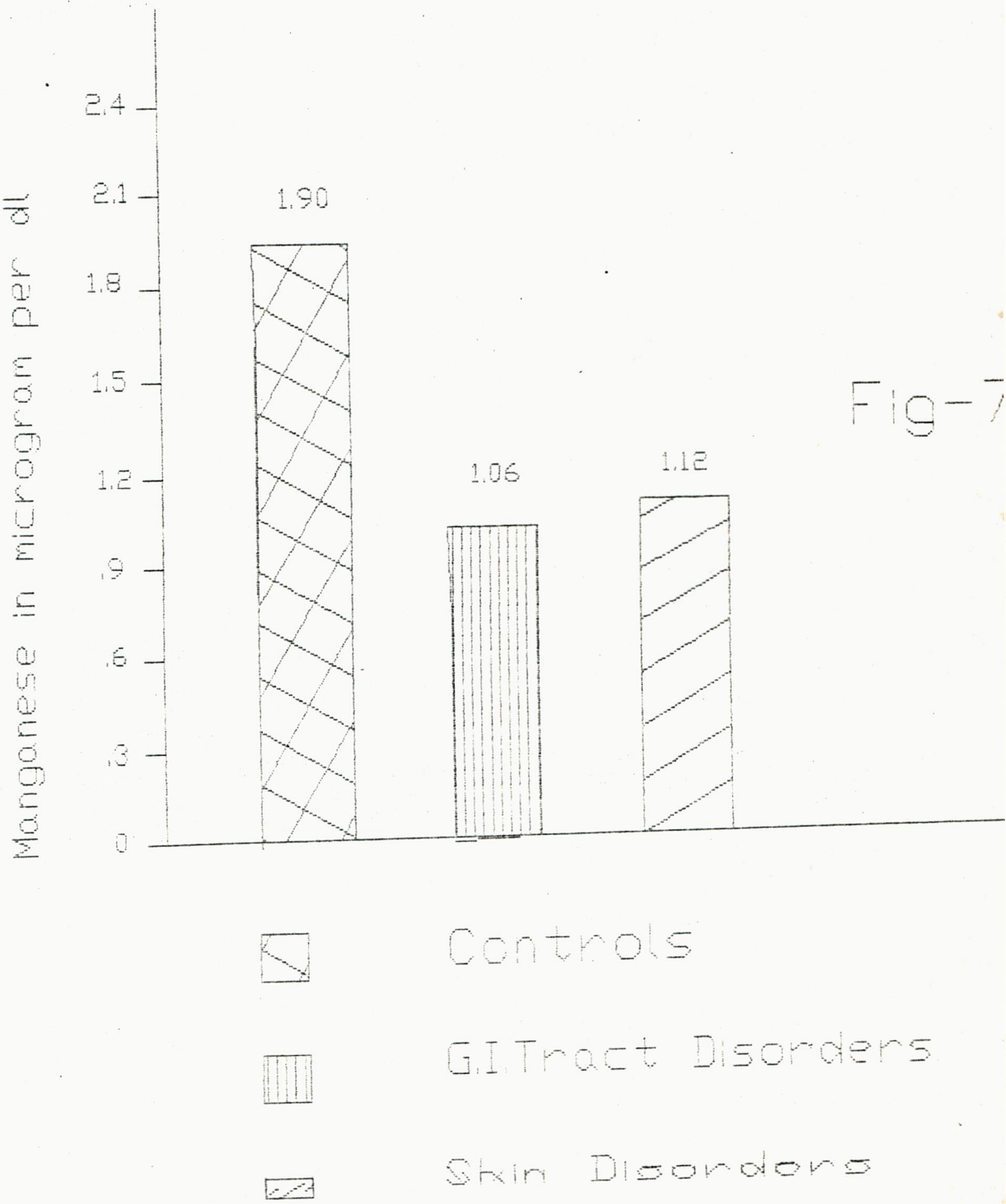

Fig-7

9. Mean Urinary levels of manganese in GI Tract and skin disorders

Table IX represents the Urinary levels of manganese in controls and GI Tract and skin disorders.

TABLE IX

MEAN URINARY LEVELS OF MANGANESE IN CONTROLS AND GI TRACT AND SKIN DISORDERS

Group	Number Selected	Manganese in µg/dl Mean ± S.D.	Groups Compared	't' value
Controls (1)	20	0.570 ± 0.280	1 vs 2	7.74**
GI Tract disorders (2)	20	0.080 ± 0.020	1 vs 3	7.77**
Skin disorders (3)	20	0.078 ± 0.004	2 vs 3	0.63^{NS}

** $P < 0.01$

NS Not significant

The mean value of Urinary excretion of manganese in controls were found to be 0.570 ± 0.280 µg per dl with a range of 0.290 to 0.850 µg per dl, where as the same was found to be 0.080 ± 0.020 µg per dl with a range of 0.060 to 0.100 µg per dl in GI Tract disorders and 0.078 ± 0.004 µg per dl with range of 0.074 to 0.082 µg per dl in skin disorders.

The normal excretion of manganese in urine is 0.2 - 1.4 µg per dl (WHO, 1981).

In GI Tract and skin disorders the Urinary excretion of manganese was found to be decreased from the control value and this decrease was significant at 1% level.

The excretion of manganese between the two groups of disorders show no significant differences.

Fig 8 indicates the mean Urinary levels of manganese in controls and GI Tract and skin disorders.

MEAN URINARY LEVELS OF MANGANESE IN CONTROLS AND G.I.TRACT AND SKIN DISORDERS

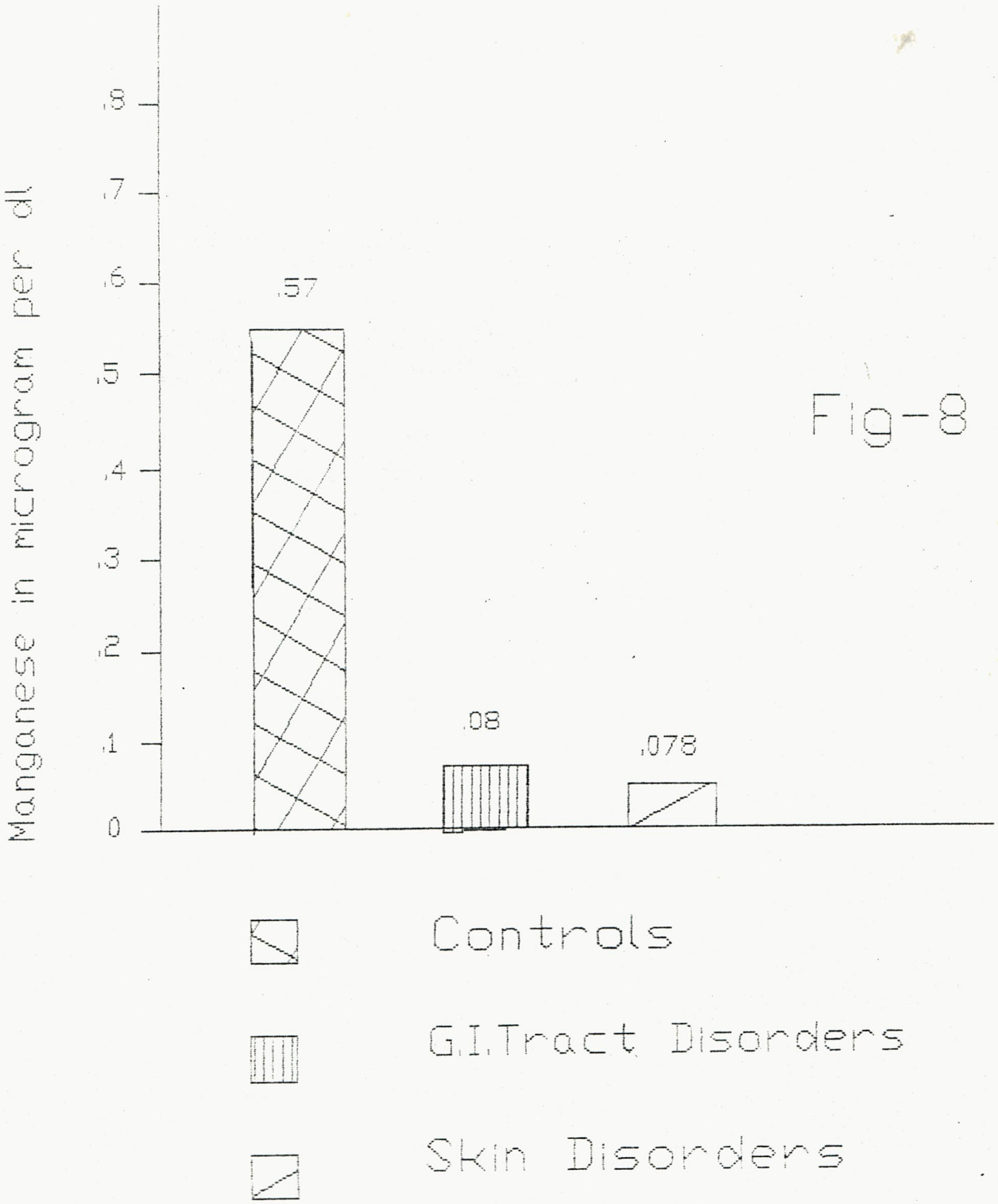

Fig-8

Chapter 5

Summary and Conclusion

V. SUMMARY AND CONCLUSION

This study "Assessment of trace elements in Gastrointestinal Tract and skin disorders" was undertaken to evaluate the mineral metabolic status in human GITract and skin disorders. Forty patients of both sexes suffering from different types of GITract and skin disorders undergoing treatment in various hospitals (G.H, K.G. Hospital, Kovai Medical Centre and Lalitha Hospitals) at Coimbatore) were selected for the study. The patients who had been diagnosed with a histologically confirmed GITract and skin disorders only were included for the investigation. Of the fourty patients studied half of them were found to be affected by GITract disorders and the remaining half of them were affected by skin disorders. Those selected with GITract disorders belong to the age group of 35 to 65 years. Among the twenty patients selected with skin disorders, one was a 5 year old child and the remaining nineteen were between 35 to 55 years. Twenty healthy persons of matching age and sex and free from diseases were selected as controls.

The levels of iron, zinc, copper and manganese were estimated in both serum and urine by piper's method (1966) using atomic absorption spectrophotometer.

The serum samples were collected from all the patients before they underwent treatment. The serum samples were also collected from the controls. The iron, zinc, copper, and manganese levels of serum samples were determined.

The mean values of serum iron in controls were found to be 66.5 ± 4.45 µg per dl whereas in GITract disorder the mean value was found to be 30.55 ± 5.03 µg per dl and in skin disorder 18.6 ± 7.68 µg per dl. In both the group of patients the serum levels of iron were decreased when compared to the control values and this decrease was found to be statistically significant at 1% level.

The iron deficiency might be due to the cancer of the stomach, chronic diarrhoea and abdominal pain.

The mean levels of zinc in serum of controls, GI Tract and skin disorders were found to be 71.36 ± 3.75, 37.14 ± 9.77 and 34.1 ± 8.53 µg per dl respectively. The level of zinc in serum of both the disorders were found to be decreased to one half when compared to the control and this decrease was statistically significant at 1% level.

The decreased levels of zinc might be due to failure to reabsorb from the gut as a result of acrodermatitis and various GI Tract disorders.

The mean serum levels of copper in controls, GI Tract and skin disorders were found to be 72.85 ± 3.34, 40.00 ± 4.45 and 48.75 ± 2.55 µg per dl respectively. The mean levels of copper were decreased in GI Tract and skin disorders when compared to the respective controls and the decrease was statistically significant at 1% level.

The decreased copper level in serum might be due to defective absorption from the intestine as a result of skin depigmentation, chronic diarrhoea and short bowel syndrome.

The mean serum levels of manganese in controls were found to be 1.90 ± 0.05 µg per dl where as in the case of GITract and skin patients the same was found to be 1.06 ± 0.23 and 1.12 ± 0.21 µg per dl respectively.

The manganese depletion might have occured due to dermatitis and GITract disorders in which there was a deposition of manganese in liver, pancrease, kidney and intestine.

When the mean serum levels were compared between the two groups of disorders, it was found that there was a significant difference in the levels of iron and copper where as the zinc and manganese have shown no significant difference.

The urine samples were also collected from all the patients before they underwent treatment. The urine samples were also collected from the controls. The iron, zinc, copper and manganese content of the urine samples were determined.

The mean levels of urinary iron in controls were found to be 5.44 ± 1.05 µg per dl whereas in GITract patients the same was found to be 2.48 ± 0.30 µg per dl and in skin patients 1.37 ± 0.25 µg per dl. In this study it was noticed that the excretion of iron in skin disorders were half that of GITract disorders and the difference in excretion was found to be statistically significant.

The mean urinary levels of zinc in controls, GITract and skin disorders were found to be 23.99 ± 6.24, 8.32 ± 3.51 and 6.33 ± 0.63 µg per dl respectively. There was a decrease in the excretion of zinc to one third in the case of GITract disorders and the same was decreased to one fourth in skin disorders and the decrease was statistically significant at 1% level for both the disorders.

The mean levels of urinary copper in control, GITract and skin disorders were found to be 20.65 ± 1.42, 9.0 ± 2.85 and 10.2 ± 3.46 µg per dl respectively. There was a decrease

in the excretion of copper to one half in both GITract and skin disorders and this decrease was found to be significant at 1% level.

The mean value of urinary excretion of manganese in controls were found to be 0.570 ± 0.280 µg per dl where as in the case of GI Tract and skin disorders, the same was found to be 0.080 ± 0.020 and 0.078 ± 0.004 µg per dl respectively. In GI Tract and skin disorders the urinary excretion of manganese was found to be decreased from the control values and this decrease was significant at 1% level.

The decreased excretion of all the four elements might be due to depleted levels of the same in serum of both the groups of GITract and skin disorders.

When the mean excretion of four elements were compared between GITract and skin disorders, there was a significant difference in the excretion of iron and zinc whereas the excretion of copper and manganese were found to be insignificant.

In summary the levels of iron, zinc, copper and manganese in both serum and urine were fond to be lower than the control values in the two groups of disorders studied. Hence it may be concluded that proper supplementation of these trace elements might prevent or

reduce the chances of occurrence of the above mentioned disorders.

Recommendations for future studies:

The sectoral and integrated approaches can be made use of to assess almost all essential trace elements in serum and urinary levels of both GI Tract and skin disorders.

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Appendices

APPENDIX I

ESTIMATION OF IRON, ZINC, COPPER AND MANGANESE IN SERUM AND URINE

(Piper, 1966)

Principle:

Serum and Urine on digestion with triple acid (nitric acid, sulphuric acid and perchloric acid) in the ratio of 9:2:1 which liberates the trace elements into solution.

Procedure:

a. **Digestion of Serum:**

1.0 ml of the serum and urine were taken in a micro kjeldahl digestion flask which was previously washed with glass distilled water and dried, and to this was added 10 ml of triple acid. The mixture was shaken and digested on a sand bath with occasional shaking, the digestion was continued till no more brown fumes evolved and the solution in the flask became colorless. The digested mixture was transferred to a 25.0 ml standard flask, the washing being done with double distilled water and made upto the mark.

b. **Estimation:**

The digested serum sample was used for analysing Zinc, Copper, Iron and manganese levels using the atomic absorption

spectrophotometer (Varian-Techtron AA model) available in the soil science Department of Tamil Nadu Agricltural University, Coimbatore.

c. **The Principle and Working of Atomic Absorption spectro photometer:**

The sample in solution or suspension is heated to a high temperature by burning it in a flame. The flame breakes up the chemical bond between the molecules and enables the individual atoms to float freely in the sample area. In this condition, the atoms absorb ultra violet or visible radiation. The wave length bands in which each element can absorb are narrow. Hence, at a particular wavelength the absorption is to be measured. The amount of light absorbed gives a direct indication of the amount of metal that is present.

Technique:

1. Select the cathode lamps to be used insert them in the lamp quadrants.

2. Depress the relevant "Lamp-select" buttons for the lamp being used and set the 'matter select' to the same lamp.

3. Switch on the instrument, set the lamp at the described current and allow to stabilise for 10 - 15 min.

4. Set the indicator unit in the transmission mode with the select switch in normal

5. Set the monochromator to the wavelength required with the relevant slit opening and using again setting to give approximately 80 percent transmission reading.

6. Select the "Auto 100" mode and firm the "Set 100" to read 0.0 absorbance or 100 percent transmission.

7. Select the desired mode of operation on the indicator unit (ie.) Absorbance or transmission.

8. Light the flame

9. Nebulize the sample into the flame.

APPENDIX II

SERUM AND URINARY VAUES OF THE IRON IN CONTROLS, GITRACT AND SKIN DISORDERS

Normal		GITract Disorders		Skin Disorders	
Serum (µg/dl)	Urine (µg/dl)	Serum (µg/dl)	Urine (µg/dl)	Serum (µg/dl)	Urine (µg/dl)
62.2	4.7	34.4	2.4	22.1	1.3
62.6	9.0	24.6	2.9	23.7	2.2
63.1	5.3	25.8	2.2	23.2	1.3
62.6	6.1	35.0	2.4	23.3	1.3
62.2	5.2	36.6	2.2	13.5	1.4
71.8	6.2	35.2	2.3	13.2	1.2
71.6	6.6	25.7	3.2	14.5	1.2
72.0	6.0	25.9	2.5	12.5	1.4
72.0	5.4	25.2	2.3	38.9	1.5
62.5	4.3	26.5	2.3	13.6	1.9
61.8	5.4	25.2	2.3	22.7	1.3
62.1	5.0	24.8	2.5	12.8	1.2
63.1	5.9	37.3	2.3	23.0	1.3
64.0	4.4	25.5	2.4	13.3	1.4
62.4	5.0	35.1	2.3	22.8	1.2
71.9	4.7	28.1	2.4	21.9	1.2
62.0	4.7	34.2	2.9	21.0	1.3
63.4	5.0	34.7	3.1	22.9	1.2
72.6	5.2	35.9	2.3	12.0	1.3
62.3	4.6	35.2	2.3	13.1	1.3

APPENDIX III

SERUM AND URINARY VALUES OF THE ZINC IN CONTROLS, GITRACT AND SKIN DISORDERS

Normal		GITract disorders		Skin disorders	
Serum (µg/dl)	Uine (µg/dl)	Serum (µg/dl)	Urine (µg/dl)	Serum (µg/dl)	Urine (µg/dl)
69.5	21.8	34.3	9.0	42.3	5.5
69.0	21.0	34.7	5.2	27.8	5.7
79.0	30.7	44.8	10.4	43.5	6.0
72.1	23.1	54.4	15.3	34.7	6.3
68.2	24.4	46.7	5.8	34.3	7.2
67.8	30.9	32.5	4.6	31.5	6.0
68.2	26.6	41.7	14.4	39.0	6.0
72.5	23.0	32.3	11.1	28.5	6.4
74.6	11.3	30.5	8.3	32.4	6.0
71.2	24.7	27.5	10.9	30.5	7.2
74.5	11.4	26.0	7.5	31.0	6.5
69.2	32.5	29.5	6.5	32.5	5.9
76.4	33.0	50.3	3.3	44.1	5.5
76.5	20.3	60.3	7.1	60.2	6.5
74.7	28.9	34.5	2.5	34.4	7.0
67.0	15.0	26.0	8.5	27.0	7.9
66.3	26.2	27.0	10.6	24.3	6.0
69.2	28.4	40.4	6.8	26.0	6.7
67.0	21.3	34.5	12.8	24.0	6.5
74.3	25.3	34.8	5.7	34.0	5.8

APPENDIX IV

SERUM AND URINARY VALUES OF THE COPPER IN CONTROLS, GITRACT AND SKIN DISORDERS

Normal		GITract disorders		Skin disorders	
Serum (µg/dl)	Urine (µg/dl)	Serum (µg/dl)	Urine (µg/dl)	Serum (µg/dl)	Urine (µg/dl)
71	24	46	13	49	12
72	23	38	10	50	10
71	19	40	11	48	10
70	21	40	12	50	13
72	21	42	15	52	11
70	22	39	7	51	11
70	21	33	8	50	14
78	23	34	7	47	12
70	20	38	13	48	12
72	20	36	6	52	12
74	20	45	9	47	11
75	19	41	6	52	10
73	20	43	6	47	4
75	20	37	8	52	7
71	19	36	7	46	3
71	20	47	8	47	8
72	20	36	8	51	7
84	21	47	6	48	18
74	19	46	13	44	12
72	21	36	7	44	7

APPENDIX V

SERUM AND URINARY VALUES OF THE MANGANESE IN CONTROLS, GITRACT AND SKIN DISORDERS

Normal		GITract Disorders		Skin Disorders	
Serum (µg/dl)	Urine (µg/dl)	Serum (µg/dl)	Urine (µg/dl)	Serum (µg/dl)	Urine (µg/dl)
1.90	0.8	1.02	0.10	0.9	0.07
1.98	0.5	1.11	0.08	1.0	0.07
1.93	0.3	1.0	0.09	1.0	0.07
1.83	0.8	1.0	0.08	1.1	0.08
1.86	0.2	1.0	0.07	1.0	0.08
1.93	0.3	1.0	0.09	1.2	0.08
1.97	1.0	2.0	0.08	1.1	0.08
1.83	0.7	1.0	0.01	1.4	0.08
1.96	0.1	1,0	0.09	1.0	0.08
1.85	0.5	1.0	0.08	1.3	0.07
1.83	0.7	1.0	0.08	1.4	0.08
1.92	0.7	0.9	0.09	1.2	0.08
1.86	0.6	1.0	0.09	1.3	0.08
1.90	0.3	1.0	0.08	1.5	0.08
1.97	1.1	1.2	0.08	1.4	0.08
1.90	0.7	1.0	0.08	1.1	0.08
1.91	0.5	1.0	0.09	0.9	0.08
1.97	0.2	1.0	0.09	0.8	0.08
1.90	0.9	1.05	0.09	0.8	0.08
1.87	0.5	1.04	0.09	1.0	0.08