Serum Calcium, Copper, Zinc, Magnesium and Iron in Diabetes and Liver Diseases

by

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for her keen interest evinced throughout this study. Her thanks are also due to Dr. (Mrs.) Godavari Kamalanathan M.Sc., Ph.D., Principal, Sri Avinashilingam Home Science College for women, (Autonomous) for granting the necessary facilities.

The author records her thanks to Dr. R.V. Srinivasan, M.S., the Dean of Coimbatore Medical College, Dr. Narasimhan M.S., Government Hospital, and Dr. Umarani, M.S. Government Hospital, Coimbatore for permitting to collect the blood samples from the patients.

The author thanks to all the patients for their full co-operation in making this study sucess.

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INTRODUCTION

I. INTRODUCTION

Many elements, although present in minute quantities in man, are essential nutrients. Their presence was long over looked and it has only been in recent years that analytical techniques capable of measuring such trace levels were developed. These elements perform functions indispensable to maintainance of life, growth and reproduction. Inadequate levels of some elements may impair cellular and physiological function or cause illness while other elements, even though present in low concentration, may be toxic (Sullivan et al., 1979).

The impairment of cellular and physiological functions is caused through the altered activities of metallic enzymes. These metalloenzymes require a small and constant number of atoms of metal per mole, to attain full activity. Thus a minute variation in these metals cause significant change in the activity of these elements (Vyas et al., 1982).

The normal morning fasting level of blood glucose is 80 - 100 mg/100 ml. After the ingestion of a meal containing carbohydrate the level rises temporarily to 120 - 140 mg/100 ml. After fasting for 24 hours or more the blood glucose level is maintained at 60 -70 mg/100 ml (Keele et al., 1974).

Sullivan et al., (1979) reported that in diabetes changes in trace elements might intiate physiological alternation in glucose metabolism.

Diabetes mellitus is a disease of glucose metabolism due to an insufficient output of endogenous insulin as a result of which blood sugar is abnormally high and sugar appears in the urine (Krull., 1978). The commonest symptoms of the disease are polyuria, polydipsia, tiredness and loss of weight (Lilly., 1980).

The disturbance in carbohydrate metabolism is due to the fact that the liver and skeltal muscles can not store glycogen and the tissues are unable to utilise glucose. Protein metabolism in the liver is also deranged and an excessive amount of protein is transformed into carbohydrate. In addition, the amount of fat metabolised by the diabetic patient is excessive, and since normal fat catabolism can only proceed at a limited rate, ketone bodies are present in the blood and the urine in much larger amounts than normally. These substances are excreted in the urine as beta hydroxy-butyric acid and acetoacetic acid and as acetone in breath. Accumulation of these acids in the blood produces acidosis, furthermore aceto acetic acid has a toxic effect which leads to coma, circulatory collapse and death of the patient. (Gupta et al., 1981).

Diabetics had no significant change in serum calcium and had significantly low serum magnesium level (Vyas *et al*., 1982) Low plasma magnesium values and increased excretion in urine was most isgnificant in a group of poorly controlled diabetics, whose fasting plasma glucose was above 250 mg per 100 ml. Low magnesium values in plasma and erythrocytes were pronounced in the diabetic patients with proliferative retinopathy, even though plasma glucose values were and similar to those in the patients without retionpathy and with background retionopathy (Fujii *et al*., 1982).

According to Goyal *et al*., 1982) zinc level did not differ significantly from the controls and it did not differ with different levels of blood urea, or total and differential serum proteins. Vyas *et al*., (1982) reported that diabetics a had significantly low serum zinc level, while Mateo *et al*., (1978) reported it to be raised.

Serum copper levels were significantly higher in diabetics. Female diabetic had higher levels than the male diabetic patients. During some of the steps in intermediary metabolism certain trace elements like copper and zinc are required in an obligatory fashion. Copper and zinc are essential components of several enzymes connected with carbohydrate and protein metabolism. There are conflicting reports about serum concentration of copper and zinc in diabetic subjects partly they may be due to different methodology used for investigating them (Goyal *et al* 1982). Raised serum copper levels have been reported by various authors (Lisun Lobanova., 1963) (Sinha *et al*., 1970, and Metwootal, 1978) while Kosombo (1965) reported it to be raised.

The liver serves as a storage organ for most of the essential micronutrient elements and therefore it is not surprising that in various liver disorders one encounters metabolic changes with respect to these elements (Ananda Prasad., 1978).

The diseases of the liver are hepatic coma, viral hepatitis, serum hepatitis and cirrhosis. Diagnosis of hepatic coma is supported by the finding of increased arterial blood ammonia levels, levels of more than 200 mg/100 ml. Frequently there is metabolic alkalosis, with arterial blood PH at the upper limit of normal. Viral hepatitis is supported by histological examination. Portal hepatic cirrhosis is confirmed by histological examination of liver biopsy material. Primary biliary cirrhosis is confirmed by the finding of marked increase in serum alkaline phosphatase activity, marked increase in serum cholesterol and the total serum lipids, increased serum bilirubin.

with bilirubin and urobilinogen in excess in the urine, with serum antibodies against mitochondria (Eastham., 1973).

Serum zinc was noted to be decreased in cirrhosis of liver (Ananda prasad et al ., 1970). In some patients with viral hepatitis serum zinc was elevated and approximately one third of these subjects excreted high zinc in the urine (Khan et al ., 1980). Serum zinc Low selenium values have been demonstrated to be associated with the low zinc level in cirrhotic patients (Sullivan et al.,1979). Serum zinc was low but urinary zinc excretion was pronounced in Indian childhood cirrhosis patients (Sharda et al ., 1982). Serum zinc is decreased in alcoholic cirrhosis (Vyas et al., 1982). According to Ananda Prasad, (1983) patients with cirrhosis of liver have p hyperzincuria.

Primary biliary cirrhosis is a chronic inflammatory process involving the intrahepatic bile ducts that progresses to cirrhosis and frequently leads to death from liver failure or variceal bleeding. The cause is unknown, but there is much evidence for an autoimmune process. The classical signs and symptoms of primary biliary cirrhosis include pyuritus, jaundice, fatigability and xanthomatosis. The diagnosis is confirmed by characteristic liver-biopsy findings and by the presence in the serum of mitochondrial antibody. (Joseph et al., 1983).

Cirrhotic patients had low serum calcium level (Sullivan *et al*., 1979).

Magnesium is an intracellular cation in human body. It is an essential chelating agent in almost all cellular biological processes (Aikawa., 1971). The concentration of magnesium in liver and striated muscles is known to be the highest amongst the nonosseous tissues (14 to 16 mEq/kg) Serum magnesium estimation may prove useful in diagnosing an occult liver abscess or an abscess in the phase of formation. Serum magnesium estimation may find a definite place in the diagnosis of alkaline phosphatase (Sarin., 1963).

The magnesium content of the liver tissue per unit weight is decreased in cirrhosis (Wilke and Spielmann., 1960). This decrease appears to be due mainly to the substitution of parenchymal tissue of high magnesium content high with a connective tissue of low magnesium content. There is a good relationship between histological changes and decrease of magnesium concentration per number of cells. The actual changes in the concentration of magnesium in the parenchymal cells of the cirrhotic liver appears to be negligible (Ananda Prasad *et al*., 1976).

Low values of serum magnesium was found in patients with alcoholic cirrhosis, (Vyas *et al*., 1983). Khan *et al*., (1983) observed a significant rise in serum magnesium level with a simultaneous increase in 24 hour urinary magnesium excretion in cases of viral hepatitis.

In another study by Sharda *et al*., (1982) magnesium in serum and urine were within normal limits in Indian childhood cirrhosis.

Copper appears to be essential for the normal development of bone, the central nervous system and connective tissues. Serum copper is increased in a number of acute and chronic pathological conditions including haemochromatosis and portal and biliary cirrhosis (Ananda Prasad, 1970)

No change in serum copper level was observed in cirrhosis (Vyas *et al*., 1982). Sharda *et al*.,(1982) reported increased values of copper in serum and urine in Indian childhood cirrhosis.

The present investigation was aimed to study the changes in serum calcium, copper, zinc, magnesium and Iron levels of diabetics and of patients suffering from liver diseases and to find out whether there is any correlation between the levels of the above trace elements in serum and of the diseases concerned.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

The literature pertaining to the study 'serum calcium, copper, zinc, magnesium and iron in diabetes and liver diseases' has been described under the following headings:

1. Functions of Trace elements:
    a) Calcium
    b) Zinc
    c) Copper
    d) Magnesium
    e) Iron
2. Trace elements in diabetics:
    a) Zinc
    b) Copper
    C) Magnesium
    d) Iron
    e) Calcium
3. Trace elements in liver disease.
    a) Zinc
    b) Copper
    c) Magnesium
    d) Calcium
    e) Iron

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There are more than twenty trace elements in human tissues. Only seven of these trace elements are generally accepted as being essential to man namely iron, copper, zinc, manganese, cobalt, chromium and molybdenum. It has also been suggested that vanadium nickel, silicon, Aluminium, strontium and tin are essential (Marks *et al.*, 1976)

Functions of trace elements:

a) Calcium: Calcium is the most plentiful cation in the body which contains 25 to 35 mol (1.0 to 1.4 Kg) in the adult. Over 99% is in the bones and teeth, the former providing a large reserve which can be drawn on as required. The small part of the body calcium present in plasma and other extra cellular fluid is 2.7 mmol or 1.1.g. There is almost no calcium in red blood cells and other introcellular fluid. (Varley *et al.*, 1980)

Calcium is involved in the bilogical processes such as neuromuscular functions which involve excitability of nerve function, neural transmission and contractility of cardiac and skeltal muscle, blood coagulation, membrance function which involves transfer of inorganic ions across cell membrane, and release of the neurotransmitters at synaptic junctions, in selected enzymatic reactions which involve the release of cellular enzymes such as amylase from the parotid and the level of activity of intracellular enzymes such as isocritric debydrogenase, phosphorylase, kinase and phosphofructo kinase. Secretion of certain peptide hormones like pitutary harmone, parathyroid hormone, calcitonin and vasopression is regulated through calcium ionic concentration (Talwar., 1980)

b) Zinc: Zinc is a component of a number of enzymes particularly carbonic anhydrase, carboxy peptidase A and the debydrogenases acting on alcohol, glutamate, glyceraldehyde - 3 - phosphate and lactate. Other enzymes are activated by zinc include a variety of dipetidases and amino peptidases, (Martin et al., 1981). Zinc is important in man for growth and sexual development. Zinc seems to be important for the synthesis of RNA and DNA and for cell division, (Varley et al., 1980) Zinc appears to be necessary for the building of protein, which makes up most of the solid matter of the cells. It is a constituent of the enzyme which release carbondioxide from bicarbonate in the blood, of the one which begins the oxidation of alcohol and of other natural substances and of the splitting or portions of proteins (Schroeder., 1976). Zinc is essential to the growth of animal tissues (Mertz., 1971).

c) Copper: The metals copper and zinc are involved in several physiological processes which include the normal physiological variations which occur in copper, and zinc concentration in various body fluids, in inter relationships between copper and zinc in steroidal hormones, the inter relationships between copper and zinc in maternal fetal metabolism and the interrelationships between metals and sensory processes, particularly that of gestation. Copper is essential to the normal development of the nervous, system and plays a significant role in the maintenance of the myolin sheath (Walter Mertz., 1971) Copper appears to be essential for the normal development of bone, the central nervous system and connective tissues (Ananda Prasad et al., 1970). Copper is an excellent catalyst for oxidation - reduction systems, showing great versatility for an impressive variety of reaction, including the formation of water from oxygen and hydrogen at body temperatures, this reaction would be explosive without copper, It is an unique agent in biological systems, all living things require it and it is vital (Schroeder., 1976).

d) Magnesium: Magnesium is an important intracellular cation in human body. It is an essential chelating agent in almost all cellular biological process (Aikawa., 1971). The adult human body contains from 21 to 28g of magnesium or approximately 200 Meq in a 70Kg. man magnesium is the forth cation in terms of abundance in the human and animal body. It's high concentration in cells is like that of potassium. There are many similarities between potassium and magnesium metabolism, although magnesium is much harder to displace from the cell than potassium(Ananda Prasad., 1976).

e) <u>Iron</u>: Iron is necessary for the carrying and exchange of oxygen in the blood of mammals and for many systems involving oxidation and its converse, reduction. It is a vital element in that without it one dies (Schroeder., 1976).

TRACE ELEMENTS IN DIABETES:

a) <u>Zinc</u>: Pidduck *et al*., (1970) demonstrated that diabetics excrete more zinc in the urine then do normal subjects, and Spencer and Samachson (1970) reported that during total starvation plasma zinc levels remained unchanged despite a tenfold increase in urinary excretion. The significane of these observations is obscure at this time, as is a major role for zinc in diabetes, vascular disease, or obesity (Katsen., 1975).

A relationship exists between insulin and zinc has been known since it was shown that zinc combines with insulin to form crystalline insulin. Further work showed that the relationship between insulin and zinc in the bovine pancreas, the organ which is responsible, for insuling synthesis. Normally there is a high level of zinc in the pancrease but in diabetes mellitus this level is greatly reduced. Studies showed that zinc and insulin flucutate in a similar way in experimentally produced diabetes and substances that reduce the zinc content of he pancrease are, infact, diabetogenic Zinc injections in diabetes are not beneficial and result in a rise in blood sugar, and high blood sugar, as well as the excreation of large quantities of sugar in the urine, are symptomatic of diabetes. The injected zinc is not excreted, and it appears to be accumulated. This may result in fibrosis of the pancrease. There is not relationship between blood - plasma zinc levels and the duration of the diabetes (Schutte., 1964)

A study was conducted by Goyal et al., (1982) in which they reported that serum zinc values were not different from controls. Highest values were seen in patients with ketoacidosis. It had no correlation with age and sex of the patient, blood sugar level, serum protein or type of diabetes. Majority of the workers (Pidduck et al., 1970) and Frank et al., (1962) Buczkowski et al.,(1968) Romner et al., (1970) have reported zinc levels of blood like the normal healthy individuals while Mateo et al., (1978) reported it to be raised, Goyal et al., (1982) in his study reported that zinc level did not differ significantly from the controls. As regards the sex of patients and the serum level of the element there was no statiscally significant difference between them, Mean serum zinc levels were not significantly different in juvenile and maturity onset type of diabetic subjects. There was no correlation between serum levels and wright of diabetic patients. Also there was not correlation with the duration of diabetes. No correlation was observed between serum zinc level and severity of diabetes as judged by fasting blood sugar level. In patients with hyper cholesterolemia serum zinc level did not differ. Serum zinc did not differ with different levels of blood urea, or total and differential serum proteins.

A study was conducted by Vyas et al.(1982) in which serum zinc, copper, magnesium and calcium levels were estimated in various human diseases. They reported that diabeteics had significantly low serum zinc level.

b) Copper: Goyal et al., (1982) reported that serum copper levels were significantly higher in diabetics. Female diabetics had higher levels than the male diabetic patients with hypercholesterolemia had statistically significant higher levels of serum copper. Highest values were seen in patients with pulmonary tuberculosis. It had no correlation with age of the patient, severity, duration and type of diabetes, weight of the patient and serum protein levels. There are conflicting reports about serum concentration of copper and zinc in diabetic subjects partly they may be due to different methodology used for investigating them. Raised serum copper levels have been reported by various authors while Kosenko reported it to be diminished. Still others (Davies et al., 1968 and Pidduck et al., (1970) found no significant difference from the normal values.

c) Magnesium: Diabetics had significantly low serum magnesium level (Vyas et al., 1982) In diabetic patients the pretreatment serum magnesium levels are very high, about 9 meq/l which rapidly fall 60 0.6 meq/l on insulin thrapy. During acidosis, which occurs on insulin withdrawal large amounts of magnesium are excreated (Talwar., 1980). Increases in serum magnesium occur in diabetic coma, falling in this condition during treatment with insulin and intravenous fluids (Varley et al., 1980).

d) Iron: Iron will not play a major in vascular disease and diabetics (H.M. Katsen., 1975).

e) Calcium: Diabetics had no significant change in serum calcium. (Vyas et al., 1982)

TRACE ELEMENTS IN LIVER DISEASES:

a) Zinc: Serum zinc is decreased in alcololic cirrhosis. A fall in serum zinc occurs with increased excreation and loss from muscles as part of the metabolic response to trauma

(Varley et al., 1980). The decrease of the element in cirrhosis is well documented and is produced, inpart, by absence of elements, in diet, vomiting diarrhoea, malabsorption, the metabolism of alcolol with increased urinary loss and decreased serum levels of metal binding proteins. (Vyas., et al., 1982)

Valle et al., (1959) suggested that low plasma zinc levels in cirrhosis may be due to conditioned zinc deficiency, second factor blamed is increased urinary excretion of zinc as observed by Srivastava et al., (1978) Prasad et al., (1963). Other factors blamed are lowered dietary intake and impaired absorptive capacity of bowel, secondary to hepatic, involvement. It may be reasonably considered that zinc is related to protein synthesis and thereby induces regeneration and repair of hepatic cells and therefore zinc therapy may not only restore the plasma zinc level but may also help regeneration of liver cells. However this statement may need further confirmation (Khare et al., 1982).

In another study by Sullivan et al.,(1962) hyberzincuria was noted in patients with post alcoholic cirrhosis, in 50 per cent of chronic alcoholics without clinical or laboratory evidence of cirrhosis and some nonalcolilic subjects with cirrhosis. Certain amino acids compete effectively with serum proteins for binding of zinc (Prasad *et al.*, 1968). Abnormal metabolism of amino acid has been reported to occur in diseases of the liver (Ning *et al.*, 1967). It is therefore conceivable that hyperzincuria in cirrhosis may be related to an abnormal pattern of urinary excretion of amino acids. In some patients with viral hepatitis serum zinc was elevated and approximately one third of these subjects excreted abnormally high zinc in the urine (Khan *et al.*, 1965). These results could have been due to the release of zinc containing enzymes from damaged hepatic cells analogous to the elevation of serum iron found in acute hepatitis (Prasad *et al.*, 1970). The normal urinary excretion of zinc is low about 0.1-0.7mg/day, after a daily intake of 10-15 mg these levels rise very highly in post alcoholic hepatic cirrhosis (Talwar 1980). Low selenium values have been demonstrated to be associated with low serum zinc calcium and magnesium levels found in cirrhotic patients (Sullivan *et al.*, 1979)

Patients with cirrhosis of liver have hyperzincuria. Several of their clinical monifestations are due to zinc deficiency and are corrected by zinc supplementation. Some of the clinical features of cirrhosis of the liver such as loss of body hair, testicular hypofunction, poor apetite, mental lethargy, difficulty in wound healing and night blindess may be related to the secondary zinc dificient state in this disease (Ananda Prasad, 1983).

b) Copper : No change in serum copper level was observed in cirrhosis (Vyas et al., 1982). The urinary excretion of copper rarely exceeds 0.8 micro mol 24 hour (50 microgram/24 hour) in normal individuals. Small increase 1.6 to 3.2 micromole (100 to 200 microgram) daily but occasionally higher, may be found in proteinuria, carrhosis of the liver, including primary biliary cirrhosis and Fanconi syndrome (Varley et al., 1980)

C) Magnesium. Low values of magnesium was found in patients with alcoholic cirrhosis (Vyas et al., 1982) Serum magnesium levels remain normal in nonsupprative hepatic amoebiasic patients, hypermagnesemia roughly proportional to the extent of liver damage in observed in 86 per cent of patients of amoebic liver abscess (ALA) conventional liver function tests usually fail to differentiate these two. Serum magnesium estimation may find a definite place in the diagnosis of ALA (Sarin et al., 1982).

Khan et al., (1983) reported a significant rise in serum magesium level with a simultaneous increase in 24 hour urinary magnesium excretion were observed in cases of viral hepatitis.

The magnesium content of the liver tissue per unit weight is ~~dax~~ decreased in cirrhosis. This decrease is due to the substitution of parenchymal tissue of high magnesium constituent with a connective tissue of low magnesium content. There is a good relationship between histological changes and decrease of magnesium concentration per number of cells. The actual changes in the concentration of magnesium in the paranchymal cells of the cirrhotic liver appear to be negligible. (Anand Prasad., 1976)

d) Calcium: Cirrhotic patients had low serum calcium level (Sullivan et al., 1979)

The decreased serum calcium found in alcololic cirrhosis and is produced inpart by the absence of the elements in the diet, vomiting, diarrhoea, malabsorption, the metabolism of alcohol with increased urinary loss and decreased serum values of metal binding proteins. (Sullivan et al., 1969) The abnormal calcium balance may also contribute to the increased incidence of osteoprosis reported in alcoholic patients. (Saville et al., 1965)

e) Iron: The serum iron increases with figures up to 300 microgram per 100 ml and over have been reported in acute infective hepatitis with normal values in obstructive jaundice (Varley et al., 1980).

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURES

The experimental procedure adopted for the study on 'Serum calcium, copper, zinc, magnesium and iron in diabetes and liver diseases' is as follows:

A. Selection of patients

B. Collection of blood

C. Separation of serum

D. Analysis of biochemical parameters.

A. Selection of Patients:

30 persons suffering from diabetes mellitus and 20 persons suffering from liver disease were selected at random from the outpatient ward of Coimbatore Medical College Hospital during January to March 1984.

An equal number of persons matching in age, sex, free from all diseases and are in good health, were chosen to serve as controls.

B. Collection of blood:

The blood was collected as follows:

(Varley *et al.*, 1980)

A tourniquet was applied a few centimeter above the elbow without obliterating the arterial pulse at the wrist. The skin was sterilised over the vein and a disposable sterile needle to a disposable syring of appropriate capacity usually 10 ml was inserted into the vein. When the needle entered the vein the plunger was withdrawn slightly. The tourniquet was released when the blood appeared. When the desired amount of blood has been drawn into the syringe, the tourniquet was released. The needle was withdrawn. With the needle still in position slowly the blood was transferred to an appropriate container, using the minimum amount of pressure.

C. Separation of Serum:

Blood was transferred to clean empty tubes and allowed to clot for three hours at room temperature. The clot was allowed to retract. Then it was centrifuged and the serum was separated using a rubber-bulb pipette. It was collected in clean dry labelled test tubes and stored in the freezer until used.

D. Analysis of biochemical parameters:

ESTIMATION OF CALCIUM:

Principle: Method of clark and collip ($V_a$rley et al., 1980)

Calcium is precipitated as oxalate directly from the serum and after washing the precipitate is dissolved in acid and titrated against permanganate.

Reagents:

1. Ammonium oxalate 4% solution
2. Ammonia 2% (v/v) solution - Dilute 2 ml of ammonia to 100 ml with water.
3. Potassium permanganate 0.01 N - Prepare freshly before use by diluting stock 0.1 N. solution
4. Approximately normal sulfuric acid.

Procedure:

To 2.0 ml of serum add 2.0 ml of distilled water and 1 ml of 4% ammonium oxalate solution. Mix well and allow to stand overnight. After calcium was precipitated centrifuged and remove the supernatant fluid without disturbing the precipitate. The supernatant was then poured off. After this 3.0 ml. of 2% ammonia was run down the sides of the tube . Mixed the precipitate and centriguged again. Poured off the supernatant. Repeated this until supernatant gave no previpitate with calcium chloride solution. Added 2 ml of approximately normal sulfuric acid. Mixed the precipitate well with the acid. Warmed by placing in a beaker containing boiling water to complete the solution of oxalate. Removed and titrated with N/100 potassium permanaganate to a faint pink colour which persists for about a minute. As a blank titrated 2.0 ml of approximately 1N sulfuric acid to the same end point.

Calculation:

One ml of 0.01 N permanaganate is equivalent to 0.2 mg of calcium. Hence since 2.0 ml of serum is used.

Mg calcium per 100 ml of serum

= (Titration of Unknown-titration of blank) x 0.2 x100/2

= (Titration of Unknown - titration of blank) x 10

ESTIMATION OF MAGNESIUM

Method of Denis: (Oser., 1971)

Principle:

After removal of calcium as oxalate the magnesium is precipitated as magnesium ammonium phosphate and the latter is estimated by a colorimetric phosphate determination. In the method here described, the Fiske-Subbarow phosphate method is used.

Reagents:

Standard Phosphate solution:

Dissolved 0.560 g of pure dry monopotassium phosphate in water to make 1000 ml. Added a few drops of chloroform to prevent growth of molds. Dilute 100 ml of this stock solution to 100 ml 1 ml = 0.01 mg. of magnesium.

10 N Sulfuric acid: Carefully added 450 ml of concentrated sulfuric acid to 1300 ml of water. To check, dilute 100 ml of this solution to 100 ml in a volumetric flask, mix and titrate a 10 ml portion with standard 1N sodium hydroxide. From the titration results, adjust the original solution if necessary to make it exactly 10 N.

Molybdate solution:

Dissolved 25 g of reagent grade ammonium molybdate in about 200 ml of water. In a 1 litre volumetric flask place, 300 ml of 10 N sulfuric acid.. Added the molybdate solution and dilute with washings to one litre with water. Mixed stable indefinitely.

Aminonapthol sulphonic acid reagent:

Placed 195 ml of 15% sodium bisulfite solution in a glass stoppered cylinder. Added 0.5 g of 1,2,4 aminonahthol sulfonic acid. Added 5 ml of 20% sodium sulfite. Stopper and shake until the powder was dissolved. If solution was not complete, add more sodium sulfite, 1 ml at a time, with shanking but avoid an excess. Transferred the solution to a brown glas bottle and stored in the cold. Thos solution was unable for about four weeks, if kept as described.

<u>15 per cent sodium bisulfite:</u>

To 30g of reagent grade sodium bisulfite in a breaker, added 200 ml of water from a graduated cylinder. Stirred to dissolve, and if turbid allow to a stand stoppered for several days and filter. Keep well stopped.

<u>20 per cent sodium sulfite:</u> Dissolved 20 g of reagent grade anhydrous sodium sulfite in water, diluted to 100 ml and filtered if necessary keep well stoppered.

<u>Procedure:</u>

Precipitated the calcium from 2 ml serum. After centrifuging, pipetted 3 ml of the supernatant fluid into a 15 ml graduated centrifuge tube and added with stirring 0.5 ml of a 5 per cent solution of ammonium phosphate containing 5 ml of concentrated ammonium hydroxide.per litre, followed by 2 drops of concentrated ammonium hydroxide. Let stand overnight, centrifuge siphon off the supernatant fluid and washed the tube with 5 ml of a mixture of one part of concentrated ammonium hydroxide. (Specific gravity 0.9) and two parts of water. Centrifuged and siphon off wash liquid. Repeated the washing a second and third time and then wash finally with 5.0 ml of 75 per cent alcohol containing 10.0 ml of concentrated ammonium hydroxide per litre. Siphon off again and let stand in a warm until the ammonia has evaporated.

To be residue in the centrifuge tube added 1.0 ml of molybdate solution used in the Fiske-Subbarow phosphate method and tap to dissolved. When dissolved added 5.0 ml of water and set aside. Prepared a Standard by placing

1.0 ml of the molybdate solution in a second graduated tube and adding 3 ml of the standard phosphate solution. (equivalent to 0.03 mg of magnesium) Plus 2.0 ml of water. Prepared a blank by placing 1 ml of molybdate solution plus 5.0 ml of water in a third graduated tube when all the tubes are ready, added to each 0.4 ml of aminoaphthol sulfonic acid reagent, followed immediately by water to the 10.0 ml mark Mixed and allowed to stand 5 minutes before reading. Set the photometer to zero density at 660 millimicron with the blank.

Calculations

$$\frac{\text{Density of unknown}}{\text{Density of Std.}} \times \frac{0.03 \times 100}{1.2} = \text{Mg Magnesium per 100 ml Serum}$$

ml serum

The characteristic of the color and the conditions of photometric measurement are the same as forthe determinations of inorganic phosphate. At 660 millimicron the standard has a density of approximately 0.500 in a 1 cm cuvet. Since this standard corresponds to a serum Mg content of 2.5 up to 5 mg percent may be accurately determined under these conditions. For higher values, or for measurement at greater depth of solution, add 2 ml of molybdate solution to unknown, blank and standard instead of the 1 ml specified (use the same 3 ml potion of std) followed by water in each case to about 12 ml then add 0.8 ml of aminonapthol sulphonic acid reagent and water to a 20 ml final volume.

There is no change in the calculations. Color development at a greater dilution is recommended rather than the analysis of a smaller protein of serum because it is not known whether or not precipitation of magnesium in this procedure will be quantitative at conditions other than those specified.

DETERMINATION OF SERUM COPPER, ZINC, AND IRON BY ATOMIC ABSORPTION SPECTROPHOTOMETER:

The principle and working of Atomic Absorption spectrophotometer:

The sample in solution or suspension was heated to a high temperature by burning it in a flame. The flame broke up the chemical bonds between the molecule and enabled the individual atoms to float freely in the sample area. In this condition the atoms (unexisted) absorb UV or the visible radiation. The wave length bands, in which each elements can absorb were narrow, Hence, at a particular ~~sfxxhxxxxx~~ wave length the absorption have a direct indication of the amount of the metal that was present. Acetylene flame was used for the study.

Working of the Instrument:

1. Selected the cathode lamps to be used and inserted them in the lamp quarants.
2. Depressed the relevant LAMP SELECT button for the lamp being used and set the METER SELECT to the same lamp.
3. Switched on the instrument, set the lamp at the desired current and allow to stabilise for 10-15 minutes.
4. Set the indicate 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 used again for setting to give approximately 90 per cent transmission reading.
6. Select the "Auto 100 " Mode and turn the "Set 100" to read (0.0 absorbance or) 100 per cent transmission.
7. Select the desired mode of operation on the indicator unit (i.e., Absorbance or) transmission.
8. Lighted the flame.
9. Nebulized the sample into the flame.

Determination of Serum Copper, Zinc, and Iron:

The trace elements in serum on digestion with triple acid (a mixture in nitric acid, sulfuric acid & perchloric acid 9:2:1 respectively) get seperated into the solution (Piper's method, 1980). (piper

Procedure:

1.0 ml of serum sample was digested with 10 ml of triple acid and made up to 25 ml with glass distilled water. The above extract was used for a estimating copper, zinc and iron, using atomic absorption spectrophotometer. Althouth various methods were avilable for the estimation of serum iron, it is estimated by Atomic Absorption Spectrophotometer since the same extract which was used for the estimation of copper and zinc can be used for the etimation of iron.

DETERMINATION OF SERUM BILIRUBIN:

Colorimetric method of Malloy and Evelyn (Varley 1976).

Principle:

Serum is diluted with water and methanol is added in a amount insufficient to precipitate the proteins yet sufficient to permit all the bilirubin to react with the diazoreagent.

Reagents:

1. Absolute methanol
2. 1.5 % Hydrochloric acid
3. Diazo reagent:

Prepared freshly before use by adding 0.3 ml of solution 'B' to 10 ml of solution 'A'

Solution 'A'

Dissolved gram of sulphanilic acid in 15 ml of concentrated hydrochloric/acid and made up to 1 litre with water.

Solution 'B'.

Dissolved 0.5 gram of sodium nitrite in water and made up to 100 ml. Prepared freshly at frequent intervals.

4. Standard solution of bilirubin:

Prepared a solution containing 10 mg per 100 ml of chloroform. It may be necessary to reflux the mixture gently to dissolved the bilirubin.

Techniques:

Took two test tubes and into each place 0.2 ml of serum and 1.8 ml of distilled water. To the unknown added 0.5 ml of the diazo reagent and to the blank 0.5 ml of 1.5 per cent hydrochloric acid. Finally to each added 2.5 ml of methanol, stand for thirty minutes and read in the colorimeter using a yellow - green filter or set at 540 nm. Subtract the reading of the balnk from that of the test.

For a standard curve dilute the above standard 1 in 5 with methanol to obtain a solution containing 2 mg bilirubin per 100 ml. Set up a series of tubes and read as for the test.

mg. bilirubin per 100 ml serum	0	2.5	5.0	10	15	20	25	30
Ml standard solution	0.5/0.5		1.0	2.0	3.0	4.0	5.0	6.0
Ml ethanol	9.0	8.5	8.0	7.0	6.0	5.0	4.0	3.0
Ml diazo reagent	Add 1 ml to each tube.							

DETERMINATION OF BLOOD SUGAR:

Principle:

Blood is deproteinised and treated with alkaline copper reagent in Folin Wu tubes and heated. The cuprous oxide formed is treated with an acid molybdate solution, when a blue coloured solution is got the colour was compared with that of the standard colorimetrically at 660 millimicron.

Reagents:

1. 10% sodium tungstate
2. 2/3 N sulphuric acid
3. Alkaline copper reagent:

40 g of sodium carbonate was dissolved in 400 ml of distilled water. 7.5 g, of tartaric acid was added and dissolved 4.5. g copper sulphate was dissolved in 150 ml of water. This solution was added to sodium carbonate solution and made upto 1 litre with distilled water.

4. Phosphomolybdic acid reagent:

Dissolved 35 g of molybdicacid and 5 g of sodium tungstate in 200 ml of 10% sodium hydroxide and200 ml of water and boil for 30 to 40 minutes to remove ammonia present in the molybdic acid, cool and transfer with washings to 500 ml flask, diluting to about 350 ml. Then 125 ml of phosphoric acid (SG 1.75) was added and made up to 500 ml with distilled water.

5. Standard glucose solution Stock:

0.2 g of anhydrous glucose was dissolved in 100 ml of distilled water.

6. Working standard solution:

10 ml of stock solutionwas diluted to 100 ml with distilled water. 1.0 ml of this solution contains 200 microgram of glucose.

Technique:

To 0.2 ml of blood 3.4 ml of distilled water, 0.2 ml of 10 per cent sodium tung state and 0.2 ml of 2/3 N sulphuric acid were added to precipitate the protein. Allowed to stand for 5 minutes after thorough mixing. Centrifuged.

2.0 ml of clear filtrate was taken in a Folin-Wu tube marked (T). 1.0 ml of working standard 10 mg per 100 ml was taken in another Folin-Wu tube marked (S). The third tube had been taken as blank marked.(B). To all the tubes 2 ml of alkaline copper had been added and mixed well.

All three tubes were kept in the boiling water bath for 8 minutes. After 8 minutes the tubes were cooled to arrest further reaction. 2 ml of phosphomolybdic acid was added to each tube and made upto 25 ml with water. Mixed well.

The molybdenum blue colour developed was read at 660 or by using red filter-

Calculation

mg of glucose in 100 ml of blood

$$\frac{\text{Reading of the unknown}}{\text{Reading of the standard}} \times 0.1 \times \frac{100}{0.1}$$

$$\frac{\text{Reading of the unknown}}{\text{Reading of the standard}} \times 100$$

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

This study was aimed at assessing 'Serum, Calcium, Copper, Zinc, Magnesium and iron in diabetes and liver diseases'. The results were compared with normal healthy individuals (controls) of matching age and sex, and were free of diseases.

The Parameters studied included:

i) Blood glucose level of diabetics and controls
ii) Serum bilirubin level of patients of liver diseases and controls.
iii) Serum calcium, magnesium, zinc, copper and iron levels of the patients and controls

The result obtainedin this study are discussed under the following headings:

(a). Distribution of diabetic and liver patients and normal individuals according to age groups.
(B) Blood sugar levels of diabetics and bilirubin levels of liver patients.
(C) Blood sugar, serum calcium and magnesium levels in diabetic patients and controls.
(D) Serum zinc, copper and iron levels in diabetic patients and controls

(E) Serum bilirubin, calcium and magnesium levels in liver patients and controls.

(F) Serum zinc, copper and iron levels in liver patients and controls.

(G) Serum bilirubin, calcium and magnesium levels in patients of different liver diseases.

(H) Serum zinc, copper and iron levels in patients of different liver diseases.

(I) Serum magnesium and calcium in diabetics and liver patients.

(J) Serum zinc, copper and iron in deabetics and liver patients.

Table I presents the distribution of diabetics and liver patients and normal individuals according to age groups.

TABLE I

DISTRIBUTION OF DIABETIC AND LIVER PATIENTS AND NORMAL INDIVIDUALS ACCORDING TO AGE GROUPS.

Age groups (years)	Number of Samples: Normal	Diabetics	Liver patients
10-30	6	4	4
30-50	8	9	12
50-70	11	13	8
70-90	5	4	1
Total	30	30	25

In the present study, thirty normal individuals and fifty five patients of both sexes, with age range 10-90 years studied. Of the fifty five patients studied, thirty were diabetics and the rest were liver patients.

Among diabetics four patients were in the age group 10 to 30 years; nine in the age group 30-50 years; thirteen in the age group 50 to 70 years and four patients in the age group 70-90 years.

Among liver patients, four patients belonged to the age group 10 to 30 years, twelve to 30-50 years, eight to 50-70 years and one patient to 70+ to 90 years of age groups respectively. These patients were analysed for serum calcium, magnesium, zinc, copper, iron, bilirubin and blood glucose levels and the results were compared with healthy individuals, of who six were between 10 to 30 years, eight of them were in the age group 30-50 years; eleven in 50 to 70 years; and five of them in 70 to 90 years of age group respectively. These biochemical estimations were carried out in the controls for comparison.

Blood sugar level of diabetics and bilirubin level of liver ptients are shown in TABLE II, The individual values of blood sugar and bilirubin are given in APPENDICES I and II respectively.

TABLE II

BLOOD SUGAR LEVEL OF DIABETICS AND BILIRUBIN LEVEL OF LIVER PATIENTS.

Diabetics Blood sugar mg/100 mlE			Liver patients Bilirubin mg/100 ml.		
No.	Mean ± S.D.	Range (mg/100ml)	No	Mean ± S.D.	Range (mg/100 ml)
30	222.90 ± 78.97	132.00 to 550.00	25	7.02 ± 7.51	1.20 to 26.0

Vatley et.al., (1980) has reported the normal range of blood sugar level to be 70-120 mg per 100 ml. As per this criterion, the patients selected for this study were confirmed to be diabetics. Their mean blood sugar was found to be 222.90 ± 78.97 mg per 100 ml with a range of 132 to 550 mg per 100 ml.

Normal serum contains not more than 1.0 mg bilirubin per 100 ml. Most normal persons appear to have 0.2-0.6 mg bilirubin per 100 ml serum. (Varley etal., 1980). In this study, bilirubin level of the patients was found to be 7.02 ± 7.81 with a range of 0.2 26 mg per 100 ml. Wide variations in he serum bilirubin level of the patients were noticed.

Serum Calcium and magnesium levels in diabetic patients and controls are shown in Table III. The individual values are given in APPENDICES I and III

TABLE III

SERUM CALCIUM AND MAGNESIUM LEVELS IN DIABETIC PATIENTS AND CONTROLS.

Serial. No.	Group	No	Calcium mg/100 ml Mean ± S.D.	Range mg/100 ml	t value	Magnesium mg/100 ml Mean ± S.D.	Range mg/100 ml	t value
1	Control	30	9.87 ± 1.27	7.00 to 13.00		2.25 ± 0.37	1.76 0.03	
					0.317 N.S.			9.8 **
2.	Diabetics	30	9.97 ± 1.20	8.00 to 13.00		1.47 ± 0.23	1.11 to 1.99	

N.S. -Not significant **($P<0.01$)

With reference to Varley etal., (1980) the normal serum calcium ranges from 9.0 to 11.0 mg per 100 ml. As per this criterion the serum calcium level of the controls was found to be in the normal ranges. It ranged from 7.0 to 13.0 mg per 100 ml with a mean value of 9.87 ± 1.25 mg per 100 ml.

The serum calcium of the diabetics ranged from 8.0 to 13.0 mg per 100 ml with a mean of 9.97 $\pm$ 1.2. mg per 100 ml. There was no significant difference between calcium level of the controls, and the diabetics. This suggested that in diabetes mellitus the serum calcuim level is not affected.

Serum magnesium in the controls ranged from 1.76 to 3.03 mg per 100 ml with a mean value of 2.25 $\pm$ 0.37 mg per 100 ml. The same ranged from 1.11 to 1.88 mg per 100 ml with a mean of 1.47 $\pm$ 0.23 mg per 100 ml. Based on the normal range of serum magnesium which is 1.7 to 2.6 mg per 100 ml proposed by Varley etal., (1980) a low level of serum magnesium was noticed in the diabetics while in the controls it was within the normal range. This difference in the serum magnesium level between diabetics and the controls was found to be statisally significent ($P < 0.01$), indicating that the serum magnesium is some how decreased in diabetes mellitus. This is in agreement with the finding of Vyal et al., (1982).

Serum Zinc, Copper and iron levels of the diabetics and the controls areshown in Table IV and the individual values are given in Appendices I and III respectively.

TABLE IV

SERUM ZINC, COPPER AND IRON LEVELS OF THE DIABETOCS AND CONTROLS

Serial No.	Group	No.	Zinc mg/100 mg Mean ± S.D.	Zinc mg/100 mg Range mg/100ml	Zinc t Value	Copper mg/100 ml Mean ± S.D.	Copper mg/100 ml Range mg/100 ml	Copper t Value	Iron mg/100ml Mean ± S.D.	Iron mg/100ml Range mg/100ml	Iron t Value
1.	Control	30	0.10 ± 0.02	0.06 to 0.15	10.0**	0.11 ± 0.03	0.06 to 0.15	2.06 N.S.	0.09 ±	0.05 to	0.00 N.S.
2.	Diabetics	30	0.05 ± 0.02	0.02 to 0.08		0.13 ± 0.03	0.06 to 0.18		0.09 ± 0.02	0.05 to 0.13	

** ($P < 0.01$)

N.S.= Not Significant

Serum zinc in the controls ranged from 0.06 to 0.15 mg per 100 ml. The Serum zinc value of the control group fell within the normal range 0.07 to 0.11 mg per 100 ml as given by Varley et al., (1980) while it was 0.02 to 0.08 mg per 100 ml in the diabetics. The mean serum zinc, level was found to be decreased in diabetic patients. It was 0.05 ± 0.02 mg per 100 ml in the controls. This difference was found to be statistically significant ($P<0.01$). This result is in agreement with the observation of Vyas et al., (1982) and Sullivan et al., (1979)

The copper level in control was found to be 0.11 ± 0.03 and 0.13 ± 0.03 mg per 100 ml in diabetics. The range was 0.06 to 0.15 mg per 100 ml in the controls against 0.06 to 0.18 mg per 100 ml in diabetics. There was no significant difference between the copper level of the controls and that of he diabetics. This result is in confirmity with that of Pidduck et al., (1970).

The normal copper level is 0.075 to 0.16 mg per 100 ml (Varley et al., (1980). According to this, the copper level of the controls and the diabetics were found to be in the normal range.

The iron level in the control ranged from 0.05 to 0.16 mg per 100 ml in controls and it was 0.05 to 0.13 mg per 100 ml om diabetics. The mean level was 0.09 ± 0.02 mg per 100 ml in the controls and 0.9 ± 0.02 mg per 100 ml in diabetics. There was no significant difference between the iron level of the controls and that of the diabetics.

This indicated that the serum zinc level is lowered in diabetes mellitus, while the serum copper and iron levels remain unaffected.

Serum bilirubin, calcium, magnesium levels in liver patients and controls areshown in Table X. The individual values are given in Appendices II and III respectively.

TABLE V

LEVELS OF SERUM BILIRUBIN, CALCIUM AND MAGNESIUM IN LIVER PATIENTS AND CONTROLS

Serial No.	Group	No.	Bilirubin mg/100 ml Mean ± S.D.	Range mg/100 ml.	t Value	Calcium mg/100 ml Mean ± S.D.	Range mg/100 ml.	t Value	Magnesium mg/100 ml Mean ± S.D.	Range mg/100 ml	t Value
1.	Control	30	0.71 ± 0.19	0.3 to 1.0	** 4.21	9.87 ± 1.25	7.0 to 13.0	** 10.93	2.25 ± 0.37	1.76 to 3.03	** 16.42.
2.	Liver Patients	25	7.02 ± 7.51	1.2 to 26.00		6.80 ± 0.93	5.0 ± 8.0		1.10 ± 0.13	0.89 to 1.48	

** ($P < 0.01$)

The serum bilirubin level in the control ranged from 0.3 to 1.0 and 1.2 to 26.0 mg per 100 ml in liver patients, with a mean value of 0.71 ± 0.19 mg per 100 ml in the controls and 7.02 ± 7.51 mg per 100 ml in liver patients. Serum bilirubin level of liver patients was found to be elevated over the normal level which is 1.0 mg per 100 ml with reference to Varley *et al.*, (1980) while in this study the mean serum bilirubin level of the controls was found to be 0.71 ± 0.19 mg per 100 ml.

The serum calcium level in the controls and liver patients ranged from 7.0 to 13.0 mg per 100 ml and 5.0 to 8.0 mg per 100 ml respectively, and the mean value was found to be 9.87 ± 1.25 mg per 100 ml in the controls and 6.7 ± 0.93 mg per 100 ml in liver patients.

The mean serum calcium level of liver patients was found to be significantly (P 0.01) decreased when compared to that of the controls. This result is in agreement with that of sulliman *et al.*,(1979) who have reported that cirrhotic patients had low serum calcium level.

The serum magnesium level was found to range from 1.76 to 3.03 mg per 100 ml in the controls and it was 0.89 to 1.48 mg per 100 ml in liver patients, and the mean value was 2.25 ± 0.37 mg per 100 ml and 1.1. ± 0.13 mg per 100 ml in the controls liver patients respectively.

Serum Zinc, Copper and iron levels in liver patients and controls are represented in Table VI and the individual values are shown in Appendices II and III respectively.

TABLE VI

SERUM BILIRUBIN, ZINC, COPPER AND IRON LEVELS IN LIVER PATIENTS AND CONTROLS

Serial No.	Group	No.	Zinc mg/100 ml Mean ± S.D.	Zinc Range mg/100ml	Zinc t Value	Copper mg/100 ml Mean ± S.D.	Copper Range mg/100 ml	Copper t Value	Iron mg/100 ml Mean ± S.D.	Iron Range mg/100 ml	Iron t Value
1.	Control	30	0.10 ± 0.02	0.06 to 0.15	** 0.015	0.11 ± 0.03	0.06 to 0.15	0.001 N.S.	0.09 ± 0.02	0.05 to 0.16	. . X 0.002 N.S.
2.	Liver	25	0.03 ± 0.01	0.01 to 0.06	± 0.02	0.12 to ± 0.1[illegible]	0.08 to 0.16	[illegible]	0.08 0.08 0.01	0.06 0.06 0.12	

** (P< 0.02)

The serum Zinc level in control ranged from 0.06 to 0.15 mg per 100 ml while was 0.01 to 0.06 mg per 100 ml in liver patients. The mean serum zinc value was found to be 0.1 ± 0.02 mg per 100 ml per and 0.03 ± 0.01 mg per 100 ml in the controls and liver patients respectively.

When compared to the control value the mean serum Zinc value of the liver patients was found to be significantly ($P<0.01$) reduced and this is in confirmity with that of Valle *et al.*, (1959) who suggested that low plasma zinc levels in cirrhosis may be due to conditioned zinc deficiency. The result is also in agreement with the results observed by Srivastava *et al.*, (1978) and prasad *et al.*, (1963).

The copper level of the controls ranged from 0.06 to 0.15 mg per 100 ml while it was 0.08 to 0.16 mg per 100 ml in liver patients. The mean serum copper was found to be 0.11 ± 0.03 mg per 100 ml and 0.12 ± 0.02 mg per 100 ml in the controls and liver patients respectively.

Serum iron level in the control ranged from 0.05 to 0.16 mg per 100 ml while it ranged from 0.06 to 0.12 mg per

100 ml in liver patients. The mean iron level was found to be 0.09 ± 0.02 mg per 100 ml in control and 0.08 ± 0.01 mg per 100 ml in liver patients. This suggested that in liver patients, the serum copper and iron levels are not affected.

Serum bilirubin, calcium and magnesium levels in patients of different lever diseases are shown in the Table VII and the individual values are shown in Appendix II.

TABLE VII

LEVEL OF BILIRUBIN, CALCIUM AND MAGNESIUM IN PATIENTS OF DIFFERENT LIVER DISEASES

Serial	Group	No.	Bilirubin mg/100 ml			Calcium mg/100 ml			Magnesium mg/100 ml		
			Mean ± S.D.	Range mg/100 ml	t Value	Mean ± S.D.	Range mg/100 ml	t Value	Mean ± S.D.	Range mg/100ml	t Value
1.	Cirrhosis	18	2.56 ± 0.99	1.2 to 4.4	** 13.51	6.83 ± 0.9	5.0 to 8.0	1.3. N.S.	1.08 ± 0.14	0.89 to 1.14	.53 N.S.
2.	Obstructive Jaundice	7	18.49 ± 4.04	14.4 to 26.0	ø.	6.29 ± 0.88	5.0 to 7.00		1.04 ± 0.12	0.85 to 1.23	

** (P<0.01)

N.S. Not Significant

The mean bilirubin level in cirrhotic patients was found to be 2.56 ± 0.99 mg per 100 ml (range 1.2. to 4.4 mg per 100 ml) and in patients of obstructive jundice it was 18.49 ± 4.04 mg per 100 ml (range 14.4. to 26.0 mg per 100 ml). The elevated level of bilirubin observed in patients suffering from obstructive jaundice was statiscally siggnificant ($P<0.01$) when compared to serum bilirubin level of cirrhotics patients.

The mean srum calcium level in cirrhotic patients was found to be 6.83 ± 0.9 mg per 100 ml (range 5.0 to 8.0 mg per 100 ml) and in patients of obstructive jaundice it was 6.29 ± 0.88 mg per 100 ml (range 5 to 7.0 mg per 100 ml). No significant difference in the serum calcium level of the two categories of patients was noticed.

The mean magnesium level in cirrhosis was found to be 1.08 ± 0.14 mg per 100 ml (range 0.89 to 0.14) mg per 100 ml and it was 1.04 ± 0.12 mg per 100 ml (range 0.85 to 1.23 mg per 100 ml) in patients of obstructive jaundice.

No significant difference in the level of magnesium was noticed between cirrhotic patients and patients suffering from obstructive jaundice.

Table VIII presents the serum zinc, copper and iron levels in patients of different liver diseases and the individuals values are shown in Appendix II.

TABLE VIII

LEVELS OF ZINC, COPPER AND IRON IN PATIENTS OF DIFFERENT LIVER DISEASES

Serial No.	Group	No.	Zinc mg/100ml Mean ± S.D.	Zinc Range mg/100 ml.	Zinc t Value	Copper mg/100 ml Mean ± S.D.	Copper Range mg/100ml	Copper t Value	Iron mg/100 ml Mean ± S.D.	Iron Range mg/100 ml.	Iron t Value
1.	Cirrhosis	18	0.03 ± 0.02	0.01 to 0.06	0.00 N.S.	0.12 *± 0.02	0.05 to 0.16	1.12. N.S.	0.08 ± 0.01	0.06 to 0.09	0.00 N.S.
2.	Obstructive Jaundice	7	0.03 ± 0.01	0.02 to 0.10		0.11 ± 0.01	0.09 to 0.12		0.08 ± 0.02	0.06 to 0.12	

N.S.= Not significant

The level of zinc in Cirrhosis was found to be 0.03 ± 0.02 mg per 100 ml (range 0.01 to 0.06 mg per 100 ml) and in obstructive jaundice it was 0.03 ± 0.01 mg per 100 ml (range 0.02 to 0.04 mg per 100 ml). There was no significant difference between these two values.

The copperlevel was found to be 0.12 ± 0.02 mg per 100 ml in cirrhotic patients (range 0.05 to 0.16) and in obstructive jaundice patients it was 0.11 ± 0.01 (range 0.09 to 0.12 mg per 100 ml.). No significant difference was found between the copper level of cirrhotic patients and that of the obstructive jaundice patients.

The iron level in cirrhotic patients was 0.08 ± 0.01 mg per 100 ml (range 0.06 to 0.09 mg per 100 ml) and it was 0.08 ± 0.02 mg per 100 ml (range 0.06 to 0.12 mg per 100 ml) in patients of obstructive jaundice.

Comparison of the serum iron levels of cirrhotic patients with that of obstructive jaundice patients showed no significant difference between them.

Table IX represents serum magnesium and calcium in diabetics and liver patients. The individual values are shown in Appendices I and II respectively.

Serum Calcium and magnesium levels in diabetics, liver patients and controls are shown in Figure I and II respectively.

FIGURE I

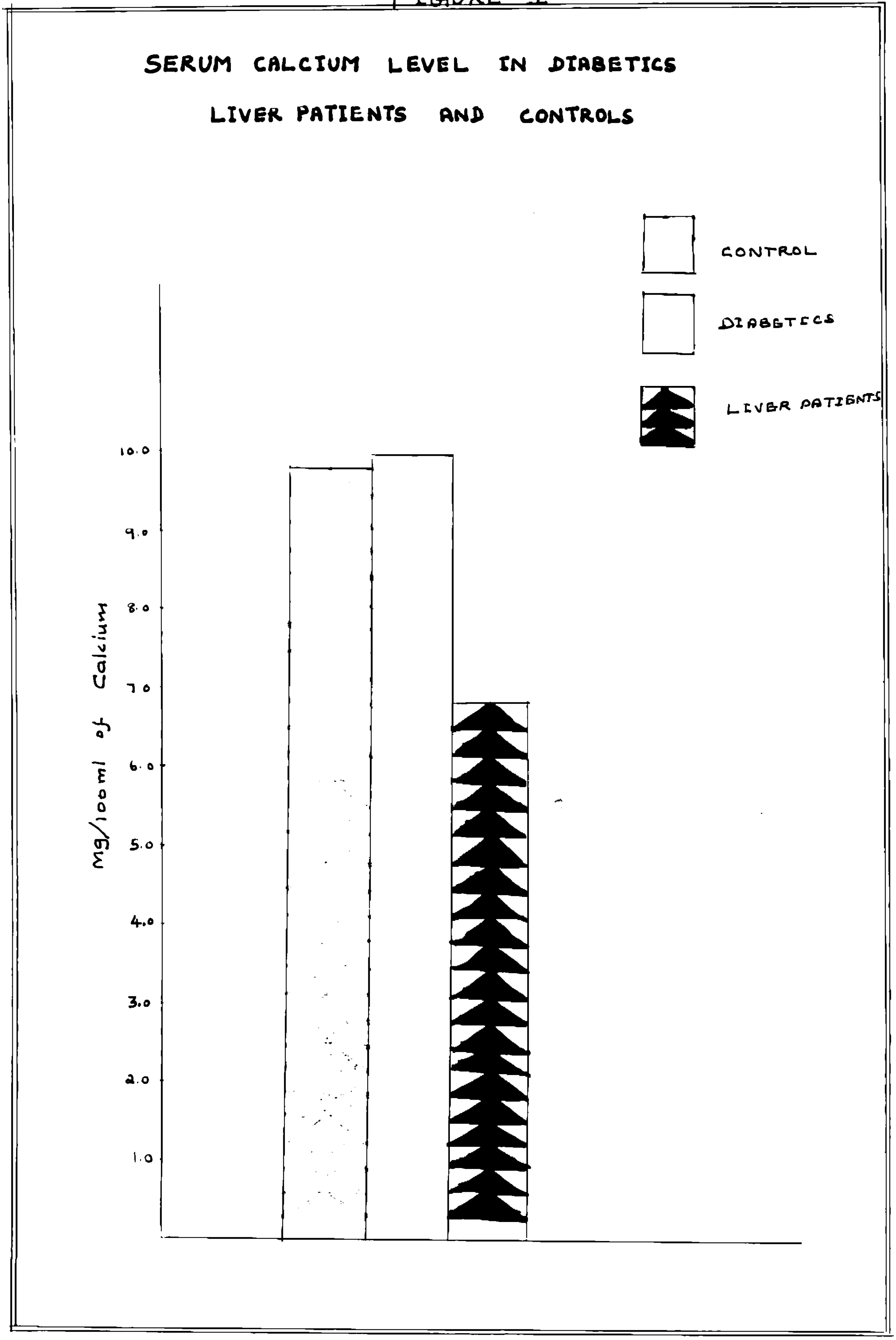

SERUM CALCIUM LEVEL IN DIABETICS
LIVER PATIENTS AND CONTROLS
CONTROL
DIABETICS
LIVER PATIENTS
Mg/100ml of Calcium
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

FIGURE II

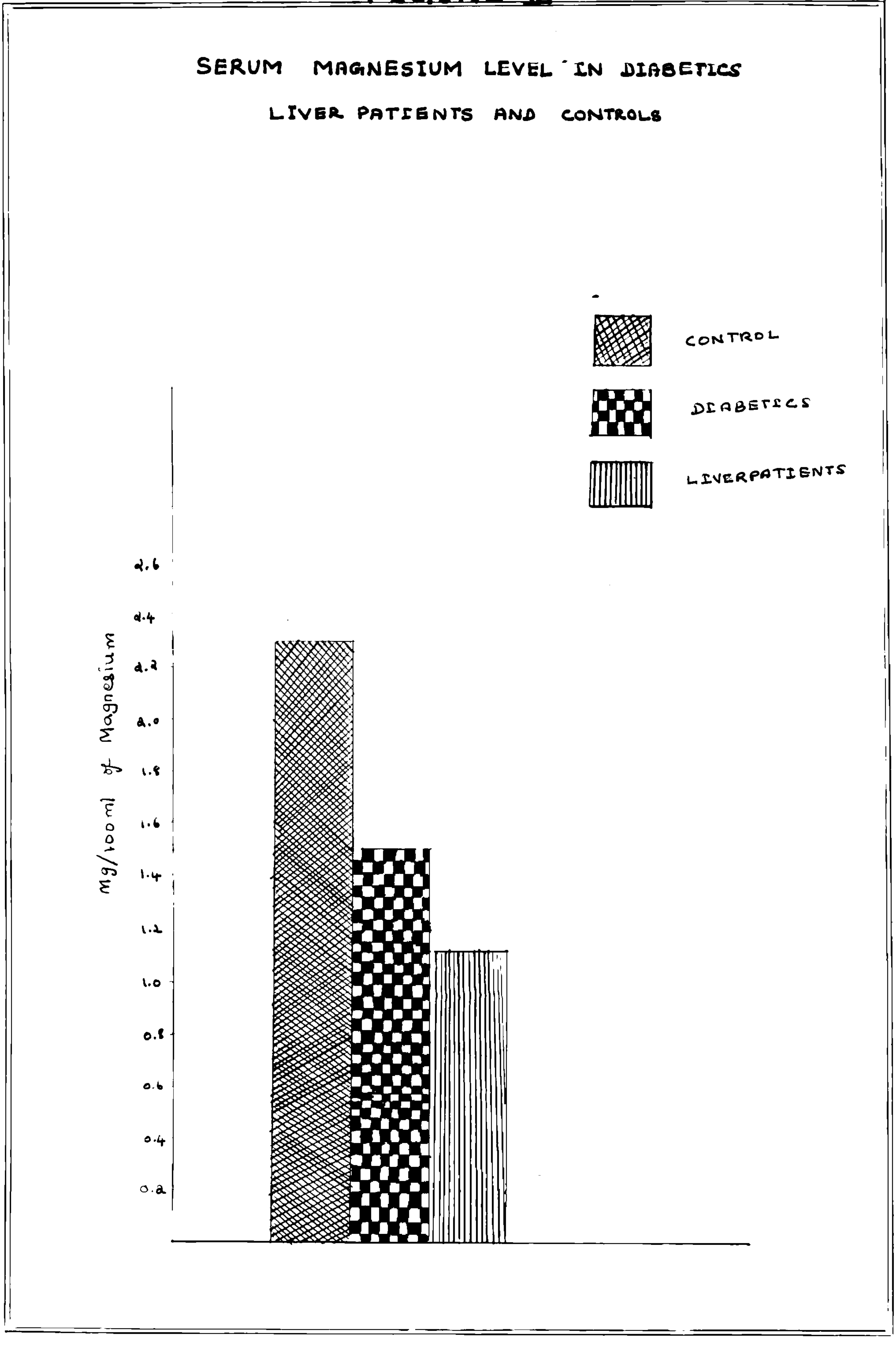

SERUM MAGNESIUM LEVEL IN DIABETICS
LIVER PATIENTS AND CONTROLS
CONTROL
DIABETICS
LIVERPATIENTS
Mg/100 ml of Magnesium
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2

TABLE XI

SERUM MAGNESIUM, CALCIUM IN DIABETICS AND LIVER PATIENTS

Serial No.	Group	No.	Calcium mg/100 ml Mean ± S.D.	Calcium Range mg/100ml.	Calcium t Value	Magnesium mg/100 ml Mean ± S.D.	Magnesium Range mg/100ml	Magnesium t Value
1.	Diabetics	30	9.97 ± 1.2	8.0 to 13.0	** 11.28	1.47 ± 0.23	1.11 to 1.99	** 7.7
2.	Liver patients	25	6.70 ± 0.93	5.0 to 8.0		1.10 ± 0.13	0.89 to 1.48	

**. ($P < 0.01$)

The mean calcium and magnesium levels in diabetes were found to be 9.97 ± 1.2. mg per 100 ml (range 8.0 to 13.0 mg per 100 ml) andi 1.47 ± 0.23 mg per 100 ml (Range 1.11 to 1.99 mg per 100 ml). In liver patients it was found as 6.7 ± 0.93 mg per 100 ml (range 5.0 to 8.0 mg per 100 ml) and 1.1 ± 0.12 mg per 100 ml (range 0.89 to 1.48 mg per 100 ml.)

Compared to the mean serum calcium and magnesium levels of liver patients, diabetics had elevated levels of calcium and magnesium levels and this increase was found to be stastically significant ($P < 0.01$).

Table X represents the levels of serum zinc, copper a and iron in diabetes and liver patients. The individual values of zinc, copper and iron are showed in Appendices I and II.

Serum, zinc, copper and iron levels in diabetics liver patients and controls are shown in figure III.

FIGURE III

SERUM ZINC, COPPER, AND IRON LEVELS IN DIABETICS LIVER PATIENTS AND CONTROLS.

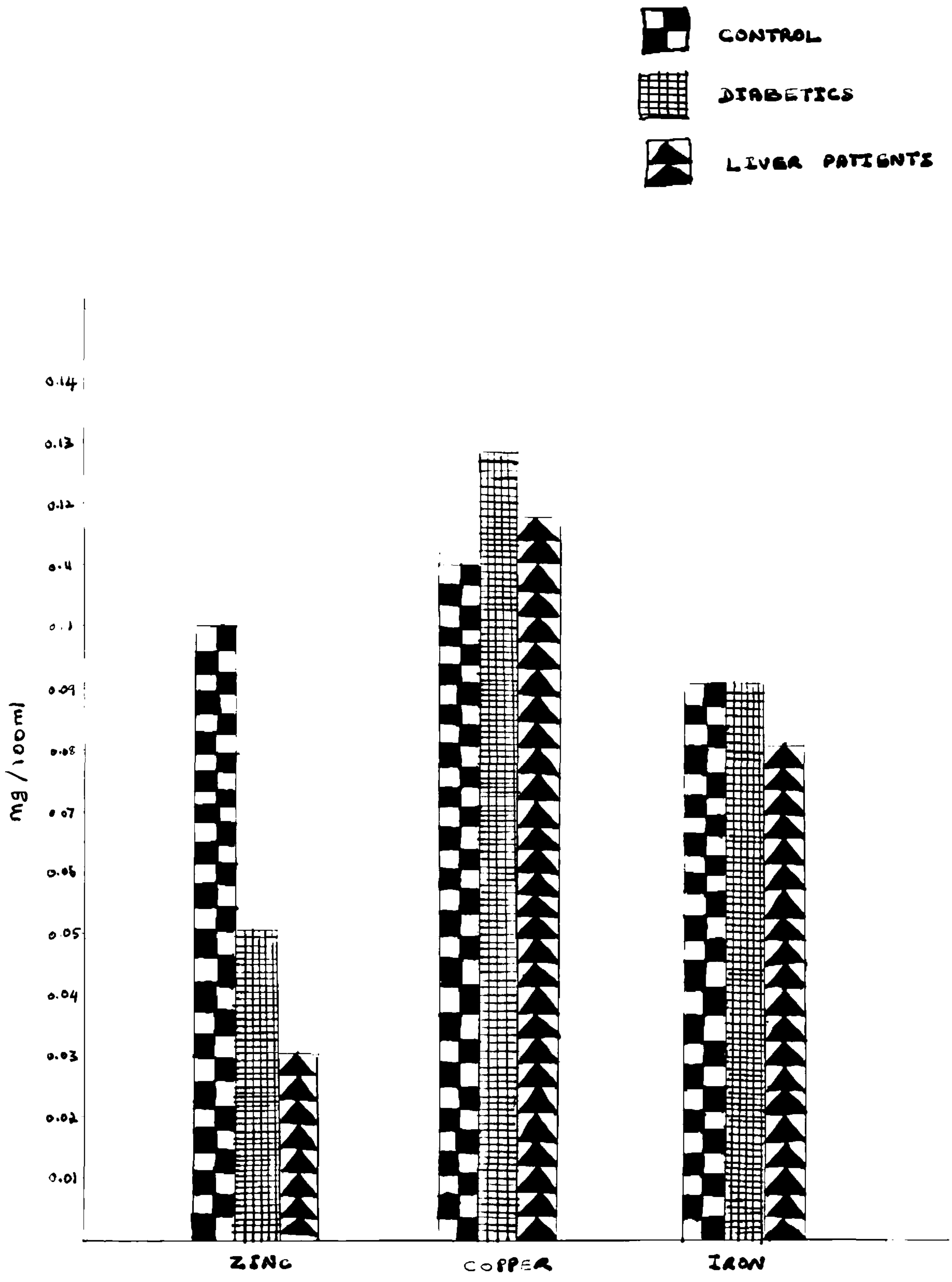


TABLE X

SERUM ZINC, COPPER AND IRON LEVELS IN DIABETICS AND LIVER PATIENTS

Serial No.	Group	No.	Zinc mg/100 ml Mean ± S.D.	Zinc Range mg/100 ml.	Zinc t Value	Copper mg/100 ml Mean ± S.D.	Copper Range mg/100ml	Copper t Value	Iron mg/100 ml Mean ± S.D.	Iron Range mg/100ml	Iron t Value
1.	Diabetics	30	0.05 ± 0.02	0.01 to 0.06	** 4.24	0.13 *± 0.03	0.06 to 0.18	1.47 N.S.	0.09 ± 0.02	0.05 to 0.13	** 2.41
2.	Liver patients	25	0.03 *± 0.02	0.01 to 0.06		0.12 ± 0.02	0.08 to 0.16		0.08 ± 0.01	0.06 to 0.12	

** (P < 0.01)
* (P < 0.05)

N.S. Not significant.

The level of zinc in diabetics ranged from 0.01 ± 0.08 mg per 100 ml with a mean of 0.05 ± 0.02 per 100 ml. The level of zinc in liver patients ranged from 0.01 to 0.06 mg per 100 ml with a mean of 0.03 ± 0.02 mg per 100 ml. A higher level of serum zinc was noticed in diabetics than in liver patients. This difference in zinc level between diabetics and liver patients was found to be significant($p < 0.01$).

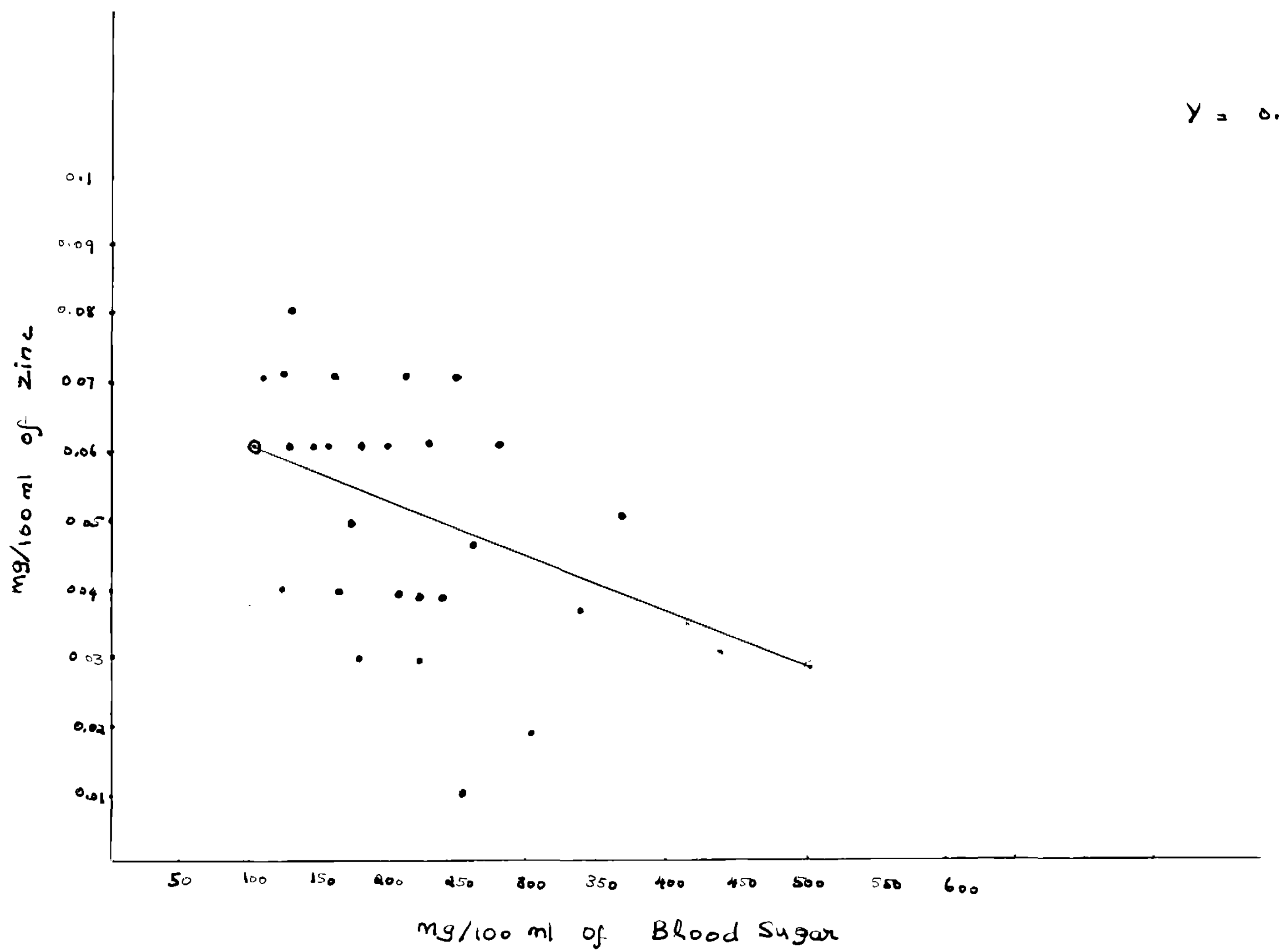
The level of copper in diabetics and liver patients ranged from 0.06 to 0.18 mg per 100 ml and 0.08 to 0.16 mg per 100mg 100 ml with a mean value of 0.13 ± 0.03 mg per 100 ml and 0.12 ± 0.02 mg per 100 ml. The difference in the copper level between diabetics and liver patients was found to be not significant.

The iron level in diabetics and liver patients ranged from 0.05 to 0.13 mg per 100 ml and 0.06 to 0.12 mg per 100 ml with a mean of 0.09 ± 0.02 mg per 100 ml and 0.08 ± 0.01 mg per 100 ml. The elevated serum iron level of diabetics was found to be significant ($P < 0.05$) when compared to the serum iron level of liver patients.

Correlation analysis was carried out between blood sugar level and the various elements studied. A positive correlation ($r = +0.2$, $r = +0.12$, $r = +0.04$, $r = +0.38$) was found between blood sugar and calcium; blood sugar and magnesium; blood sugar and copper; and blood sugar and iron respectively but they are not statistically significant. A positive correlation ($r = +0.36$) was found between blood sugar and zinc level which was statistically significant ($p < 0.05$). Correlation between blood sugar and serum zinc level in diabetic patients was shown in Figure IV.

FIGURE IV

CORRELATION BETWEEN BLOOD SUGAR AND SERUM ZINC LEVELS IN DIABETIC PATIENTS.



SUMMARY AND CONCLUSION

V. SUMMARY AND CONCLUSION

The present investigation was aimed to study the 'serum calcium, copper, zinc, magnesium and iron in diabetes and liver diseases'. The results were compared with normal healthy individuals of matching age and sex, who were free of diseases.

A total of thirty persons suffering from diabetes millitus and twenty five liver patients were selected at random from the outpatient ward of coimbatore Medical College Hospital. Serum samples of these patients were analysed for serum calcium, magnesium, zinc, copper and iron along with blood glucose levels in diabetics and bilirubin in liver patients and the data thus obtained was compared with those of the normal healthy individuals of matching age and sex, who were free of diseases and served as controls.

The summary and conclusion arrived out of this study are as follows:

1. A total of fifty five patients of both sexes, with age range 10-90 years were studied. Of the fifty five patients studied thirty were diabetics and the rest were liver patients. Among diabetics, four patients were in the age group 10-30 years, nine in the age group 30-50 years; thirteen in the age group 50-70 years; and four patients in the age group 70-90 years.

Among liver patients, four patients belonged to the age group 10-30 years; twelve to 30-50 years; eight to 50-70 years; and one to 70-90 years of age groups respectively.

Thirty normal healthy individuals of matching age and sex, free of diseases were chosen to serve as controls. Among the controls six persons belonged to the age group 10-30 years; eight to 30-50 years; eleven to 50-70 years and five to 70-90 years of age groups respectively.

2. The mean blood sugar level of patients was 222.9 ± 78.97 mg per 100 ml. This was found to be highly elevated when compared to the normal value 120 mg per 100 ml and thus the patients were confirmed to be diabetics.

   The mean serum bilirubin level of the patients was 7.02 ± 7.51 mg per 100 ml which was highly elevated than the normal level (1.0 mg per 100ml) and hence these patients were confirmed to be liver patients.

3. The mean serum calcium in the diabetics was found to be 9.97 ±1.2 mg per 100 ml while it was 9.87 ± 1.25 mg per 100 ml in controls. There was no significant difference between calcium level of the controls and the diabetics. This suggested that in diabetics mellitus the serum calcium is not affected.

4. A significant difference (P 0.01) was found in serum magnesium level between controls and diabetics. The mean serum magnesium level in control and diabetics was 2.25 $\pm$ 0.37 mg per 100 ml and 1.47 $\pm$ 0.23 mg per
1 100 ml respectively. The mean serum magnesium level was found to be decreased in diabetic patients as compared to controls.

5. The serum zinc level between control and diabetics showed a statistically significant decrease (p 0.01) in diabetics. The mean serum zinc level in control was 0.10 $\pm$ 0.15 mg per 100 ml while it was 0.05 $\pm$ 0.02 mg per 100 ml in diabetics.

6. Comparision of serum copper and iron values of controls and diabetics showed no significant difference between these two groups. The mean serum copper levels of control group was 0.11 $\pm$ 0.03 mg per 100 ml and it was 0.13 $\pm$ 0.03 mg per ml in diabetics. The mean serum iron level in control and diabetic patients was found to be 0.09 $\pm$ 0.02 mg per 100 ml and 0.09 $\pm$ 0.02 mg per 100 ml respectively.

7. A significant difference (p 0.05) was noted in serum bilirubin level between control and liver patients. The mean bilirubin level was 0.71 $\pm$ 0.19 mg per 100 ml in controls, while it was 7.02 $\pm$ 7.51 mg per 100 ml in patients.

8. The mean serum calcium and magnesium levels of liver patients was found to be significantly (p 0.01) decreased when compared to that of the controls. The mean calcium and magnesium levels expressed in mg per 100 ml, were found to be 9.87 ± 1.25 ± 0.37 in controls while 6.70 ± 0.93 and 1.10 ± 0.13 mg per 100 ml in liver patients.

9. When compared to controls, the serum zinc, level of liver patients was found to be significantly (p 0.01) reduced. The mean serum zinc level was found to be 0.1 ± 0.02 mg per 100 ml in controls, while it was 0.03 ± 0.01 mg per 100 ml in liver patients.

10. No significant difference was found in copper and iron levels between liver patients and controls. The mean serum copper and iron levels were found to be 0.11 ± 0.03 and 0.09 ± 0.02 mg per 100 ml in controls and 0.12 ± 0.02 and 0.08 ± 0.01 mg per 100 ml in liver patients.

11. The elevated level of serum bilirubin observed in patients suffering from obstructive jaundice was statistically significant (p 0.01) when compared to the serum bilirubin level of cirrhotic patients. The mean bilirubin level was 2.56 ± 0.99 mg per 100 ml in cirrhotic patients, while it was 18.49 ± 4.04 mg per 100 ml in patients suffering from obstructive jaundice.

12. No significant difference in the levels of calcium and magnesium was noted between cirrhotic patients and patients suffering from obstructive jaundice. The mean serum calcium and magnesium levels in cirrhotic patients were noted to be 6.83 ± 0.9 and 1.08 ± 0.14 mg per 100 ml respectively. In patients suffering from obstructive jaundice the levels of serum calcium and magnesium were found to be 6.29 ± 0.88 and 1.04 ± 0.12 mg per 100 ml.

13. Comparison of the serum zinc, copper and iron levels of cirrhotic patients with that of obstructive jaundice patients showed no significant difference between them. The mean serum zinc, copper and iron levels of cirrhotic patients were 0.03 ± 0.02, 0.12 ± 0.02, and 0.08 ± 0.01 mg per 100 ml respectively. The meal level of zinc, copper and iron expressed in mg per 100 ml were 0.03 ± 0.01, 0.11 ± 0.01 and 0.08 ± 0.02 mg per 100 ml in patients with obstructive jaundice.

14. Compared to the mean serum calcium and magnesium levels of liver patients, diabetics ahd elevated levels of calcium and magnesium values and this increase was found to be statistically significant (p 0.01) The mean calcium and magnesium levels in diabetics were found to be 9.97 ± 1.2 and 1.47 ± 0.23 mg per 100 ml while they were 6.7 ± 0.93 and 1.1 ± 0.13 mg per 100 ml in liver patients.

15. A higher level of serum zinc was noticed in diabetics

than in liver patients and this difference was found to be statistically significant (p 0.01). The mean level of zinc in diabetics was 0.05 ± 0.02 mg per 100 ml and it was 0.03 ± 0.02 mg per 100 ml in liver patients.

16. There was no significant difference noted in copper level between liver patients and diabetics. The mean level of copper in diabetics and liver patients were 0.13 ± 0.03 and 0.12 ± 0.02 mg per 100 ml respectively.

17. The elevated serum iron level in diabetics was found to be significant (p 0.05) when compared to the serum iron level of liver patients. The mean serum iron level of diabetics and liver patients were 0.09 ± 0.02 and 0.08 ± 0.01 mg per 100 ml respectively.

18. Correlation analysis was carried out between blood sugar level and the various elements studied. A positive correlation (r = +0.2, r = +0.12, r = +0.04, r = +0.38) was found between blood sugar and calcium, blood sugar and magnesium, blood sugar and copper and blood sugar and iron respectively but they are not statistically significant. A positive correlation (r = +0.36) was found between blood sugar and zinc level which was statistically significant (p 0.05).

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Aikawa, J.K., 1971 — The relationship of magnesium to diseases in domestic animals and human, publishers charles C. Thomas. P.280

2. Ananda Prasad., Donald oberleas., and Rajeskaran, G. 1970 — "Essential Micronutrient elements-Biochemistry and changes in liver disorders". The American Journal of clinical Nutrion, 23:5, 581.

3. Ananda Prasad, S., and Donald oberleas. 1976 — Trace elements in human health and disease - Essential and toxic elements, Academic press, Newyork; A subsidiary of Harcourt Brance, Jovanovich, publishers, II:1-2.

4. Ananda Prasad, 1983 — "Clinical, biochemical and nutritional spectrum of zinc deficiency in human subjects, An update". Nutrition Reviews, 41:7,197.

5. Bucakowski, M., Slominska, P.T., and Perlinski, B. 1968 — " Serum zinc level in patients of diabetes" Pol-med.J. 7: 1357.

6. Davies, I.J.T., Musa, M., and Dormondy, T.L. 1968 — "Measurement of plasma zinc part I in health and disease". Journal of clinical pathology, 21:359.

7. Eastham, R.D. 1973 — A laboratory guide to clinical Diagonosis, III edition, Bristol: John wright and sons Ltd., 134.

8. Frank, A. and Graig. 1962 — "Role of zinc and other trace elements in diabetes mellitus." Journal of Medicine, 62:75.

9. Fujii, S., Takomura, T., Wada, M., Akai, T., and Okvda, K. 1982 — "Magnesium levels in plasma, erythocyte and urine in patients with diabetes mellitus". Hormone and metabolic Research, 14:63, 161-162.

10 Goyal, V.K., Kaushik, S.K., Jain, S.K., and Bh.., N. 1982 — "Studies on serum copper and zinc in patients with uncontrolled diabetes mellitus". Journal of diabetic association of India, 22:4, 102.

11 Gupta, L.C. Ram, A.K. and (Mrs) Ram, R. 1981 — " Common Anti Diabetics". Indian Medical Gazette, 7:225

12. Joseph Roll, James, L., Boyer., Daniel Barry., Klatskin, G. 1983 "The prognostic importance of clinical and Histologic features in Asymptomatic and Primary biliary cirrhosis". The new England Journal of Medicine, 308:1, 1.

13. Katsen, M.K. and Richard Mahler, J. 1975 "Advances in modern nutrition". Diabetes, obesity and cascular disease, John Willey and Sons., Newyork, 2:577.

14. Keile, C.A., and Neil, E. 1974 Samson wrights applied physiology. 12th edition, oxford Medical Publications:438.

15. Khan, A.M., Helwig, H.L., Redeker, A.G., and Reynold, T.B., 1965 "Urine and serum zinc abnormalities in diseases of liver". American journal of clinical pathology, 44:426.

16. Khare, V.M., 1982 "Significance of plasma zinc in cirrohosis of Liver". Indian Medical Gazette, 2:38.

17. Khan, A.S., Tewar, S.G., Tyagi, S.P., Khan, R.V., Ahamad, J., and "Serum magnesium in viral hepatitis". Indina Journal of Medical Reasearch, 78:91.

Siddigui, M.
1983

18. Kosenko, L.G., 1965 — "Concentration of trace elements in blood of patients with diabetes mellitus". Fed.Proc.(Transl-suppl) 24:237.

19. Krall, L. 1978 — Joslin diabetes Manual, XII edition, Joslin Dia. Foundation, Inc:16.

20. Lisun Lobanova, V.P. 1963 — "Trace elements (Manganese, Copper and Zinc) in patients with diabetes mellitus". English translation read, Zdravokr Bethorussa, 9:49.

21. Lilly, E. 1980 — Diabetes Mellitus, Modern Concepts and treatment. published for the medical profession of lilly, research, laboratories. Indian polis, Indiana Ist division, 8th edition, 7.

22. Marks, V., and Hales, C.N., 1976 — "The clinical value of trace metal measurements". Essays in Medical Biochemistry, The bi chemistry Society and the association of clinical biochemistry, 2:37.

23. Mateo, M.C.H., Bustamante, J.B., Cantalapiedra, M. 1978 — "Serum zinc, Copper and insulin in diabetes mellitus" Biomedicine., (Express) 29:2:56.

24.

24. Martin, D.W., Mayes, P.A., and Rodwell, V.W., 1981 — Harper's review of biochemistry, 18th edition, Maruzen Asian edition; 555

25. Marts,W. and cornatser, W.E. 1971 — "Copper and zinc metabolism". Newer trace elements in Nutrition. Marcen Deckler, Inc; 256

26. Ning, M., Lowenstein, L.M. and Davidson, S. 1967 — "Serum amino acid concentrations in alcoholic hepatits". Journal of laboratory and clinical Medicine, 70:554.

27. Oser, L.B., 1971 — Hawks physiological chemistry, 18th edition, Macraw Hill Boot Co., 1193.

28. Piper K.G., and Higgins, G. 1967 — "Estimation of trace metals in biological material by Atomic Absorption spectrophotometer". Proc.Ass.Clin.Bioch. 4:190

29. Pidduck, H.G., Wren, P.J.J. and Price Evans, D.A. 1970 — "Plasma zinc and copper in diabetes mellitus". Diabetes, 19:234.

30. Pidduck, H.G., Wren, P.J.J. and price Evans, D.A. 1970 — "Hyperzincuria of diabetes mellitus and possible implications of this observation" Diabetes, 19: 240-247.

31. Prasad, A.S., Miale, Farid, J., Sand stead, H.H., and schulert, A.R. 1963 — "Zinc metabolism inpatients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism and hypogonadism". Journal of Laboratary and clinical Medicine, 61:535 - 547.

32. Prasad, A.S., and Oberleas, 1969 — "Zinc in human serum:evidences, for an amino acid bound fraction". Journal of laboratory and clinical Medicine, 73:1036.

33. Rosner, F., and Gorfien, P.C., 1970 — "Zinc and magnesium levels in diabetes." J.A.M.A., 211:2156

34. Saville, P.D. 1965 — "Changes in bone mass with age and alcoholism". Journal of bone joint surgery, 47: 492 - 499

35. Sarin, S.K., Mathur, D., and Mehta, S.R. 1982 — "Diagonstic significance of serum magnesium hepatic amoebiasis". Indian Medical Gazette, 4:100

36. Schutte, K.H., 1964 — The biology of the trace elements-their role in nutrition, Crosby Lockwood and Son Ltd, P-C

37. Schroeder, H.A., 1976 — Trace elements and nutrition, Faber and Faber, 3 quen square, London:41.

38. Shine, S.N., Gabrielli, E.R., 1970 — "Serum copper and zinc level in various pathologic states". American Journal of clinical pathology, 54:470

39. Sharda, B., Bhandari, B., and Bhandari, L.M. — "Study of copper, zinc, magnesium and calcium in Indian childhood cirrhosis patients, parents, and siblings". Transactions of the Royal society of Tropical Medicine and Hygine, 76:6:747-750.

40. Spencer, H. and Samachson, J. 1970 — "Studies of zinc metabolism in man". Trace element metabolism in animals, edition, C.F.Mills, Edinburg, Livingstone P.1312-320.

41. Srivastava, B.M., Mehta, G.S., Mathur, P.C., Rathone and Goswami, C.L., 1978 — "Plasma and urine zinc in cirrhosis liver". Summary., A.P.I.26;11.

42. Sullivan, J.F., and Lankfrd, H.G., 1962 — "Urinary excretion of zinc in alcoholism and post alcoholic cirrhosis". American Journal of Clinical Nutrition, 10:153.

43. Sullivan, J.F., Wolpert, F.W., Williams, R., and Egoan, J.D. 1969 — "Serum magnesium in chronic alcoholism". Ann. Ny. Acad. Sci. 162: 947-962.

44. Sullivan,R., Blotcky, A.J., Mary Jetton, M., Henry Hahr, K.J., and Robert Burch, E. 1979 — "Serum levels of selenium, Calcium, copper, magnesium, manganese and zinc in various human diseases". Journal of Nutrition, 109 8:1432.

45. Talwar 1980 — Text book of Biochemistry and Human biology, Prentice - Hall of Indian Private Ltd., P.474.

46. Valle, B.L., 1959 — "Biochemistry, Physiology and pathology of Zinc". Physiological Review, 39: 443-490.

47. Verley, H., Alan Gowenlock, H., and Maurice Bell. 1980 — Practical clinical Biochemistry, 5th edition, William Heinemann Medical Books Ltd., London, I :850.

48. Vyas, R.K., Gupta, A.P., Gupta, A., and Aeson, A.K., 1982 — " Serum copper, zinc, magnesium, and calcium levels in various human diseases". The Indina Journal of Medical Research, 76:301

49. Wilke, H., and Spielmann, H., 1968 — Unter suchungen uber das Magnesium gehalt der Leber bei der cirrhose. Klin wochenschr, 46: 1162-1164.

APPENDICES

APPENDIX I

LEVELS OF BLOOD SUGAR, SERUM CALCIUM, MAGNESIUM AND IRON IN DIABETICS

No.	Sex	Age	Blood sugar mg/100 ml	Calcium mg/100 ml	magnesium mg/100 ml	zinc mg/100 ml	Copper mg/100 ml	Iron mg/100 ml
1.	Male	18	140	8.0	1.999	0.072	0.069	0.080
2.	Female	22	170	9.0	1.777	0.079	0.081	0.073
3.	Male	24	150	9.0	1.616	0.080	0.079	0.079
4.	Male	25	170	9.0	1.111	0.060	0.060	0.060
5.	Female	33	186	10.0	1.500	0.063	0.099	0.078
6.	Male	36	200	9.0	1.387	0.060	0.072	0.092
7.	Male	42	190	9.0	1.832	0.052	0.143	0.070
8.	Female	43	210	9.0	1.277	0.076	0.158	0.060
9.	Female	45	230	10.0	1.197	0.048	0.139	0.053
10.	Male	46	285	11.0	1.500	0.028	0.110	0.058
11.	Male	47	300	9.0	1.332	0.087	0.100	0.050
12.	Male	45	163	11.0	1.222	0.043	0.123	0.072
13.	Male	49	280	10.0	1.555	0.060	0.138	0.089
14.	Male	50	178	12.0	1.616	0.073	0.096	0.092
15.	Female	52	262	9.0	1.277	0.058	0.155	0.091

LEVELS OF BLOOD SUGAR, SERUM, CALCIUM, MAGNESIUM,

ZINC, COPPER AND IN DIABETES

No.	Sex	Age	Blood Sugar mg/ 100 ml	Calcium mg/100 ml	Magnesium mg/100 ml	Zinc mg/100 ml	Copper mg/100 ml	Iron mg/100 ml
16.	Male	52	332	10.0	1.273	0.053	0.149	0.089
17.	Female	55	180	11.0	1.111	0.032	1.179	0.096
18.	Male	56	220	10.0	1.147	0.043	0.148	0.092
19.	Male	59	224	9.0	1.839	0.40	0.127	0.071
20.	Male	60	302	11.0	1.417	0.040	0.120	0.066
21.	Female	60	190	9.0	1.332	0.042	0.140	0.110
22.	Male	62	550	11.0	1.500	0.035	0.112	0.102
23.	Female	64	249	13.0	1.410	0.016	0.182	0.108
24.	Male	65	208	9.0	1.611	0.030	0.108	0.100
25.	Male	60	220	10.0	1.666	0.060	0.129	0.120
26.	Female	65	198	9.0	1.277	0.069	0.179	0.132
27.	Male	72	243	10.0	1.417	0.040	0.118	0.098
28.	Female	78	150	10.0	1.66	0.061	0.152	0.072
29.	Female	75	132	10.0	1.389	0.073	0.169	0.189
30.	Female	74	173	13.0	1.734	0.063	0.158	0.110

APPENDIX II

LEVELS OF SERUM BILIRUBIN, CALCIUM, MAGNESIUM, ZINC, AND IRON IN PATIENTS

(a) Cirrhosis

No.	Sex	Age	Bilirubin	Calcium mg/100 ml	Magnesium mg/100 ml	Zinc mg/100 ml	Copper mg/100 ml	Iron mg/100 ml
1.	Male	26	3.0	5.0	1.120	0.060	0.096	0.070
2.	Female	28	1.6	5.0	0.920	0.042	0.090	0.070
3.	Male	28	2.8	7.01	1.140	0.038	0.080	0.070
4.	Male	30	3.84	7.0	1.030	0.010	0.091	0.081
5.	Male	32	3.0	6.0	1.0	0.012	0.108	0.089
6.	Male	35	1.8	8.0	1.38	0.010	0.100	0.090
7.	Male	40	2.0	7.0	0.98	0.042	0.110	0.080
8.	Male	42	2.87	7.0	1.11	0.060	0.128	0.060
9.	Male	43	1.6	8.0	1.117	0.020	0.129	0.072
10.	Male	44	1.2	8.0	0.91	0.28	0.140	0.070
11.	Male	47	4.4	6.0	0.93	0.058	0.132	0.060
12.	Female	48	2.7	7.0	1.2	0.047	0.139	0.0 91
13.	Male	50	2.6	7.0	1.12	0.021	0.140	0.091
14.	Female	50	1.6	6.0	1.22	0.032	0.160	0.089

No.	Sex	Age	Bilirubin	Calcium mg/100 ml	Magnesium mg/100 ml	Zinc mg/100 ml	Copper mg/100 ml	Iron mg/100 ml
15.	Male	54	3.75	7.0	1.09	0.01	0.148	0.092
16.	Male	56	4.4	5.0	0.99	0.041	0.135	0.088
17.	Male	62	1.54	7.0	1.32	0.038	0.12	0.091
18.	Male	63	1.59	7.0	1.00	0.03	0.125	0.096
			(b) Obstructive Jaundice					
19.	Female	20	15.6	7.0	1.23	0.026	0.098	0.092
20.	Female	30	16.0	7.0	1.08	0.025	0.109	0.07
21.	Male	33	26.0	7.0	1.12	0.028	0.103	0.078
22.	Male	42	23.0	7.0	1.10	0.02	0.11	0.065
23.	Male	55	14.4	0.5	1.00	0.03	0.122	0.081
24.	Male	60	18.4	6.0	0.92	0.037	0.119	0.062
25.	Male	82	16.0	5.0	0.85	0.04	0.128	0.120

APPENDIX III

LEVELS OF BLOOD SUGAR, SERUM BILIRUBIN, CALCIUM, MAGNESIUM,

ZINC, COPPER AND IRON IN NORMAL INDIVIDUALS

No.	Sex	Age	Blood Sugar mg/100 ml	Bilirubin mg/100 ml	Calcium	Magnesium	Zinc	Copper	Iron
1.	Female	16	80	0.4	9.0	2.326	0.097	0.080	0.072
2.	Female	19	107	0.5	9.0	2.000	0.082	0.152	0.078
3.	Female	20	100	0.9	9.0	2.111	0.090	0.190	0.070
4.	Male	26	125	0.9	9.0	2.055	0.092	0.092	0.069
5.	Female	28	90	0.7	9.0	2.000	0.098	0.098	0.083
6.	Female	23	85	0.3	9.0	2.125	0.112	0.093	0.092
7.	Male	30	127	0.7	10.0	1.932	0.099	0.091	0.072
8.	Male	36	110	0.6	10.0	1.860	0.100	0.102	0.080
9.	Male	32	96	0.6	10.0	1.388	0.102	0.104	0.080
10.	Male	33	120	0.8	9.0	2.055	0.092	0.101	0.082
11.	Male	42	108	0.5	9.0	2.777	0.090	0.093	0.062
12.	Male	45	93	0.5	11.0	2.432	0.111	0.142	0.060
13.	Female	43	100	0.6	11.0	2.000	0.072	0.148	0.053
14.	Male	49	94	0.8	12.0	2.124	0.083	0.121	0.069
15.	Male	50	82	0.9	9.0	1.982	0.128	0.140	0.092

No.	Sex	Age	Blood Sugar mg/100 ml	Bilirubin mg/100 ml	Calcium mg/100 ml	Magnesium mg/100 ml	Zinc mg/100 ml	Copper mg/100 ml	Iron mg/100 ml
16.	Female	52	86	1.0	8.0	1.999	0.112	0.130	0.093
17.	Male	52	106	0.4	10.0	2.600	0.090	0.123	0.089
18.	Female	57	120	0.6	9.0	3.030	0.142	0.115	0.078
19.	Male	59	112	0.7	11.0	2.980	0.100	0.100	0.089
20.	Male	60	95	0.8	11.0	1.766	0.138	0.115	0.091
21.	Male	62	116	1.0	11.0	2.666	0.086	0.110	0.090
22.	Female	62	103	0.9	13.0	2.124	0.063	0.091	0.096
23.	Male	64	115	0.9	7.0	2.632	0.145	0.100	0.090
24.	Male	65	80	0.8	9.0	2.100	0.090	0.082	0.090
25.	Male	6.	75	0.8	9.0	1.888	0.112	0.120	0.128
26.	Female	70	110	0.7	11.0	1.932	0.110	0.110	0.160
27.	Female	78	130	0.9	10.0	2.866	0.150	0.132	0.128
28.	Male	74	95	0.9	11.0	2.325	0.082	0.148	0.099
29.	Female	74	100	0.8	11.0	2.055	0.073	0.060	0.087
30.	Male	80	86	0.4	10.0	2.900	0.100	0.096	0.100