THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI

M.D. BRANCH XIII

BIOCHEMISTRY

Dissertation on

A STUDY OF SERUM ZINC, COPPER AND CALCIUM IN PREGNANCY AND PREECLAMPSIA

INSTITUTE OF BIOCHEMISTRY
MADRAS MEDICAL COLLEGE
CHENNAI - 600 003

SEPTEMBER 2006

SPECIAL ACKNOWLEDGEMENT

I gratefully acknowledge and sincerely thank **Dr.KALAVATHY PONNIRAIVAN, B.Sc., M.D., DEAN**, Madras Medial College, Government General Hospital, Chennai - 600 003, for granting me permission to utilize the facilities of this institution for the study.

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled "***A STUDY OF SERUM ZINC, COPPER AND CALCIUM IN PREGNANCY AND PREECLAMPSIA*** submitted by **Dr.D.PONNUDHALI,** is a work done by her during the period of study in this department from August 2003 - September 2006.

.....

Dr.A.MANAMALLI,M.D.,
Director and Professor
Institute of Biochemistry
Madras Medical College
Chennai - 600 003.

.....

Dr.KALAVATHY PONNIRAIVAN,
B.Sc., M.D.
DEAN
Madras Medical College
Chennai - 600 003.

ACKNOWLEDGEMENTS

It is my privilege to express my heartfelt gratitude to **Dr.T.S.ANDAL, DCH, M.D.**, former Director and Professor, Institute of Biochemistry, Madras Medical College, Chennai, for her expert advice, thoughtful suggestions and constant encouragement throughout the course of study, which had played a pivotal role in its success.

I am extremely grateful to **Dr. A. MANAMALLI, M.D.**, Professor of Biochemistry, Institute of Biochemistry, Madras Medical College, Chennai, for her scholarly and perceptive criticisms which had been indispensable for this study. I would also like to make a special mention of her skillfulness in integrating the varied sentences, to order.

I am especially thankful to **Dr. PRAGNA B DOLIA, M.D.**, Additional Professor, Institute of Biochemistry, Madras Medical College, Chennai for her good spirits and intelligent suggestions which had been quite enriching for the study's progress.

I would like to thank **Dr. CHANDRASEKAR**, Reader in Biochemistry for his guidance.

It is my pleasure to thank the Assistant Professors **Dr. SHYAMRAJ, M.D.,** and **Dr.I. PERIYANDAVAR, M.D**. for their relevant suggestions, constant guidance and cheerful motivation during the course of study.

I am thankful to **Dr.V. BALAKRISHNAN, MVSC. Ph.D**, Professor and Head of Department, Department of Animal Nutrition, Madras Veterinary college, Chennai for granting me permission to analyse the blood samples in the Atomic absorption spectrophotometer present in their department.

I owe special thanks to **Dr. D. RAJENDRAN, MVSC Ph.D**, Department of Animal Nutrition, Madras Veterinary College for his dedication and involvement in helping me with the analysis of blood samples in the Atomic absorption spectrophotometer and also with the statistical analysis of the various parameters involved in the study.

I am indebted and thankful to my husband, **Dr. P. NAGARAJAN, M.D(Ped),D.M(Nephro)** but for whose immense support and critical suggestions, this study wouldn't have been possible.

I am also thankful to my colleagues and other staff of the Institute of Biochemistry for their help.

I would also like to thank my family members for their support.

I thank all the individuals who have donated their blood samples willingly, for the study.

CONTENTS

INTRODUCTION

Deficient or excessive levels of minerals can be an adverse factor in human and animal pregnancies, which for a long time have been correlated with many maternal complications. Minerals like zinc, copper and calcium have been documented to play an important role in pregnancy and fetal outcomes.

Trace elements like zinc and copper are essential for the normal growth and development; they have been implicated in various reproductive events like preeclampsia, infertility, pregnancy wastage, congenital anomalies, placental abruption, premature rupture of membranes, neurological abnormalities, fetal deaths and low birth weights. Calcium is the macronutrient that has been best studied in relationship to preeclampsia.

Preeclampsia is a critically important disease of pregnancy, one of the major causes of fetal and maternal morbidity and mortality throughout the world. It is diagnosed by new onset of increased blood pressure, proteinuria occurring after 20 weeks of gestation and remission of these signs after delivery.

Preeclampsia is associated with substantial risks. For the fetus these include intra-uterine growth retardation, death and prematurity whereas the mother is at risk for seizures (eclampsia), renal failure, pulmonary edema, stroke and death. Despite considerable research, the cause or causes of preeclampsia remain unclear and there are no clinically useful screening tests to identify women in whom it will develop. The cause of preeclampsia is multifactorial and involves both genetic and other factors.

The role of various minerals like zinc, copper and calcium in relation to preeclampsia

have been studied in different populations, all over the world. There are many studies showing a lowering of serum zinc along with the rise in serum copper and ceruloplasmin levels. Low serum calcium levels have often been associated with preeclampsia. Several calcium supplementation trials have shown that the incidence of preeclampsia can also be reduced by increasing the calcium intake.

Hence the interest has developed to assess the above minerals in preeclampsia, in our population. Therefore the present study has been undertaken with the view of establishing the role of zinc, copper and calcium in preeclampsia by determining their levels in serum in nonpregnant, normal pregnant and preeclamptic women.

REVIEW OF LITERATURE

Preeclampsia is a pregnancy specific disorder characterized by high blood pressure, headache, protein in urine and edema of the face, hands and feet. It can progress to eclampsia, which is marked by convulsions and can lead to coma, brain damage and death. More recently, increased attention to the multisystemic nature of the syndrome with involvement of almost all organs, activation of coagulation cascade and increased sensitivity to pressor agents has expanded the understanding of the disorder.[1] Preeclampsia has been dubbed the "disease of theories" because of the multiple hypotheses proposed to explain its occurrence.[2]

CLASSIFICATION OF HYPERTENSIVE DISORDERS COMPLICATING PREGNANCY

There as 5 types of hypertensive diseases in pregnancy. The diagnostic criteria of each group based on *National high blood pressure education programme working group report*[3] is elaborated below

1. **Gestational Hypertension** (formerly pregnancy induced hypertension (or) Transient hypertension)

- BP ≥ 140/90 mmHg for first time during pregnancy
- No proteinuria
- BP return to normal < 12 weeks' postpartum
- Final diagnosis made only during postpartum period
- May have other signs of preeclampsia, for example, epigastric

discomfort or thrombocytopenia.

2. **Preeclampsia** (gestational hypertension plus proteinuria)

Minimum Criteria

- BP ≥ 140/90 mmHg after 20 weeks' gestation
- Proteinuria ≥ 300mg / 24 hours or ≥ 1 + dipstick

Increased certainty of preeclampsia

- BP ≥ 160/110 mmHg.
- Proteinuria 2.0 g/24 hours or ≥ 2 + dipstick.
- Serum creatinine > 1.2 mg/dl unless known to be previously elevated.
- Platelets < 100,000/$mm^{3.}$
- Microangiopathic hemolysis (increased LDH).
- Elevated ALT or AST.
- Persistent headache or other cerebral or visual disturbance.
- Persistent epigastric pain.

3. **Eclampsia**

Seizures that cannot be attributed to other causes in a women with preeclampsia.

4. **Superimposed Preeclampsia (on chronic hypertension)**

1. New-onset proteinuria ≥ 300mg/24 hours in hypertensive women but no proteinuria before 20 weeks' gestation.

2. A sudden increase in proteinuria or blood pressure, or platelet count < 100,000/mm^3, in women with hypertension and proteinuria before 20 weeks' gestation.

5. Chronic hypertension

BP ≥ 140/90 mmHg before pregnancy or diagnosed before 20 weeks' gestation.

(or)

Hypertension first diagnosed after 20 weeks' gestation and persistent after 12 weeks' postpartum.

CURRENT CONCEPTS OF PATHOGENESIS OF PREECLAMPSIA

Preeclampsia is characterized by a generalized dysfunction of the maternal endothelium and impairment of endothelium - dependent relaxation in maternal resistance arteries. The placenta appears to be the pregnancy component that leads to the disorder. Fetus is not the contributor. Important placental feature in preeclampsia is poor perfusion.[4]

Role of placenta in preeclampsia

Evidence points to the placenta as the key source of factors that lead to the maternal endothelial cell dysfunction in preeclampsia.[5] The genesis of preeclampsia is clearly related to deficient trophoblast invasion and failure of uterine artery remodeling.[6] This defective spiral artery remodeling results in reduced uteroplacental perfusion and foci of placental hypoxia or ischemia. Placental infarcts occur with increased frequency in preeclampsia, which results in the excess production of placental tumor necrosis factor-α (TNF-α).[7] Increased production of TNF-α from the placenta and/or maternal adipose tissue could contribute to insulin resistance, dyslipidemia and oxidative stress in preeclampsia. Lipid peroxidation products namely malondialdehyde and 4 hydroxy nonenal are increased due to placental oxidative stress leading to increase in syncytiotrophoblast membrane shedding into the maternal circulation. Placental lipid peroxidation products, TNFα and syncytiotrophoblast membrane fragments are candidate blood borne agents with the potential to cause endothelial cell dysfunction.

Current thinking characterizes preeclampsia into two stages.[8]

Stage -1	:	Reduction in perfusion
Stage - 2	:	Maternal syndrome

Reduced perfusion of almost all the organs is due to vasoconstriction, micro thrombi formation and reduced circulatory plasma volume. Vasoconstriction is secondary to an increased sensitivity of the vasculature to any pressor agent (ex

Angiotensin II). Normal pregnant women develop refractoriness to infused vasopressors. Activation of coagulation cascade produces microthrombi.The reduced plasma volume, reflecting an endothelial leak with fluid loss from the intravascular compartment, further compromises perfusion.

Oxidative stress is proposed as the linkage of the two stages of preeclampsia.[9] It is posited that reduced placental perfusion generates free radicals which in the appropriate maternal environment generate systemic oxidative stress. This hypothesis is supported by the evidence of oxidative stress in the circulation and tissues of women with preeclampsia.[10]

Endothelial cell dysfunction in preeclampsia

Preeclampsia is characterized by a generalized dysfunction of the maternal endothelium, as demonstrated by increased levels of factors VIII, total and cellular fibronectin, thrombomodulin, endothelin, growth factor activity, and a disturbance of the prostacyclin / thromboxane A_2 balance.[11] There are also increased levels of VCAM-1 (Vascular Cell Adhesion Molecule).[12]Evidences indicate that adverse changes in structure and function of the maternal vascular endothelium account for the altered vascular reactivity, activation of the coagulation cascade and the multisystem damage that occurs in preeclampsia. Maternal endothelial cell dysfunction is the key event resulting in diverse clinical manifestations of preeclampsia.

MATERNAL CONSEQUENCES

In preeclampsia, cardiovascular changes are basically related to increased cardiac afterload caused by hypertension. Maternal thrombocytopenia can be induced by preeclampsia - eclampsia. The cause of thrombocytopenia results from platelet activation and consumption. Plasma levels of renin, angiotensin II and aldosterone are increased during normal pregnancy. In preeclampsia there is a decrease of these values towards the normal non-pregnant range. There is extra cellular fluid volume expansion manifesting as edema. There is no appreciable change in the electrolyte values.

There should be some degree of proteinuria to establish the diagnosis of preeclampsia. This is due to increased permeability to large molecular weight proteins like hemoglobin, globulins and transferrin, along with albumin.

With severe preeclampsia, at times there are alterations in tests of hepatic function like delayed excretion of bromosulfophthalein and elevation of serum aspartate amino-transferase levels. Hyperbilirubinemia is uncommon. Liver involvement is frequently accompanied by other organ involvements resulting in HELLP syndrome (Hemolysis, Elevated liver enzymes and Low platelets).

Retinal artery vasospasm may be associated with visual disturbances, but blindness is uncommon. Symptoms of cerebral edema are lethargy, confusion, blurred vision and coma.

CAUSES OF PREECLAMPSIA

Among the many proposed causes are increased oxidative stress, dyslipidemia, inflammatory factors, increased insulin resistance, prostaglandin imbalance (Increased ratio of thromboxane levels to prostacyclin levels), immunologic derangements (a maternal immune reaction to paternal antigen in the placenta), genetic factors and nitric oxide. Thus preeclampsia is likely to be multifactorial in origin and characteristics of the mother and the placenta may interact to lead to its development.

Oxidative stress

In normal pregnancy free radical activity is increased either by increased cell turnover, increased cell damage or a decline in antioxidant free radical scavenging mechanisms.[13] There is increased attention for the hypothesis that placental and maternal free radical reactions promote a cycle of events that compromise the defensive functioning of the vascular endothelium, in preeclampsia.

Thus oxidative stress may be the point at which multiple factors converge resulting in endothelial cell dysfunction and the consequent clinical manifestations of preeclampsia. As in atherosclerosis, in preeclampsia also the endothelial cells are important targets and the dyslipidemia predisposing to atherosclerosis, occurs in preeclamptic pregnancies. Oxidative stress interacting with the dyslipidemia (formation of lipid peroxidation products) has been hypothesized to be important in the altered endothelial function that results in the clinical manifestations.

Women with preeclampsia have increased triacylglycerols, increased LDL, increased LDL cholesterol and reduced HDL cholesterol.[14]

Inflammatory factors

Although the pathophysiology of preeclampsia is not understood completely, there is an interest in a possible link between inflammation and preeclampsia. Preeclampsia can result when there is a generalized perturbation of the normal, generalized maternal intravascular inflammatory adaptation to pregnancy.[15]

The maternal syndrome of preeclampsia has previously been ascribed to generalised maternal endothelial cell dysfunction. Redman et al[15] have suggested that the endothelial dysfunction is part of a more generalized intravascular inflammatory reaction involving intravascular leukocytes as well as the clotting and complement systems. They also suggest that such an inflammatory response is already well developed in normal pregnancy and hence they argue that preeclampsia arises when a universal maternal intravascular inflammatory response to pregnancy is too strong or if the stimulus is too strong to induce such a response. Normal pregnancy is associated with activation of peripheral blood leukocytes, a response more marked in women with preeclampsia.[16] Before going into the details of this inflammatory state in preeclampsia, the acute phase response can be discussed as follows.

Acute phase response

The "acute phase response" is a prominent systemic reaction of the

organism to local or systemic disturbances in its homeostasis caused by infection, tissue injury, trauma or surgery, neoplastic growth or immunologic disorders.[17]

Local inflammation is the major reaction of the body upon tissue injury. At the site of tissue damage, a number of responses of the tissue itself are initiated. Proinflammatory cytokines are released, and the vascular system and inflammatory cells are activated.[18] These responses lead to the production of more cytokines and other inflammatory mediators which circulate in the blood resulting in a systemic acute phase reaction with the following laboratory findings.

1. Increased values of adrenocorticotrophic hormone (ACTH) and glucocorticoids.

2. Activation of the complement system and blood coagulation system.

3. Decreased levels of calcium, zinc, iron, vitamin A and α - tocopherol.

4. Change in concentration of several plasma proteins, the acute phase proteins (APP), largely due to a changed hepatic metabolism[19].

Cytokines and acute phase response

At least 15 different low molecular weight peptide mediators are known to be secreted by activated leukocytes (Interleukins) and other cells. They are collectively termed cytokines and are involved in triggering the acute phase response. For ex TNFα, IL - 1 & IL 6.

The next important aspect of the acute phase response is the altered biosynthetic profile of the liver. Liver synthesizes a characteristic range of plasma proteins called the acute phase proteins (or) acute phase reactants, following an inflammatory stimulus. In this hepatic acute phase response, TNFα , IL-1 and IL-6 play a key role.[20] The above cytokines activate hepatocytic receptors and the synthesis of various acute phase proteins starts. IL-6 is the major mediator for the hepatic secretion of most of the acute phase proteins.[21] IL-6 stimulates the liver to produce acute phase proteins, stimulates the proliferation of B lymphocytes and increases neutrophil production. TNF- α is the principle cytokine that mediates acute inflammation.

Other effects of the acute phase response include induction of the metallothionein synthesis[22] leading to decreased serum zinc levels. Calcium levels are also reduced.[23] Hence these are also included under acute phase reactants.

Acute phase reactants[17, 23]

Positive acute phase reactants

1. Major acute phase reactants - serum amyloid A and P components, C-reactive protein.

2. Ceruloplasmin, complement factor -3

3. Haptoglobin, fibrinogen, α globulins with antiprotease - activity and lipopolysaccharide binding protein.

Negative acute phase reactants

1. Transthyretin, Retinol binding protein.

2. Albumin and Transferrin

3. Serum zinc, iron and calcium

Acute phase response in preeclampsia

Redman et al[15] have suggested that preeclampsia is attributable to an excessive maternal inflammatory response to pregnancy secondary to a combination of placental and maternal factors related to phenotype and genotype. Inflammatory cells are activated in preeclampsia and localized to the site of vascular injury. This white cell activation is associated with higher levels of proinflammatory markers like cytokines.[24] This inflammatory response contributes to the wider syndrome of endothelial dysfunction and thrombotic and metabolic

disturbances seen in preeclampsia.[25] Recent studies have demonstrated that cytokines - mediators of inflammatory response may cause endothelial dysfunction through different mechanisms such as oxidative stress and endothelial cell damage.[26] Dilys J Freeman et al[25] and many others[24,27,28,29] have observed TNF-α and IL-6 levels to be higher in preeclamptic women.

Endothelial cells have been demonstrated to have increased IL-6 production in severe preeclampsia.[30] The elevation of IL-6 levels may indicate a greater degree of endothelial dysfunction.

As we have already seen, increase in IL-6 levels results in increased hepatic synthesis of the acute phase proteins, as part of the acute phase response. According to Vince GS et al[29] increases in plasma ceruloplasmin, complement activity, α_1 antitrypsin and haptoglobin and reduction in albumin and transferrin in preeclampsia are characteristic of the acute phase reaction that may be related to increase in IL-6 levels.

Hence the inflammatory response in preeclampsia may be denoted as excessive acute phase response.

Role of other factors

Hyperinsulinemia and increased insulin resistance are seen in preeclamptic women. Carl A hubel[31] has stated that TNF-α contributes to the development of insulin resistance. Thromboxanes are increased and prostacyclin and prostaglandin E_2 are decreased resulting in vasoconstriction and increased sensitivity to pressor

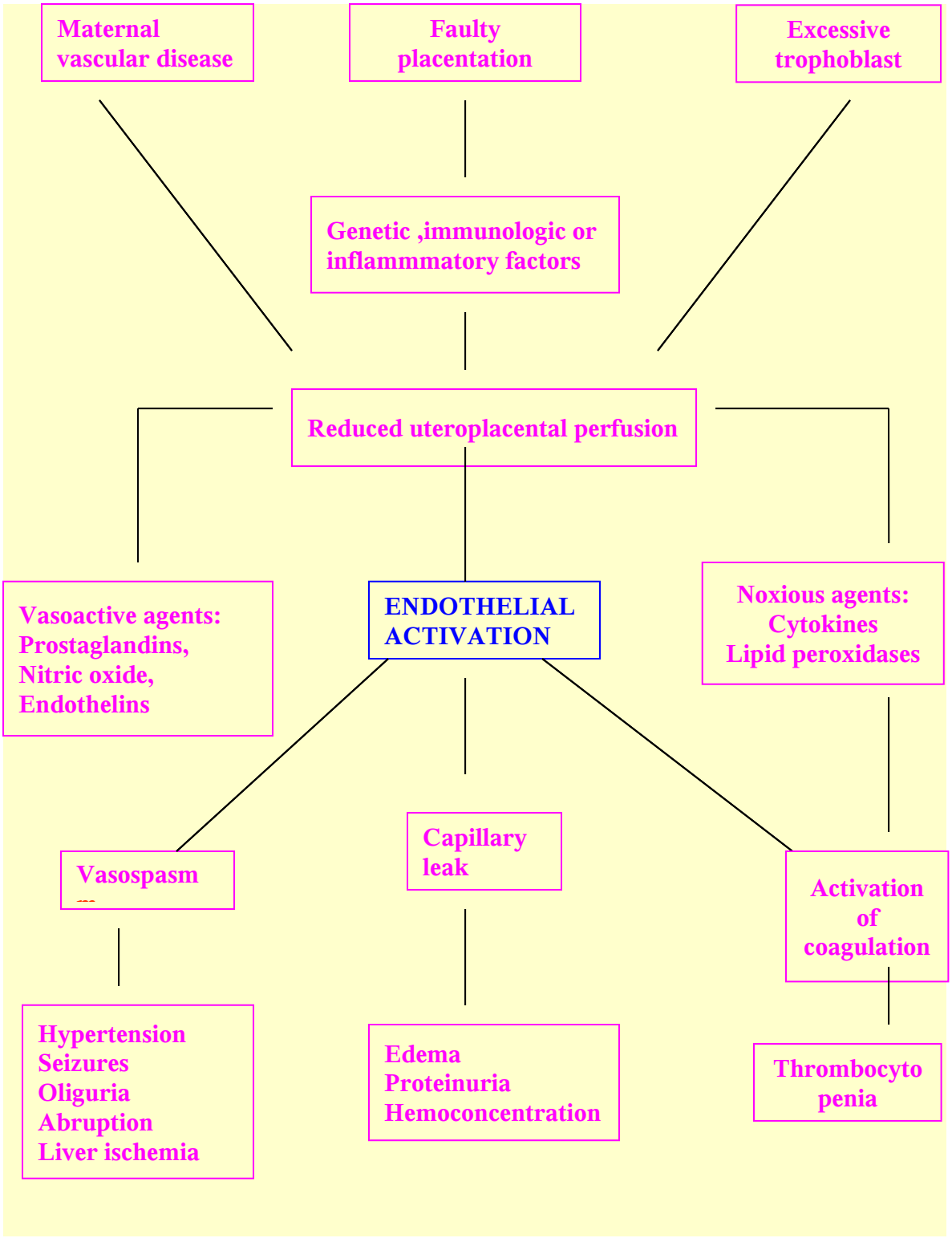
agents.

The tendency for preeclampsia is inherited. The susceptibility to preeclampsia is dependent upon a single recessive gene. There is an association between the histocompatibility antigen HLA-DR4 and proteinuric hypertension. Nitric oxide (NO) is a potent vasodilator. It has been proposed that deficient nitric oxide production or availability may contribute to the patho physiology of preeclampsia.

The risk of hypertensive disorders due to pregnancy is enhanced in circumstances where formation of blocking antibodies to antigenic sites on the placenta might be impaired. In preeclamptic women, there are lower levels of T-helper cells compared with normotensive women.

The pathophysiology of preeclampsia has been illustrated below as a chart.

PATHOPHYSIOLOGY OF PREECLAMPSIA



MINERAL INVOLVEMENT IN PREECLAMPSIA

The epidemiological and biological evidence suggest that minerals play an important role in maternal and fetal outcomes. Various minerals like zinc, copper, calcium and magnesium have been implicated in preeclampsia. There are many studies showing a decrease in the levels of zinc and calcium; increase in the levels of copper and either an increase or decrease in the levels of magnesium, in the serum of preeclamptic women.

As the study has been restricted to the former three minerals a review of them follows.

ZINC

Zinc is an important element performing a range of functions in the body, as it is a cofactor for the synthesis of a number of enzymes, DNA and RNA. Zinc is an integral component of nearly 300 enzymes in different species. Important zinc containing enzymes are carbonic anhydrase, alkaline phosphatase, RNA polymerase, DNA polymerase, thymidine kinase, carboxypeptidases and alcohol dehydrogenase.

Approximately 20-30% of ingested dietary zinc is absorbed. Absorption occurs mostly in the duodenum and proximal jejunum. The absorbed zinc is transported in blood plasma by albumin (60-70%) and by α2-macroglobulin (30-40%). A small amount is associated with transferrin and free amino acids. Major route of zinc excretion is via the faeces. Urinary losses of zinc are about

0.6mg/day in an adult consuming about 2mg/day of zinc.

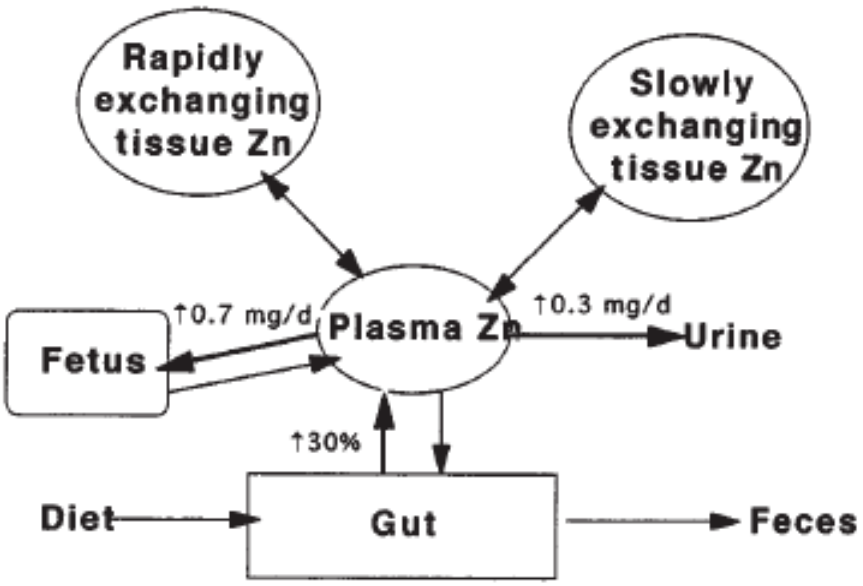
The additional need for zinc in human pregnancy is estimated to be approximately 100mg.[32] This represents approximately 5-7% of the whole body zinc concentration in a non-pregnant woman. The additional need for zinc during pregnancy can be met either by an increase in zinc intake or by adjustments in zinc homeostasis. Intestinal zinc absorption appears to be the primary means by which zinc retention is increased to meet the fetal demands.

Other mechanisms for adjustment of zinc metabolism include reduced endogenous gastrointestinal zinc excretion, renal conservation and release of maternal tissue zinc. The concentration of urinary zinc increases during gestation, reaching a value nearly twice that of preconception. This is possibly because of an increase in glomerular filtration rate. Hence renal conservation does not appear to be a mechanism for retaining zinc during pregnancy and lactation.

The demand for zinc to support fetal growth may also be met by the release of maternal tissue zinc, as is observed in some experimental animals. However in humans, there is not much evidence to prove the release of maternal tissue zinc during pregnancy. Hence it appears that adjustments in zinc absorption are the primary means by which additional zinc needs are met during pregnancy.

Normal serum level of zinc = 0.7 – 1.2 mg/L

MODEL OF ZINC METABOLISM IN LATE PREGNANCY



Rapidly exchanging tissue zinc – liver

Slowly exchanging tissue zinc – muscle

SERUM ZINC IN PREGNANCY

Zinc is 60-85% bound to albumin and low levels of plasma zinc have been reported to occur when serum albumin is lowered. Giroux et al[33] showed that 75% of the decrease in total serum zinc in pregnancy was due to a decrease in albumin bound to zinc and postulated that hypozincemia of pregnancy was secondary to the hypoalbuminemia. They also suggested that there was a decreased affinity of albumin for zinc.

Sheena tuttle et al[34] have said that the apparent hypozincemia and hypoalbuminemia of normal pregnancy are largely due to increasing plasma

volume after the first trimester. Christine et al[35] in their study have suggested that the decrease in serum zinc in the late gestation could reflect maternal fetal zinc transfer in response to fetal growth. The decline represents a physiological adaptation to pregnancy reflecting in part, maternal fetal transfer of zinc and expansion of maternal plasma volume.

Janette Taper et al[36] have suggested that blood volume expansion in pregnant women contributed to a greater diluting effect on serum zinc and the decrease could also be attributed to increased levels of estrogen. Isabelle et al[37] have said that severely depressed levels of zinc in serum indicate inadequate zinc status in pregnancy.

Pathak Priyali & Kapil Umesh[38] in their study have stated that during pregnancy there is a decrease in circulating zinc and also a decline occurs as pregnancy progresses possibly due to decrease in zinc binding and increased transfer of zinc from mother to the fetus. Tamura et al[39] have stated that the decline in serum zinc concentration during pregnancy has been well documented and could be due to a normal physiologic adjustment to pregnancy, a response to hormonal changes, hemodilution or a combination of these.

Conditions that may induce a secondary zinc deficiency during pregnancy[40]

- Factors that may limit the net absorption of zinc

    - High intake of dietary inhibitors of zinc absorption, such as phytate, liver and calcium.

- High doses of supplemental iron

- Gastrointestinal diseases that limit zinc absorption such as intestinal bypass, crohn's disease, bacterial overload, and viral or bacterial infections.

- Factors that may interfere with placental transport of zinc are

  - Cigarette smoking
  - Alcohol abuse
  - Acute maternal infections
  - Strenuous exercise
  - Therapy with certain drugs

Zinc deficiency in India

In India there is occurrence of milder grades of zinc deficiency in malnourished population subsisting on rice based diets, which could be qualitatively poor in meeting the zinc requirements. Bioavailability of zinc in any of the cereal based diets is poor due to the presence of phytate and fiber. Hence zinc intake and absorption is low in the diets of poor socio-economic population.[41]

SERUM ZINC IN PREECLAMPSIA

Several workers in the field of preeclampsia have suggested an association between zinc and preeclampsia which they have confirmed by the reduced serum zinc concentrations in women with preeclampsia.[42, 43, 44, 45]

A secondary zinc deficiency and low maternal plasma zinc concentrations can arise through a variety of mechanisms including poor availability of dietary zinc, genetic defects in zinc metabolism, physiological stress, disease or drug or toxicant induced changes in zinc metabolism. Thus hypozincemia can be a complication of numerous disease states that includes hypertension.[46]

In preeclampsia there is an excessive acute phase response with increased levels of TNF-α and IL-6. One effect of the TNF-α and IL-6 is a marked stimulation of the synthesis of the acute phase protein, metallothionein. Metallothionein is a low-molecular weight cystine-rich protein that can avidly bind zinc and other divalent metals. The functions of this protein include protection against metal toxicity and short-term storage of zinc and copper and the scavenging of hydroxyl radicals.[47] One consequence of cytokine induced increases in liver metallothionein, is hypozincemia.[46]

Gordon et al[48] showed that zinc deficiency is associated with a defect in platelet aggregation which appears to be related to altered metabolism of arachidonate and possibly to changes in the synthesis of prostaglandins.

Cherry FF et al[49] conducted a study with 272 adolescent pregnant women to ascertain the relationship of pregnancy outcome to plasma zinc levels. They found that women experiencing hypertension/toxemia were found to have significantly lower plasma zinc levels. Kumru S et al[50] have said that the serum zinc levels were 43% lower in the preeclamptic group compared with the normal pregnant women.

COPPER

Copper is an essential trace element important for growth and development. Copper dependent functions in our body include formation of melanin and keratin, synthesis of connective tissue and myoglobin, hemoglobin synthesis, energy production, synthesis of neurotransmitters (catecholamines), free radical scavenging (superoxide dismutase), immune function, formation of the myelin sheath of nerves, fertility and maintenance of pregnancy.

Copper is absorbed maximally in the duodenum with 50-80% of ingested copper being absorbed. Absorbed copper is bound primarily by albumin and transported to the liver. In the liver 95% of the copper is incorporated into ceruloplasmin (Ferroxidase I). In addition, a portion of copper is also incorporated in bile. A third portion of copper is incorporated into intracellular enzymes, such as superoxide dismutase and cytochrome oxidase. Copper is excreted primarily in faeces and its losses in urine and sweat amount to less than 3% of the dietary intake.

In the liver, copper is stored mostly as metallothionein like cuproproteins. Metallothionein is a copper and zinc binding protein present in most, if not all, tissues of higher eukaryotes. The regulation of copper metabolism is quite complex. The liver is the key organ, with copper being stored there and released as ceruloplasmin to maintain the blood levels.

Normal serum copper level = 0.8 – 1.55 mg/L

Ceruloplasmin

Ceruloplasmin is an α_2 - globulin containing copper, generated in liver. Its content in the blood of normal individuals is maintained at 300-400mg / litre of blood plasma. It is an enzyme protein containing 6 copper atoms.

It is the major extracellular antioxidant. Its antioxidant property is through its oxidase activity.[51] Ceruloplasmin is one of the varied agents that produces non enzymatic detoxification of reactive oxygen species like H_2O_2.[52] The antioxidant is characterized as an " *acute phase reactant"* and is one of the essential factors of natural organism defense. So ceruloplasmin levels are high in various neoplastic and inflammatory states.

Since ceruloplasmin contains a major portion of plasma copper, changes in its concentration determine the copper concentration in serum with very few exceptions.[53]

SERUM COPPER IN PREGNANCY

Increased concentration of serum copper and ceruloplasmin occur in pregnancy and with estrogen therapy. The serum copper concentration was approximately twice that of normal non pregnant value and these high levels persisted throughout the gestation and into early postpartum. Elevation in serum copper levels has been ascribed to increased estrogen levels and progesterone concentration.[54]

It has been earlier reported that the increase in serum copper during pregnancy is mainly in bound form due to increase in the carrier protein, ceruloplasmin, in response to stimulation by elevated levels of maternal estrogens.[55] Estrogens increase serum copper levels probably by increasing hepatic ceruloplasmin synthesis.

Pathak Priyali and Kapil Umesh[38] have said that pregnancy is associated with increased copper retention, which may be partly due to decreased biliary copper excretion induced by hormonal changes, typical during pregnancy.

Increased copper levels may also be due to the mobilization of copper from maternal tissues[56], especially from the liver. This in turn has been associated with an increase in blood estrogens.[57]

Okonofua FE et al[45] have found that in Nigerian woman there is a 100% increase in copper levels in pregnant women over non - pregnant levels at the end of pregnancy. Martin Lagos F et al[58] have proclaimed that serum copper levels in the second and third trimesters of pregnancy were significantly higher ($P<0.05$) than those determined during the first trimester and for non pregnant controls. Anand S et al[59] have said that serum copper levels rose with the progress of pregnancy and the increase was statistically significant ($P<0.005$) in the second trimester.

SERUM COPPER IN PREECLAMPSIA

Acute phase responses found in preeclampsia are complicated by

perturbations in copper metabolism resulting in hypercupremia. The hypercupremia is a hallmark of the acute phase response.[47] The increase in serum copper levels may be related to increased levels of ceruloplasmin, a positive acute phase reactant.[17,23]

Many evidences point to the increased levels of serum copper and ceruloplasmin in preeclamptic women as compared with that of normal pregnant women.[54, 60, 61, 62] Zehra Serdar et al[63] have reported in their study that copper and ceruloplasmin levels in serum were significantly increased in women with severe preeclampsia. Aksov et al[64] have stated that serum ceruloplasmin levels were increased along with an increase in malondialdehyde levels in the preeclamptic group compared with the normotensive pregnant group.

CALCIUM

Calcium is the most prevalent cation found in our body and its deficiency is related to hypertensive disorders of pregnancy and related maternal and fetal adverse outcomes.

Calcium exists in three physico-chemical states: free ionized calcium (50%), protein bound calcium (40%) and calcium combined with small anions (10%).Of the protein bound calcium fraction, 80% is associated with albumin and

20% with globulin.[65] Physiologically calcium is classified as intracellular or extra cellular calcium.

The daily intake of calcium averages 25 mmol (1g). Of this only 6 mmol is absorbed by active transport in the proximal small bowel. Normally 98% of the calcium filtered by the kidneys is reabsorbed in the proximal tubule.

Total serum calcium - 8.6 - 10.3 mg/dl

CHANGES IN CALCIUM HOMEOSTASIS DURING PREGNANCY

Calcium homeostasis is an important aspect of maternal and fetal physiology during gestation and recent evidence implicates alterations in calcium metabolism, in the pathogenesis of hypertension during pregnancy. Deficiencies in calcium intake have been linked to preeclampsia / eclampsia, and hypocalciuria and deviations in both 1,25 dihydroxy vit D3 and parathyroid hormone have been shown in women with preeclampsia.[66]

The physiologic demand for calcium is elevated by as much as 200 - 300mg / day during pregnancy and lactation. According to Lorrene D Ritchie et al[67] fetal calcium demand is met by increased maternal intestinal absorption, early breast - milk calcium was provided by maternal renal calcium conservation and loss of spinal trabecular bone, a loss that was recovered post menses.

In pregnancy there is approximately twofold increase in the circulating levels of 1,25 Dihydroxy vitamin D[68], produced in maternal kidneys along with contributions from maternal decidua, placenta and fetal kidneys. 1,25 Dihydroxy

vitamin D promotes the absorption of calcium from small intestine and also enhances the reabsorption of calcium from the proximal convoluted tubules of the kidneys. Other hormones contributing to the regulation of calcium metabolism are prolactin, placental lactogen and PTH- related protein. Hence in pregnancy, there is an increase in calcium absorption.

Though there is an increase in calcium absorption during pregnancy, the total calcium levels fall in pregnancy.[69] This reduction in total calcium may be due to the normal expansion of maternal blood volume and the pregnancy induced increase in urinary calcium excretion.[70] Pitkin RM et al[71] have stated that the major portion of decline in maternal serum calcium reflects changes in the non ionized (albumin - bound) fraction while the ionic portion declines only slightly. According to Reddy G S et al[72] increased calcium transfer from maternal circulation to the developing fetus may result in lowered serum calcium levels in pregnancy.

SERUM CALCIUM IN PREECLAMPSIA

Considerable interest has developed regarding the role of calcium in the regulation of blood pressure. The effect of calcium on blood pressure may influence the incidence and /or gestational age of development of preeclampsia.

The precise factors involved in the pathogenesis of pregnancy induced hypertension /preeclampsia is unclear, but several associated alterations in calcium metabolism have been identified. They include decrease in serum 1,25 dihydroxy vitamin D concentration, decrease in serum ionized calcium concentration[73] and a

decrease in urinary calcium excretion. Whether these biochemical abnormalities are a consequence of PIH/preeclampsia or result from impaired calcium absorption, inadequate dietary calcium intake, or both is unclear.

There are several studies showing a significant relationship between low serum calcium levels and preeclampsia.[50,61,62,74,75] According to Kumru S et al[50], the serum calcium is 10% lower in the preeclamptic women compared to that of normal pregnant women. Borella P et al[61] has stated that in EPH gestosis (edema, proteinuria, hypertension), total calcium was reduced with a reduction in the plasma calcium / magnesium ratio.

There are some studies showing an inverse relationship between calcium intake and the incidence of hypertensive disorders of pregnancy.[70,76] This fact has also been supported by many calcium supplementation studies like the one done by Jose Villar et al.[77]

Role of calcium in blood pressure regulation

Women with preeclampsia have elevated intracellular calcium concentrations in erythrocytes, platelets and vascular smooth muscle cells.[78] In the prospective study by Zemel et al[79], although no significant difference in basal platelet intracellular calcium concentration was found, preeclamptic patients had significantly greater intracellular calcium concentrations in platelets in response to stimulation by arginine vasopressin than did normotensive women. If established, however, an increase in intracellular calcium in vascular smooth muscle cells during pregnancy is consistent with development of vasoconstriction and resultant

hypertension.

Alternatively it has been hypothesized that calcium affects smooth muscle cell contractility indirectly by influencing the production of other vasoactive agents such as nitric oxide, prostacyclins, or angiotensin (via the renin-angiotensin- aldosterone metabolic pathway).[78] Calcium supplementation appears to affect circulating concentrations of parathyroid hormone and renin, which may modulate intracellular ionized calcium, resulting in the observed effect on smooth muscle relaxation.[80]

The biochemical mechanism responsible for the possible increase in intracellular calcium and concomitant decrease in extracellular calcium is presently unclear.

AIM OF THE STUDY

Deficient or excessive levels of minerals have for a long time been associated with various pregnancy outcomes, preeclampsia being one among them. The information available on the involvement of minerals like zinc, copper and calcium in preeclampsia has been reviewed and the present study was undertaken with the following objectives.

- To determine the serum zinc and copper levels in non pregnant, normal pregnant and preeclamptic women and to analyse whether their levels in the three groups differed statistically.

- To determine the serum calcium levels in non pregnant, normal pregnant and preeclamptic women and to analyse whether their levels in the three groups differed statistically.

- To determine the serum albumin levels in the three groups, analyse its variation in the groups and to correlate its level with that of the minerals.

MATERIALS AND METHODS

The present cross sectional study was carried out on one hundred and fifty female subjects in the age group of 20-32 years. Out of the hundred and fifty women, fifty were non pregnant, fifty were normal pregnant and the other fifty were preeclamptic women. The preeclamptic and normal pregnant women were from the wards and out patient Departments of Kasturba Gandhi Hospital, Triplicane and Institute of Obstetrics and Gynaecology, Egmore. Friends, relatives and their family members who formed the non pregnant group, constituted the control group for the study.

The subjects selected for this study were not supplemented for zinc and copper. Low dose calcium was supplemented to the pregnant and preeclamptic groups, but with poor compliance.

NON PREGNANT GROUP (50 members)

This group consisted of non pregnant women in the age group of 20-32 years without any preexisting medical illnesses like diabetes, hypertension, renal and cardiovascular disease

NORMAL PREGNANT GROUP (50 members)

This group consisted of

1. Normotensive primigravidae in the age group of 20-32 years and gestational age ≥ 28 weeks of pregnancy (3rd trimester).

2. No complications of pregnancy.

PREECLAMPTIC GROUP (50 members)

Inclusion criteria

Primigravidae in the age group of 20-32 years and gestational age ≥ 28 weeks of pregnancy (3rd trimester) presenting with

1. Persistent elevation of blood pressure ≥140/90 mmHg confirmed by two measurements (in the sitting posture at least six hours apart) or an increase of at least 30mmHg of systolic or 15mmHg diastolic over baseline value.

2. Proteinuria ≥ 0.3g / 24 hours urine specimen (or) ≥ 1+ dispstick.

Exclusion criteria

Women with history of preexisting hypertension, diabetes, renal or heart disease.

SAMPLE COLLECTION

The syringes, centrifuge tubes and eppendorfs were all washed well with detergent solution and rinsed thoroughly with tap water. They were then soaked in 5% HNO_3 solution overnight and afterward rinsed individually for six times, with deionised water. After this they were all dried in a hot air oven. These treated syringes, centrifuge tubes and eppendorfs only were used for sample collection, transport and storage. This was done to avoid any pre analytical error due to contamination.

8cc of venous blood (from antecubital vein) was collected from each of the

one hundred and fifty subjects selected for the study. The blood was immediately transferred to two tubes. First one was the pretreated centrifuge tube and the second one was a glass tube containing the anticoagulant mixture (Potassium oxalate and sodium fluoride in the ratio of 3:1). The blood in the second tube was analysed for glucose to confirm the exclusion of diabetic subjects.

The blood in the pretreated centrifuge tubes were allowed to clot and the serum separated. The serum from each sample was again transferred to two eppendorfs. The serum in one eppendorf was for estimation of zinc and copper; the serum in the other eppendorf was for estimation of calcium and albumin. The serum samples in the eppendorfs were stored at -20^{o}C until the analysis.

ESTIMATION OF ZINC AND COPPER

The estimation of zinc and copper was done in the Atomic Absorption Spectrophotometer - PERKIN ELMER - 3110.

PRINCIPLE

Atomic absorption

The ground state atom absorbs light energy of a specific wavelength as it enters the excited state. As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made.

Here in atomic absorption, the only function of the flame is to convert the sample aerosol into atomic vapour. Here the element is not appreciably excited but is merely dissociated from its chemical bonds and placed in an unexcited ground state (Neutral atom), which is capable of absorbing light from the primary light

source. The neutral atom is at a low energy level in which it is capable of absorbing radiation at a very narrow bandwidth corresponding to its own line spectrum.

ATOMIC ABSORPTION INSTRUMENTATION

There are 5 basic components of an atomic absorption instrument.

1. The light source that emits the spectrum of the element of interest.
2. An "absorption cell" in which atoms of the sample are produced. (Flame, graphite furnace, MHS Cell, FIAS cell, FIMS cell).
3. A monochromator for light dispersion.
4. A detector, which measures the light intensity and amplifies the signal.
5. A display that shows the reading after it has been processed by the instrument electronics.

INSTRUMENT CALIBRATION

Quantitative measurements in atomic absorption spectrophotometer are based on Beer's Law, which states that concentration is proportional to absorbance. If the analytic concentration of all the samples to be analysed falls with in the linear range, one calibration standard should be used.

ESTIMATION OF ZINC

Stock standard solution - 1000 mg/L in 1% HCl

Working standard - 0.8 µg/ml is prepared in 5% glycerol

Wavelength used (nm)	Slit width (nm)	Standard concentration (mg/L)	Linearity range (mg/L)	Sensitivity (mg/L)

213.9	0.7	0.8	1	0.018

Recommended flame - Air acetylene

Zinc bulb - 0303 – 6081

Method

First the atomic absorption spectrophotometer was calibrated with the standard. Then the samples were fed into the machine and the displayed concentrations were read and documented. For every 10 samples fed, the machine was calibrated once with the standard.

Reference range: 70 – 120 µg/dl (or) 0.7-1.2 mg /L

ESTIMATION OF COPPER

Stock standard solution - 1000 mg/L in 1% HNO_3

Working standard - 4 mg/L of working standard is prepared in 10% glycerol

Wavelength used (nm)	**Slit width (nm)**	**Standard concentration (mg/L)**	**Linearity range (mg/L)**	**Sensitivity (mg/L)**
324.8	0.7	4	5	0.077

Recommended flame - Air acetylene

Copper bulb - 0303 - 6024

Method

First the atomic absorption spectrophotometer was calibrated with the standard. Then the samples were fed into the machine and the displayed concentrations were read and documented. For every 10 samples fed, the machine was calibrated once with the standard.

Reference range: 80 – 155 µg/dl (or) 0.8-1.55 mg /L

ESTIMATION OF CALCIUM (OCPC method)

Serum total calcium has been measured photometrically using the semi auto analyser by O - cresolphthalein complexone method.

Principle

Calcium in an alkaline medium combines with O-cresolphthalein complexone to form a purple coloured complex [O-cresolphthalein complexone $(CA^{++})^2$]. The intensity of the coloured complex produced is directly proportional to the concentration of calcium present in the sample.

Calcium + O-cresolphthalein complexone $\rightarrow$ Purple coloured complex

Sample *Fresh clear unhemolysed serum.*

Reagents

L_1	:	Buffer reagent	(75 ml)
L_2	:	Colour reagent	(75ml)
S	:	Calcium standard (10mg/dl)	(5 ml)

Reagent preparation *Reagents are ready to use.*

Procedure

Wavelength / Filter	:	570nm / yellow
Temperature	:	Room temp
Light path	:	1 cm

Pipette into clean dry test tubes labeled as Blank (B), Standard (S), and Test (T).

Addition sequence	B (ml)	S (ml)	T (ml)
Buffer Reagent (L_1)	0.5	0.5	0.5
Colour Reagent (L_2)	0.5	0.5	0.5
Distilled water	0.02	-	-
Calcium standard (S)	-	0.02	-
Sample	-	-	0.02

Mix well and incubate at room temperature (25°C) for 5 minutes. Measure the absorbance of the standard (Abs.S) and Test sample (Abs.T) against the Blank, within 60 minutes.

Calculations

$$\text{Calcium in mg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 10$$

Linearity *This procedure is linear up to 18 mg/dl*

Reference range

Serum / plasma: 8.7 - 11.0 mg/dl.

System Parameters

Reaction	End point
Wavelength	570nm
Zero setting	Reagent blank
Incubation temperature	room temp.

Incubation time	5 minutes
Sample volume	0.02 ml
Reagent volume	1.00 ml
Standard	10mg/dl
Reaction slope	Increasing
Linearity	18mg/dl
Units	mg/dl

ESTIMATION OF ALBUMIN

Serum albumin has been measured photometrically using the semi auto analyser by Bromocresol green dye binding method.

Principle

Determination of albumin in serum or plasma is based on the specific binding of the dye bromocresol green to albumin in the presence of other plasma or serum proteins. The binding affinity between the dye and albumin is very high. At pH 3.68, albumin acts as a cation and binds to the anionic dye, forming a green complex, the absorbance of which is measured at 630 nm.

Reagents

Reagent 1(100 ml) - Albumin Reagent (100 ml)
(Bromocresol green, buffer pH 3.68)

Reagent 2(3 ml) - Albumin standard (4 gm/dl)

Working Reagent preparation

Reagent 1 (Albumin Reagent) and Reagent 2 (Albumin standard) are ready for use as supplied.

Specimen

Unhaemolysed serum or plasma serum is preferred.

System Parameters

Mode	End point
Wavelength	630 nm (600 - 630nm)
Temperature	Room temperature
Optical path length	1 cm
Zero setting	Reagent blank
Incubation time (minutes)	1
Sample volume	10 µl
Reagent volume	1000 µl
Concentration of the Standard	4gm/dl
Linearity	Up to 6 gm/dl
Maximum absorbance limit	2.00
Units	gm/dl

Procedure

Pipette into clean dry test tubes and perform the tests as given below:

Pipette into tubes marked	**Blank (ml)**	**Standard (ml)**	**Test (ml)**
Serum or plasma	-	-	0.01
Albumin standard	-	0.01	-
Albumin Reagent	1.0	1.0	1.0

Mix well. Incubate at room temperature for 1 minute.

1. Blank the analyser with the reagent blank.
2. Aspirate standard followed by tests.

Calculation

$$\text{Albumin (gm/dl)} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 4$$

Reference range

Adults : 3.5 - 5.0 gm/dl

Children : 2.4 - 4.8 gm/dl

RESULTS

In the present study the serum levels of zinc, copper, calcium and albumin, in non pregnant, normal pregnant and preeclamptic women were assessed, analysed and tabulated.

The results of the above said parameters obtained in the three groups are given in Tables 1-3. Table -1 signifying 50 non pregnant women, Table - 2 that of 50 normal pregnant women and Table-3 that of 50 preeclamptic women. The mean and standard deviation (S.D) for each parameter analysed in the different groups are also given in the respective tables. The same has also been illustrated as bar diagrams from figures 1 to 4.

To determine how far the mean levels of the various biochemical parameters obtained in the normal pregnant group and the preeclamptic group varied from the corresponding means of the control group, each of the former 2 groups were compared with that of the latter in Tables - 4 & 5 respectively. Similarly to obtain at the difference between the normal pregnant and preeclamptic groups, the mean and standard deviation of the parameters in the 2 groups were compared in Table - 6. The statistical significance of the variation in levels were obtained from the p value which has been calculated using the students `t' test. The obtained p value and the corresponding statistical significance on comparison of the 3 groups with one another are also shown in the tables. The correlation between serum albumin and the minerals zinc, copper and calcium are given in Tables - 7 to 9 respectively.

TABLE -1 Serum zinc, copper, calcium and albumin levels in non pregnant women

S.No.	**Zinc** (mg/L)	**Copper** (mg/L)	**Calcium** (mg/dl)	**Albumin** (g/dl)
1	0.67	1.2	10.6	4.6
2	0.48	1.2	9.9	4
3	0.62	1.7	9.5	4
4	0.53	2	9.5	3.1
5	0.36	1.8	9.4	3.7
6	0.51	1.3	8.8	4.1
7	0.58	1.3	9.3	4.1
8	0.58	1.4	9.2	3.8
9	0.47	1.3	8.8	4
10	0.5	1.6	9.9	3.9
11	0.49	1.2	10.3	3.9
12	0.44	1.4	9.9	4
13	0.46	1.6	10	4
14	0.29	2	8.3	3.2
15	0.45	2.6	10.4	3.6
16	0.4	1.8	10.1	4.1
17	0.39	1.8	10.5	3.7
18	0.41	1.3	9.1	3.8
19	0.31	1	8.8	3.5
20	0.49	1.3	8	4.1
21	0.74	1.4	9.9	3.8
22	0.52	1.5	10.4	3.9
23	0.68	1.7	9.9	3.3
24	0.57	1.4	9.5	3.9
25	0.6	1.7	10.3	4
26	0.64	1.3	10.5	4
27	0.47	1.2	9.7	4.1
28	0.61	1.8	10.4	4
29	0.53	1.9	9.9	2.8
30	0.36	1.8	8.3	3.3
31	0.52	1.4	8.8	3.9
32	0.61	1.4	8.1	4
33	0.65	1.5	8.6	3.8
34	0.53	1.3	9.3	3.9
35	0.54	1.7	10.1	3.8
36	0.47	1.1	10.2	4.1
37	0.45	1.3	10.5	3.9
38	0.49	1.6	10.3	4.1
39	0.3	2.1	9.6	3.1
40	0.43	2.6	10.4	3.8
41	0.58	1.9	10	4.1
42	0.36	1.8	10.2	3.7
43	0.41	1.4	10	3.9
44	0.27	1.1	9	3.5
45	0.43	1.4	9	4.3
46	0.48	1.3	8.2	4
47	0.46	1.4	9.5	4.2
48	0.64	1.6	9	3.3
49	0.5	1.4	9.4	4.6
50	0.6	1.6	8.7	4
Mean	**0.50**	**1.55**	**9.56**	**3.85**
S.D.	**0.11**	**0.34**	**0.73**	**0.35**

TABLE -2 Serum zinc, copper, calcium and albumin levels in normal pregnant women

S.No.	**Zinc** (mg/L)	**Copper** (mg/L)	**Calcium** (mg/dl)	**Albumin** (g/dl)
1	0.57	2.5	7.4	3.1
2	0.46	2.6	7.3	3.2
3	0.41	2.7	8.6	3.2
4	0.47	1.8	7.1	3.1
5	0.43	2.4	7.6	3.1
6	0.43	2.2	8.5	3.4
7	0.26	2.9	9.3	3.1
8	0.28	1.8	7.8	3.3
9	0.33	2.3	8.3	3.1
10	0.55	1.9	8.1	3.2
11	0.44	2.7	10.4	2.9
12	0.39	2.1	8.6	3.2
13	0.53	1.9	8.3	3.1
14	0.41	2.8	10.3	3.1
15	0.37	1.8	10.5	3
16	0.54	2.9	10.3	3
17	0.55	2.5	8.3	3.1
18	0.53	2.4	9.6	2.9
19	0.51	3.5	9	3.2
20	0.53	2.4	7.8	3.1
21	0.53	2.2	7.6	3
22	0.46	2.1	7.7	3.2
23	0.59	2.6	8.2	3
24	0.37	2.4	8.7	3.4
25	0.35	3.3	9.1	3.2
26	0.49	2.4	8.1	3.6
27	0.35	2.4	9.4	3
28	0.31	2.6	8.6	3.3
29	0.48	2.6	8.7	2.6
30	0.41	1.9	7.6	3.4
31	0.47	2.5	8.1	3.3
32	0.4	2.7	8.4	3.3
33	0.54	1.8	9	3.4
34	0.5	2.2	8.7	3.5
35	0.43	1.9	8	3.2
36	0.37	2.4	8.5	3.3
37	0.45	1.9	10.4	3.1
38	0.55	1.8	8	3.4
39	0.61	1.7	9.4	3.3
40	0.34	2.7	9.7	3.1
41	0.39	2.8	9.8	3.3
42	0.36	2.7	8.8	3.2
43	0.34	2.1	7.8	3.5
44	0.37	2.4	9.4	3.4
45	0.42	2.5	10	3.6
46	0.38	2.2	9.8	3.3
47	0.32	2.3	10	3.6
48	0.4	1.7	7.9	3
49	0.4	2.5	7.8	3.2
50	0.41	2.4	9.1	3.1
Mean	**0.44**	**2.36**	**8.71**	**3.20**
S.D	**0.08**	**0.40**	**0.92**	**0.19**

TABLE -3 Serum zinc, copper, calcium and albumin levels in preeclamptic women

S.No.	**Zinc** (mg/L)	**Copper** (mg/L)	**Calcium** (mg/dl)	**Albumin** (g/dl)
1	0.43	3.3	10	3.2
2	0.44	3.2	8.6	2.8
3	0.4	3.2	8.5	3.4
4	0.41	2.7	8.7	3
5	0.55	3.4	8.9	3.3
6	0.44	2.4	8.6	3.2
7	0.32	3	10.1	3.1
8	0.36	3	8.8	3
9	0.39	3.8	9.1	3.2
10	0.45	2.7	8.7	3.5
11	0.48	3.3	8.2	3.3
12	0.33	2.5	8.1	3.2
13	0.33	2.4	8.2	3
14	0.3	2.1	8.1	2.8
15	0.39	3.3	10.1	2.9
16	0.37	2.6	7.9	2.9
17	0.33	2.8	8.6	3
18	0.23	2.9	10	3.1
19	0.32	2.4	8.8	2.9
20	0.33	2.7	8.9	3.1
21	0.31	2	8.7	3.2
22	0.6	2.6	8.9	3.1
23	0.45	2.3	7.8	3.1
24	0.47	2.5	8.2	3.2
25	0.41	2.8	7.9	3.3
26	0.45	3.1	9.8	3.1
27	0.59	2.8	7.9	3
28	0.41	2.8	7.4	2.9
29	0.33	1.7	7.6	3.2
30	0.48	2.1	7.9	3.3
31	0.39	3	8.4	3
32	0.3	2.5	9.7	3.1
33	0.33	3.3	8.8	3.3
34	0.32	3.1	8.5	3.2
35	0.38	2.1	9.3	3.3
36	0.44	3	9.6	3.2
37	0.29	2.6	8.8	3.3
38	0.33	1.8	9.7	3.2
39	0.38	2	7.9	3.1
40	0.42	2.7	8.4	3.4
41	0.46	1.9	7.8	3.3
42	0.5	3.3	9.1	3.2
43	0.28	1.9	8.2	3.1
44	0.39	2.8	9	3.2
45	0.33	2.9	8.3	3.4
46	0.31	2.8	7.9	3.2
47	0.35	2.5	8.5	3.1
48	0.36	2.8	8.4	3
49	0.45	3.7	8.2	3.1
50	0.41	3.4	8.4	2.9
Mean	**0.39**	**2.73**	**8.64**	**3.14**
S.D	**0.08**	**0.5**	**0.7**	**0.16**

FIGURE - 1

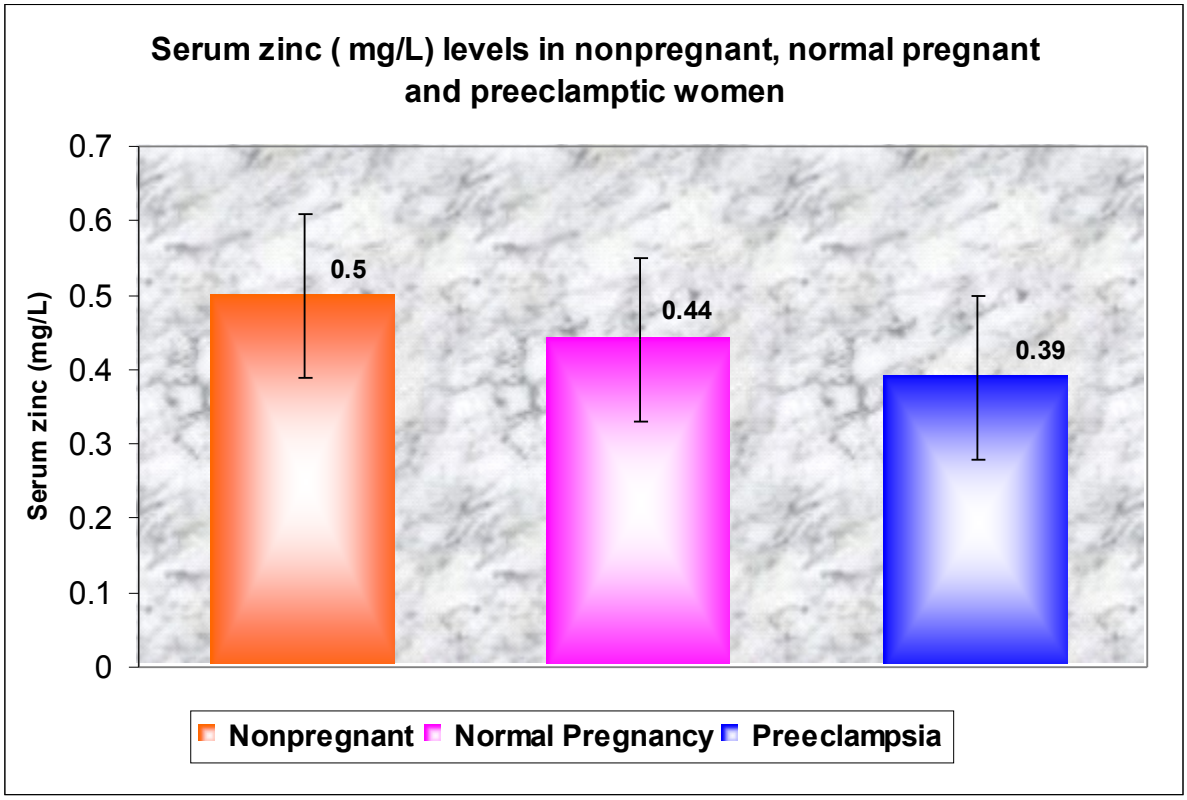


FIGURE -2

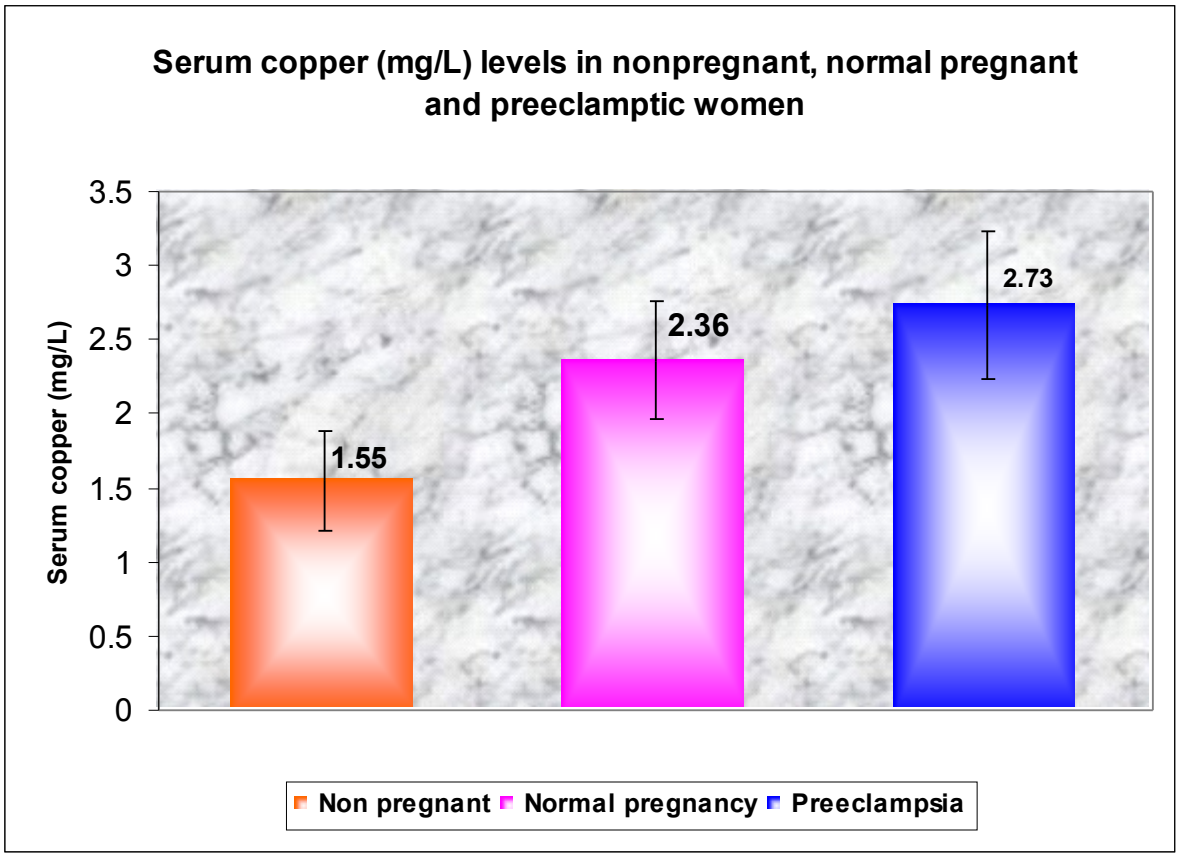


FIGURE - 3

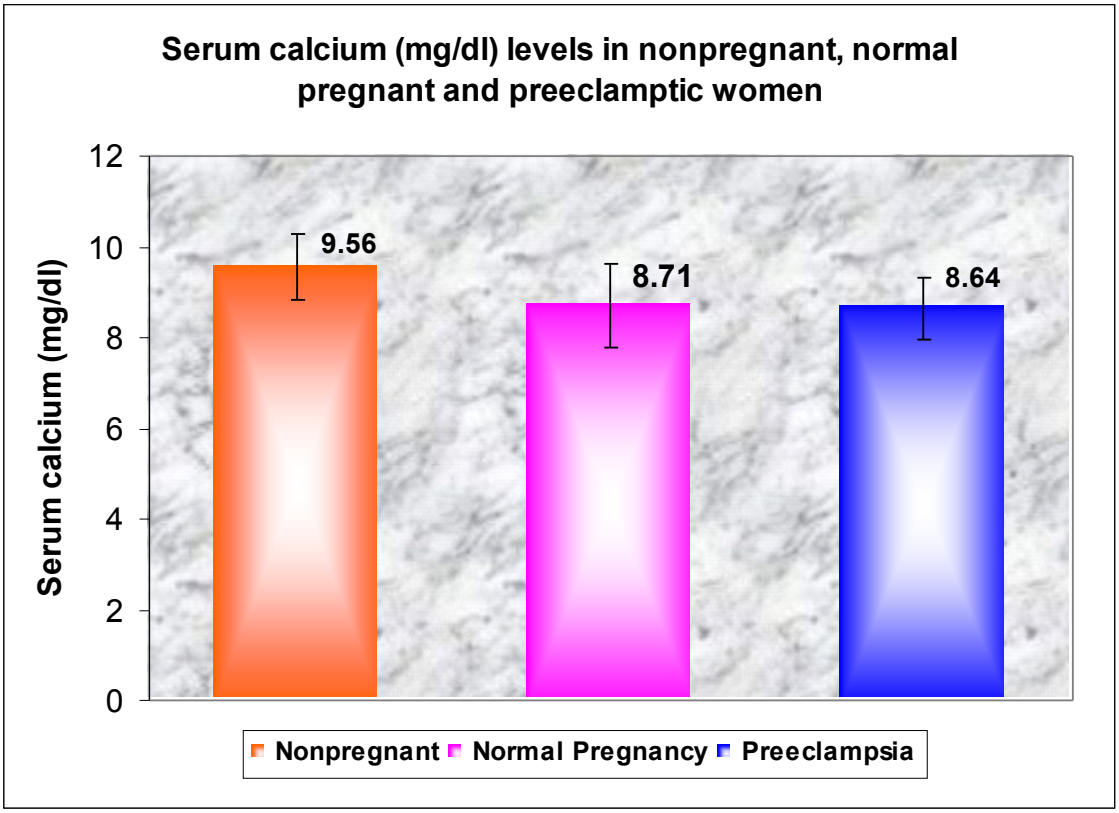


FIGURE - 4

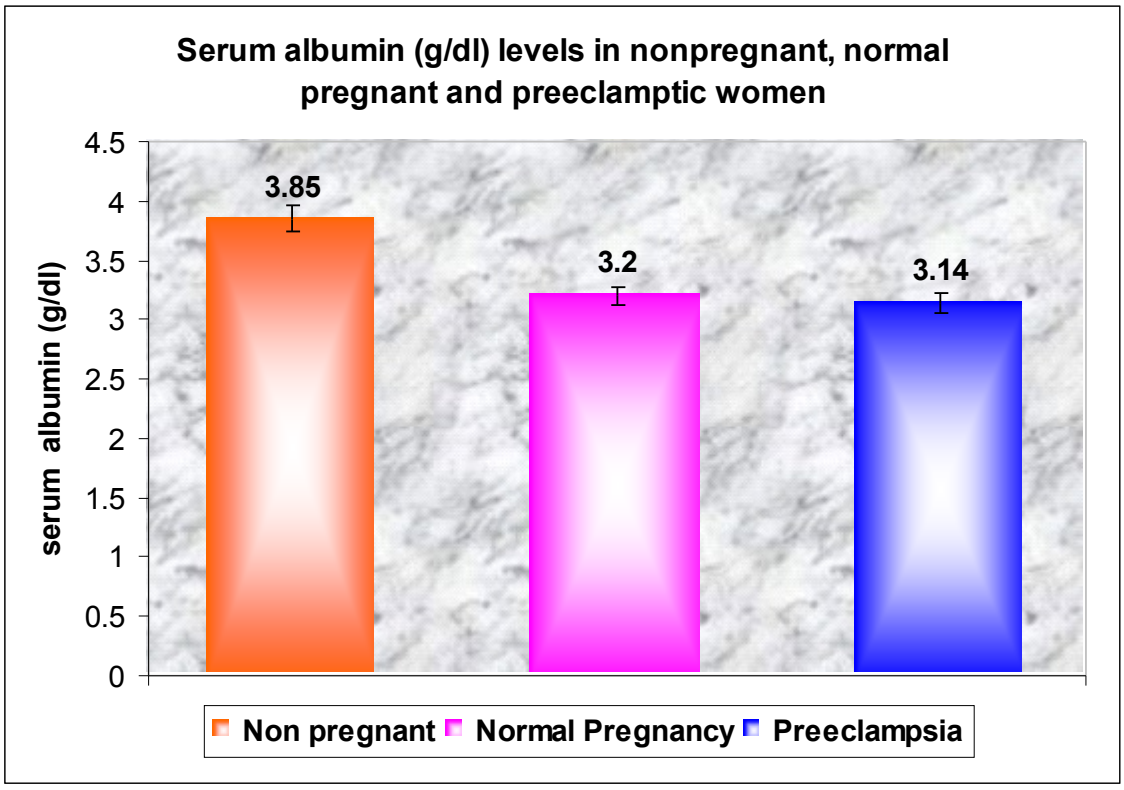


TABLE – 4

Comparison of serum zinc, copper, calcium and albumin levels between non-pregnant and normal pregnant women

Groups	**Zinc** (mg/L)	**Copper** (mg/L)	**Calcium** (mg/dl)	**Albumin** (g/dl)
Non-Pregnant	0.5 ± 0.11	1.55 ± 0.34	9.56 ± 0.73	3.85 ± 0.35
Normal Pregnant	0.44± 0.08	2.36 ± 0.40	8.71 ± 0.92	3.20 ± 0.19
P value	P<0.01	P<0.001	P<0.001	P<0.001
Significance	MS↓	HS↑	HS↓	HS↓

MS-Moderately significant HS-Highly significant

TABLE - 5

Comparison of serum zinc, copper, calcium and albumin levels between non-pregnant and preeclamptic women

Groups	**Zinc** (mg/L)	**Copper** (mg/L)	**Calcium** (mg/dl)	**Albumin** (g/dl)
Non-Pregnant	0.5 ± 0.11	1.55 ± 0.34	9.56 ± 0.73	3.85 ± 0.35
Pre-eclamptic	0.39 ± 0.08	2.73 ± 0.5	8.64 ± 0.7	3.14 ± 0.16
P value	P<0.001	P<0.001	P<0.001	P<0.001
Significance	HS↓	HS↑	HS↓	HS↓

HS-Highly significant

TABLE - 6

Comparison of serum zinc, copper, calcium and albumin levels between normal pregnant and preeclamptic women

Groups	**Zinc** (mg/L)	**Copper** (mg/L)	**Calcium** (mg/dl)	**Albumin** (g/dl)
Normal Pregnant	0.44 ± 0.08	2.36 ± 0.4	8.71 ± 0.92	3.20 ± 0.19
Pre-eclamptic	0.39 ± 0.08	2.73 ± 0.5	8.64 ± 0.7	3.14 ± 0.16
P value	P<0.05 (P=0.012)	P<0.001	P=0.68	P<0.05 (P=0.047)
Significance	S↓	HS↑	NS	S↓

S -Significant HS -Highly significant NS -Non significant

TABLE - 7

Correlation between serum zinc and albumin levels

	Zinc	**Albumin**
Pearson Correlation	1.000	.393
Sig. (2- tailed)		0.01
N	150	150

Positive significant correlation (P<0.01) exists between serum zinc and albumin levels (r = .393).

TABLE - 8

Correlation between serum copper and albumin levels

	Copper	Albumin
Pearson Correlation	1.000	-.674
Sig. (2- tailed)		0.01
N	150	150

Negative significant correlation (P<0.01) exists between serum copper and albumin levels (r = -.674).

TABLE – 9

Correlation between serum calcium and albumin levels

	Calcium	Albumin
Pearson Correlation	1.000	.396
Sig. (2- tailed)		0.01
N	150	150

Positive significant correlation (P<0.01) exists between serum calcium and albumin levels (r = .396).

DISCUSSION

In the present study, serum zinc, copper, calcium and albumin levels have been analysed among three groups (nonpregnant, pregnant and preeclamptic women).

To start with, the reference range obtained in the study from apparently normal nonpregnant females is scrutinized.

The mean serum zinc level of 0.50± 0.11 mg/L though higher to 0.36 ± 0.06 mg/L obtained by Ajose A et al[81], is well within the reference range obtained by Pathak Priyali et al[82] who have conducted their study in New Delhi in populations with high calorie intake and low calorie intake. In the high calorie intake group the zinc level was 0.63 ± 0.15 mg/L while in the low calorie intake group it was 0.59 ± 0.13 mg/L. However the reference range for zinc in this study and that of Pathak Priyali et al are much lower to 0.947 ± 0.265 mg/L of Martin Lagos et al[58] and that of 0.7 to 1.2 mg/L quoted by David B.Milne[83] in Tietz text book of Clinical Chemistry.

It should be taken into account that the latter 3 persons have given the reference range for serum zinc by studying the Western population, compared to the work of Pathak Priyali et al and the present study which were undertaken with Indians, who consume cereals as their staple diet. According to Bhaskaram P[41], in Indians, there is occurrence of milder grades of zinc deficiency in malnourished population subsisting on rice based diets. This could be due to the presence of factors like phytate and fiber in cereals, which form insoluble complexes with

zinc, leading to its reduced absorption. Hence the reference range of zinc is lower in this study, compared to that of western population.

On the other hand, the serum copper mean of 1.55 ± 0.34 mg/L obtained for the controls of this study is at the upper limit of the reference range of 0.8 to 1.55 mg/L given in Tietz text book of Clinical Chemistry.[83] It is also higher to the level of 1.092 ± 0.362 mg/L of Martin Lagos et al.[58] This difference in the level of copper in the control group when compared to the values given in Tietz textbook of clinical chemistry and that given by Martin Lagos et al can be once again explained in terms of racial variation of the population studied.

The mean calcium level of 9.56 ± 0.73 mg/dl falls within the reference range of 8.7 to 11mg/dl of the kit method selected for the study and that of 9.5 ± 1.6 mg/dl obtained by Malas NO et al.[75] It also falls within the reference range of 8.6 - 10 mg / dl given in Tietz text book of Clinical Chemistry.[65]

Among the 4 parameters, it is found that calcium and albumin levels in controls fall well within the reference range quoted by previous workers in the field and that in standard textbooks. The lower mean levels of zinc and the higher mean levels of copper in controls of this study compared to several previous studies have been attributed to the racial variation. Therefore the mean levels obtained for all the 4 parameters from apparently normal nonpregnant women can be accepted as the valid reference range for this study.

Comparison of the serum zinc levels obtained in the normal pregnant and preeclamptic women, with the above reference range of the minerals reveal the

following. From table- 4 it is evident that serum zinc levels are lowered to significant levels ($P<0.01$) in the normal pregnant women, from its reference range. This reduction of serum zinc levels in pregnant women from their non pregnant counterparts correlates well with the previous studies of Giroux et al[33], Sheena Tuttle et al[34], Christine et al[35], , Janette Taper et al[36], Isabelle et al[37] , Pathak Priyali et al[38] and Tamura et al.[39]

Various reasons can be attributed to this decrease in serum zinc level in pregnancy. In pregnancy 75% of the decrease in total serum zinc can be due to the decrease in albumin bound zinc leading to hypozincemia secondary to hypoalbuminemia as suggested by Giroux et al.[33] It may also be due to plasma volume expansion resulting in hypozincemia and hypoalbuminemia as indicated by Sheena Tuttle et al[34] or it may be due to a normal physiological adjustment to pregnancy in response to hormonal changes, hemodilution or a combination of the above, as pointed out by Tamura et al.[39]

The serum zinc levels in preeclamptic women are lowered to highly significant levels ($P<0.001$) compared to the reference range which is evident in Table-5. Preeclampsia is characterized by excessive acute phase response (inflammatory response) as indicated by Redman et al.[15] In acute phase response cytokines like IL-1, IL-6 and TNF-α are released which in turn induces the hepatic synthesis of various acute phase proteins, one of them being metallothionein.[22] Metallothionein causes increased sequestration of zinc in maternal liver, resulting in hypozincemia, as indicated by Carl L. Keen et al.[46] This decrease in serum zinc may also be associated with a defect in platelet aggregation which appears to be

related to alteration in the metabolism of arachidonate and to possible changes in the synthesis of prostaglandins, as suggested by Gordon et al.[48]

Hence it is clear that significant lowering of zinc from its reference range occurs in both the normal pregnant and the preeclamptic women. But when Table - 6 is scrutinized, it is obvious that in preeclamptic women, serum zinc levels are even lower to that in normal pregnant women. This difference which is statistically significant ($P<0.05$) correlates to the observations of James Roberts et al[1], Bassiouni et al[44], Carl L Keen et al[46], Gordon et al[47], Cherry et al[49] who have obtained significantly lower levels of serum zinc in preeclamptic women compared to their normal counterparts. Thus the greater degree of decrease in serum zinc levels of preeclamptic women can probably be due to the excessive acute phase response in preeclampsia, compared to the proinflammatory stimulus associated with normal pregnancy.[15]

On analysis of serum copper in normal pregnant women, it is clear that the level is increased to a highly significant degree ($P<0.001$) from the reference range (Table-4). This correlates well with the studies by Pathak Priyali et al[38], Okunofua PE et al[45], Sheila et al[54], Martin Lagos et al[58] and Anand S et al.[59]

The increase in serum copper in normal pregnancy can be attributed to the increase in bound form of the mineral as a result of the increase in the carrier protein ceruloplasmin, in response to stimulation by elevated maternal estrogens, as indicated by Pathak Priyali[38] et al. It can also be due to increase in both estrogen and progesterone levels, as pointed out by Sheila et al.[54]

Increase in the level of serum copper to highly significant levels ($P<0.001$) from the reference range is also evident in preeclamptic women (Table-5). This increase can be related to the excessive acute phase response in preeclampsia which is characterized by increases in plasma ceruloplasmin, complement activity, α_1-antitrypsin, haptoglobin and reduced albumin and transferrin, as suggested by Vince GS et al.[29] Ceruloplasmin, a copper binding carrier protein, is the major circulating form of copper. It is also a positive acute phase reactant. The increased level of serum ceruloplasmin may be related to the increased levels of serum copper as stated by Carl L.keen et al[46] or Zehra Serdar et al.[63]

On scrutinizing Table-6 regarding the elevation of serum copper levels in both preeclamptic women and normal pregnant women, it is clear that the elevation of the mineral in preeclamptic women is higher to that of the pregnant women resulting in a highly significant difference ($P<0.001$) between the two elevated levels. This finding correlates with the studies by Vince GS et al[29] , Sheila et al[54], Fattah MM et al[60], Borella P et al[61], Zhao F et al[62], Zehra Serdar et al[63] and Aksov et al[64] who have shown a greater increase in serum copper levels in preeclamptic women compared to the normal pregnant women. The higher elevation of serum copper in preeclamptic women can be viewed in similar terms to that of zinc and therefore attributed to the excessive acute phase response of preeclampsia.

The serum calcium levels in normal pregnant women are lowered to highly significant levels ($P<0.001$) from its reference range (table - 4). The reduction in serum calcium during pregnancy can be due to the normal expansion of maternal

blood volume and to increase in excretion of urinary calcium as reported by Lorrene D Ritchie et al.[70] The decrease of total calcium can also be a reflection of the fall in serum albumin and the albumin bound to total calcium as stated by Pittin RM and Gebhardt MP.[71] It may also be due to the increased calcium transfer from maternal circulation to the developing fetus as suggested by Reddy GS.[72]

As per Table-5 the serum calcium levels in preeclamptic women are also lowered to highly significant levels ($P<0.001$) from its reference range. The decrease in serum calcium as suggested by Lorrene D Ritchie et al[70], may be due to reduced dietary intake of calcium because the subjects analysed belong to the lower economic strata. It can also be the result of the excessive acute phase response seen in preeclampsia because it has already been reviewed that calcium is a negative acute phase reactant. As a result of this decrease in serum calcium i.e. extracellular calcium, intracellular calcium may be increased as suggested by Lorrene D Ritchie et al.[67] This intracellular calcium in preeclampsia has been found to be more pronounced in RBCs, platelets and vascular smooth cells as stated by Jorge A. Prada et al.[78] He has suggested that the increase in calcium in vascular smooth muscle cells will have a direct effect by causing abnormal contraction of smooth muscle cells and an indirect effect by influencing the production of other vasoactive agents like nitric oxide, prostacyclins and angiotensin which will increase the vascular resistance and the blood pressure, resulting in preeclampsia. Hence the decrease of serum calcium and associated increase of intracellular calcium can also be the reason for preeclampsia in the subjects analysed.

In Table-6 we find that statistically there is no difference between the lowered calcium levels of preeclamptic women (p=0.68) and those of normal pregnancy. This finding of the study does not correlate with those of Kumru S et al[50], Borella P et al[61], Zhao F et al[62] , Huang HM et al[74] and Malas NO et al[75] who have shown significantly lower serum calcium levels in preeclamptic women to that in normal pregnancy.

This is rather surprising because albumin and calcium, negative acute phase reactants get reduced when there is an acute phase response.[17,23] Therefore in preeclampsia where there is albuminuria along with exaggerated acute phase response it is natural to expect a lower level of serum albumin and calcium in the condition in comparison to normal pregnancy where plasma volume expansion is the only contributory factor. As per expectation the serum albumin level is significantly lower ($P<0.05$) in preeclampsia than to its level in normal pregnancy (Table-6).Since 40% of the calcium is normally bound to albumin and since it is by self a negative acute phase reactant, theoretically there should be a greater decrease of serum calcium level in preeclampsia unlike that of our study where though the serum calcium is lower to its level in normal pregnancy, the lowering is not sufficient to produce any statistically significant difference.

As Lorrene D Ritchie et al[67] has suggested that there is decreased urinary excretion of calcium in preeclampsia unlike that of albumin, the absence of statistically lower level of calcium in preeclampsia can be attributed to this decrease of its excretion.

The serum albumin levels in normal pregnancy are lowered to highly significant levels ($P<0.001$) compared to its reference range (Table-4). This hypoalbuminemia of pregnancy could be the result of plasma volume expansion as suggested by Sheena Tuttle et al[34] and Lorrene D Ritchie et al.[67]

The serum albumin levels in preeclamptic women are also lowered to highly significant levels ($P< 0.001$) compared to its reference range (Table-5). This decrease in serum albumin in preeclampsia could be the result of acute phase response plus albuminuria characteristic of preeclampsia.

Comparison of the lowered levels of serum albumin in preeclampsia and normal pregnancy reveals a significantly lower ($P<0.05$) level of albumin in preeclampsia to that of normal pregnancy (Table-6). The greater lowering of serum albumin in preeclampsia can be attributed to 2 factors namely acute phase response and albuminuria contributing to the lowering in the above condition against the single cause of plasma volume expansion in normal pregnancy.

When the level of albumin is compared with that of the minerals, it is seen that zinc and copper have a positive significant correlation with albumin (Table-7 and Table-9), while copper has a negative significant correlation with it (Table-8).

Among the minerals evaluated, as only serum zinc and copper have revealed statistically significant difference in their decrease and increase respectively in normal pregnant and preeclamptic women, attempt has been made to establish cut off levels of zinc and copper to demarcate preeclampsia from normal pregnancy. For this purpose line graphs of zinc and copper by plotting

their levels in 50 subjects each, of normal pregnancy and preeclampsia have been made in graphs 1 and 2 respectively.

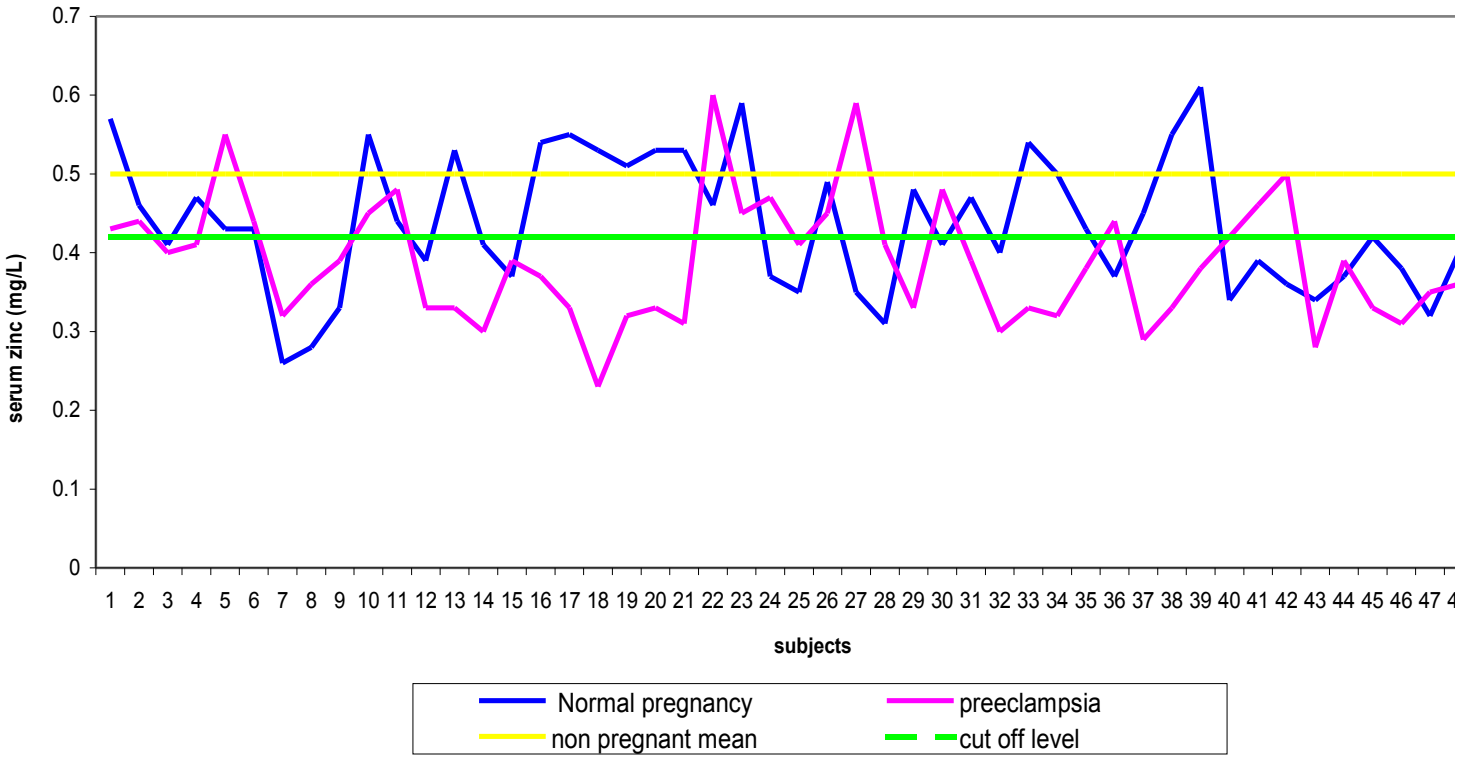
TABLE – 10

Serum zinc

S.No.	Cutoff values (mg/L)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
1.	0.35	38	82	67.8	57
2.	0.4	58	60	59	58.8
3.	0.42	68	50	57.6	61
4.	0.45	84	40	58.3	71.4
5.	0.48	92	30	56.7	79

GRAPH - 1

Serum zinc (mg/L) in normal pregnant and preeclamptic women



GRAPH - 2

Serum copper (mg/L) in normal pregnant and preeclamptic women

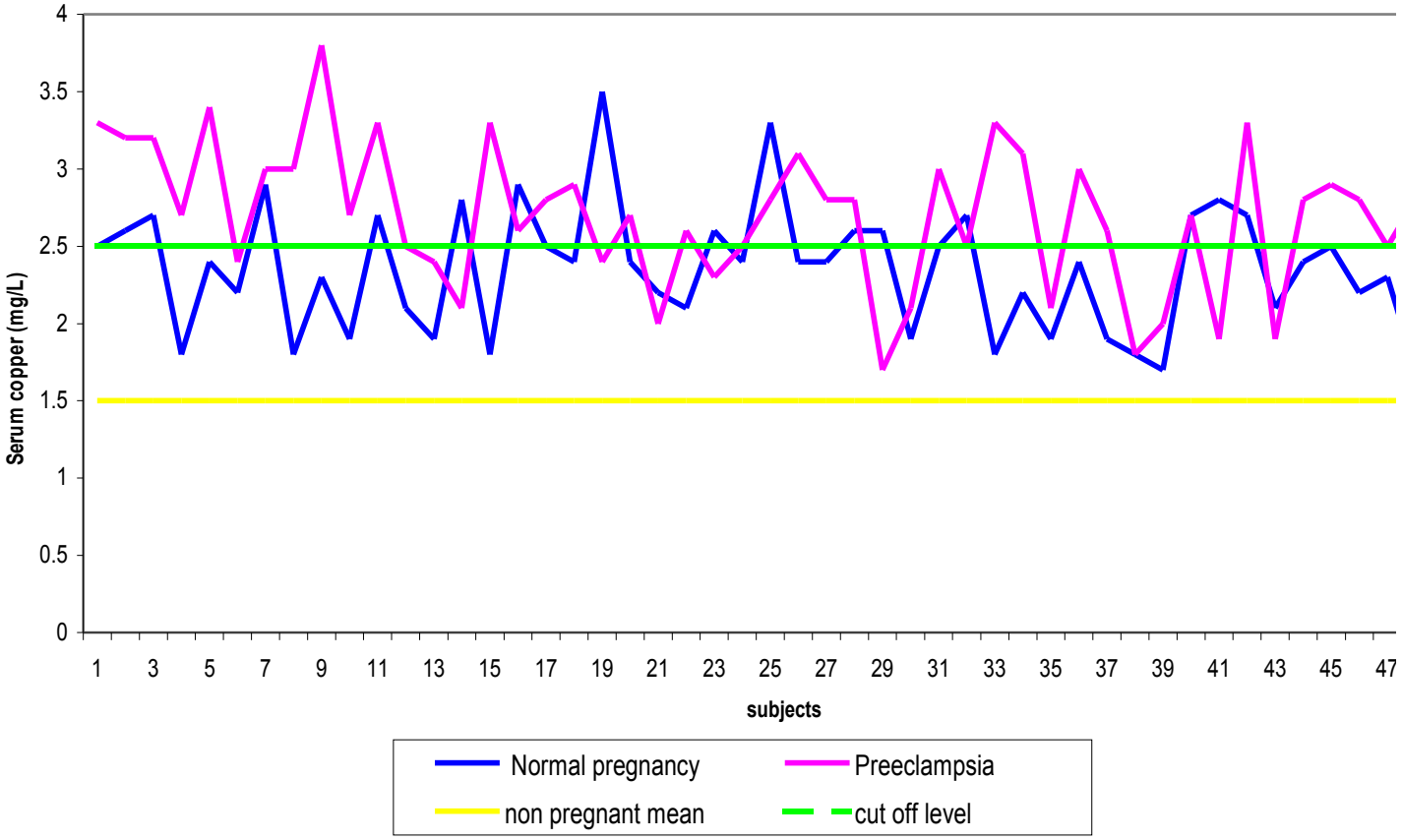


TABLE - 11

Serum copper

S.No.	Cutoff values (mg/L)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)

1.	2.4	80	42	58	67.7
2.	2.5	74	60	65	70
3.	2.6	66	70	68.75	67.3
4.	2.7	60	78	73	66
5.	2.8	52	88	81.25	64.7
6.	2.9	38	92	82.6	60

Table -10 and table -11 reveal the calculated sensitivity, specificity, positive predictive value and negative predictive value for the various cut-off levels selected from the respective graphs for zinc and copper. It is clear from Table-10 that the cut off level of 0.42 mg/L for zinc, which has a sensitivity of 68%, specificity of 50%, positive predictive value of 57.6% and negative predictive value of 61%, can be the most appropriate one to demarcate preeclampsia from normal pregnancy. Similarly from table-11, 2.5 mg/L of copper having a sensitivity of 74%, specificity of 60%, positive predictive value of 65% and negative predictive value of 70% is selected as the most suitable cut off level for the purpose.

The arrived appropriate cut off values of serum zinc and copper of the study, namely 0.42 mg/L and 2.5mg/L are also shown in the respective graphs. However since the above cut off values of both the minerals don't have 100% sensitivity or specificity, their validity is rather minimal in the diagnosis of preeclampsia.

CONCLUSION

From the study on serum zinc, copper, calcium and albumin conducted on a total of 150 subjects comprising of 50 non pregnant women, 50 normal pregnant and 50 preeclamptic women, the following inferences were arrived at. The conclusions were made after analysing the reference range obtained from the control group constituted by the 50 non pregnant women and on comparing the mean levels of the biochemical parameters of the other groups with the reference range and with one another.

I. In normal pregnant women

1. Serum zinc level is lower than in non pregnant women.
2. Serum copper level is higher than in non pregnant women.
3. Serum calcium level is lower than in non pregnant women.
4. Serum albumin level is lower than in non pregnant women.

II. In preeclamptic women

1. Serum zinc level is lower than in non pregnant women and to that in normal pregnant women.
2. Serum copper level is higher than in non pregnant women and to that in normal pregnant women.
3. Serum calcium level is lower than in non pregnant women but similar to that in normal pregnant women.

4. Serum albumin level is lower than in non pregnant women and to that in normal pregnant women.

III. Serum zinc and calcium have a positive correlation with serum albumin while serum copper has a negative correlation.

IV. Most appropriate cut off level of zinc to demarcate preeclampsia is 0.42 mg/L i.e. a level of 0.42 mg/L or below signifies preeclampsia.

V. Most suitable cut off level of copper to demarcate preeclampsia is 2.5 mg/L i.e. a level of 2.5 mg/L or above signifies preeclampsia.

BIBLIOGRAPHY

1. James M. Roberts, Judith L. Balk, Lisa M. Bodnae, Jose M. Belizan, Eduardo Bengal and Anibal Martinez. Nutrient involvement in Preeclampsia. American Society for Nutritional Sciences 2003; 1684S - 1692S.

2. Chesley, L.C. Hypertensive disorders of Pregnancy, Appleton Century - croffs, New York.

3. Gifford R.W, August P.A. Cunningham G. Green LA, Lindheimer, McNellis D, Roberts JM, Sibai BM & Taler SJ. Report of the National High Blood Pressure Education Program working group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol; 183 : S1 - S22.

4. Page, E.W. The relation between hydatid moles, relative ischemia of the gravid uterus, and the placental origin of eclampsia. Am J Obstet. Gynecol; 37 : 291 – 293.

5. Redman CW. Current topic : Preeclampsia and the placenta. Placenta 12 1991: 301 – 308.

6. Sheppard BL, Bonnar J. An Ultrastructural study of uteroplacental spiral arteries in hypertensive and normotensive pregnancy and fetal growth retardation. Br J Obstet Gynaecol 1981; 88 : 695 – 705.

7. Benyo DF, Miles TM, Courad KP. Hypoxia Stimulates cytokine production by villous explants from the human placenta. J Clin Endocrinol Metab 1997; 82 : 1582 – 1588.

8. Roberts, J.M. & Cooper, D.W. Pathogenesis and genetics of Preeclampsia. Lancet 2001; 357 : 53 – 56.

9. Roberts, J.M. & Hubel, C.A. Is oxidative stress the link in the two - stage model of Preeclampsia? Lancet 1999; 354 : 788 – 789.

10. Hubel, C & Robert's J. Lipid metabolism and oxidative stress. In Chesley's Hypertensive Disorders in Pregnancy (Lindheimer, M. Roberts, J. and Cunningham, F., eds), PP 453 – 486 Appleton & lange, New York.

11. Roberts JM, Redman CWG. Preeclampsia more than pregnancy induced hypertension. Lancet 1994; 341 : 1447 – 54.

12. KraussT, Juhn W, Lakoma C, Augustin HG. Circulating endothelial cell adhesion molecules as diagnostic markers for the early identification of pregnant women at risk for development of preeclampsia. Am J Obstet Gynecol 1997; 177 : 443 – 449.

13. Wang Y, Walsh SW. Placental mitochondria as a source of oxidative stress in preeclampsia. Placenta 1998; 19 : 581 – 586.

14. Knopp RH, Bonet B, Lasuncion MA, Montelongo A, Herrara E. Lipoprotein metabolism in pregnancy. In : Herrara E, Knopp R, Eds. Perinatel Biochemistry. Boca Raton, FL:1992; CRC Press, Inc., pp 20-51.

15. Redman CW, Sacks GP, Sargent IL. Preeclampsia : an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol. 1999 Feb;180(2 Pt 1):499-506.

16. Sacks GP, Studena K, Sargent II, Redman CWG. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am J Obstet gynecol, 1998; 179:80-86.

17. Gruys.E,Toussaint, M.J.M. Landman, W.J.M. et al. Infection, Inflammation and stress inhibit growth. Mechanisms and non-specific Assessment of the processes by Acute phase proteins, 10th International Conference, ISBN 1999 : 90-74134-60-2.

18. Gruys.E,Toussaint, M.J.M.,Niewold T.A et al. Acute phase reaction and acute phase proteins. J Zhejiang Univ SCI 2005 6B(11):1045-1056.

19. Dinarello, C.A., Pathogenesis of fever during hemodialysis. Contr Nephrol 1983; 36 : 90-99.

20. Heinrich, P.C.,Castell,T.A.,Andus.T. Interleukin - 6 and the acute phase response. Biochem J 1990; 265:621- 636.

21. Heinrich, P.C., Behrmann, I, Muller - Newen, G. et al. Interleukin - 6 - type

cytokine signalling through the gp 130/Jak/STAT pathway. Biochem J , 1998; 334:297-314.

22. Disilvestro.R.A.,Carlson,G.P. Inflammation an inducer of metallothionein, inhibits carbon- tetrachloride induced hepatotoxicity in rats. Toxicol Lett , 1992;60:175-181.

23. Gruys.E. Obwolo, M.J. Toussaint, M.J.M. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry : a review. Vet Bull,1994; 64:1009-1018.

24. Greer IA, Lyall F, ,Perara T et al.Increased concentrations of cytokines IL-6and IL-1 receptor antagonist in plasma of women with preeclampsia: a mechanism for endothelial dysfunction? Obstet Gynecol 1994;84:937-940.

25. Dilys J. Freeman, Frances McManus, Elizabeth Ann brown et al. Short and long term changes in plasma Inflammatory markers associated with preeclampsia, hypertension 2004; 44:708-714.

26. Nawroth PP,Stem DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 1986;163:740-745.

27. Conrad K.P. Miles TM, Benyo DF. Circulating levels of immunoreactive cytokines in women with preeclampsia. Am J. Reprod Immunol,1998;40:102-111.

28. Bartha JL, Romoro - Carnona R, Escobar - LIompart M etal. The relationships between leptin and inflammatory cytokines in women with preeclampsia. BJOG 2001;108:1272-1276.

29. Vince G.S, Starkey PM, Austgulen R, et al Interleukin 6. Tumor necrosis factor and soluble tumor necrosis factor receptors in women with preeclampsia. Br J Obstet Gynaecol 1995;102 : 20- 5.

30. Takacs P,Green KL, Nikaeo A Kauma SW.Increased vascular endothelial cell production of IL- 6 in severe preeclampsia. Am J Obstetric Gynecol 2003;188:740-744.

31. Carl A. Hubel .Oxidative stress in the pathogenesis of preeclampsia P.S.E.B.M.

1999,Vol 222.

32. Swanson CA, King JC. Zinc and Pregnancy Outcome. Am.J clin Nutr 1987; 46 : 763 – 71.

33. Giroux E, Schechter PJ, Shoun J. Diminished albumin binding of zinc in serum of pregnant women. Clin Sci Mol Med 1976;51: 45 – 9.

34. Sheena Tuttle, Peter J Aggett, Doris Campbell and Ian Macgillivray. Zinc and copper nutrition in human pregnancy a longitudinal study in normal primigravidae and in primigravidae at risk of delivering a growth retarded baby. Am J Clin Nutr 2000; 71 (Suppl): 1334S - 43S.

35. Christine A Swanson and Janet C King. Zinc and Pregnancy Outcome. Am J Clin Nutr 1987; 4 : 763 – 71.

36. Janette Taper, Jean T Oliva and SJ Ritchey. Zinc and copper retention during pregnancy: the adequacy of prenatal diets with and without dietary supplementation. Am J Clin Nutr 1985 ; 41: 1184 – 1192.

37. Isabelle F Hunt Norma J Murphy, Amitra E Cleaver, Bahram Faraji, Marian Eswendseid, Ben L Browdy, Anne H Coulson, Virginia A Clark, Robert H Settlege and J Cecil smith. Zinc supplementation during pregnancy : effects on selected blood constituents and on progress and outcome of pregnancy in low income women of Mexican descent. Am J Clin Nutr 1984;40:508-21.

38. Pathak Priyali, Kapil Umesh. Role of trace elements zinc, copper and magnesium during pregnancy and its outcome. Symposium on micro nutrient deficiency disorders 2004; Vol 71 : 1003 - 1005.

39. Tamura T, Goldenberg RL. Zinc nutriture and pregnancy outcome. Nutr Res 1996; 16 : 139 – 81.

40. Janet C King. Determinants of maternal zinc status during pregnancy. Am J Clin Nutr 2000; 71 : 1334S - 43S.

41. Bhaskaram P. Zinc deficiency. Indian Pediatr; 32:1153-1156.

42. Hunt, I.F. Murphy, N.J., Cleaver, A.E. Faraji, B., Swendseid, M.E., Browdy, B.L.,

Caulson, A.H., Clark, V.A., Selttage, R.H. & Smith, J.C., Jr. Zinc supplementation during pregnancy in low-income teenagers of maxican descent : effects on selected blood constituents and on progress and outcome of pregnancy. Am J Clin Nutr 1985; 42(5) : 815-28.

43. Lazebnik, N., Kuhnert, B.R. & Kuhnert, P.M. Zinc, Cadmium and hypertension in parturient women. Am J Obstet Gynecol 1989;161:437- 440.

44. Bassiouni, B.A., Foda; A.I. & Rafei A.A. Maternal and fetal plasma zinc in preeclampsia. Eur J Obstet Gynecol Reprod Biol 1979; 9:75-80.

45. Okonofua FE, Amole FA, Emofurieta WD Ugwu NC . Zinc and Copper concentration in plasma of pregnant women in Nigeria. Int J Gynaecol Obstet 1989; 29(1) : 19-23.

46. Carl L.Keen, Michael S.Clegg, Lynn A.Hanna, Louise Lanoue, John M.Rogers, Geoge P Daston, Patrica oteiza and Janet Y.Uriu Adams. The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. J Nutr 2003; 133:1597S-1605S.

47. Coyle, P.Philcox, J.C. Carey, L.C. & Rofe, A.M. Metallothionein: the multipurpose protein. Cell. Mol. Life Sci 2002; 59 : 627-647.

48. Gordon PR, woodruff CW, Anderson HL, O' Dell BL. Effect of acute zinc deprivation on plasma zinc and platelet aggregation in adult males. Am J Clin Nutr 1982; 35 : 113 – 19.

49. Cherry FF, Bennett EA, Bazzano GS, Johnson LK, Fosmire GJ, Batson HK. Plasma zinc in hypertension / toxemia and other reproductive variables in adolescent pregnancy. Am J Clin Nutr 1981; 34(11) : 2367-75.

50. Kumru S, Adyni S, Simsek M., Sahin K, Yaman M, AyG. Comparison of serum copper, zinc, calcium and magneisum levels in preeclamptic and normal pregnant women. Biol Trace Elem Res 2003;94(2) :105-12.

51. V.K.Verma, V.Ramesh, Satyendra Tewari, R.K.Gupta, Nakul Sinha and C.M.Pandey. Role of Bilirubin, vitamin C and ceruloplasmin as antioxidants in coronary artery disease (CAD) .Indian Journal of Clinical Biochemistry 2005;

20(2) : 68-74.

52. Cochrane C G. Mechanisms of oxidant injury of cells, Mol Aspects Med 1999; 12 : 137 – 147.

53. John G.Reinhold. Trace Elements - A selective survey. Clin Chem 1975; 21/4 : 476-500.

54. Sheila C.Vir, A.H.G Love and W. Thompson, serum and hair concentrations of copper during pregnancy. The American Journal of Clinical Nutrition, 1981; 2382 – 2388.

55. Priyali Pathak, Suresh Kumar Kapoor, Umesh Kapil, Yogendra Kumar Joshi, Sada Nand Dwivedi. Copper nutriture amongst pregnant women in a rural area of India. Eastern Journal of Medicine 2003; 8(1) : 15-17.

56. Fay J, Carturight GE, Wintrobe MM. Studies on free erythrocyte protoporphyrin. Serum iron binding capacity and plasma copper during normal pregnancy. J clin Invest 1949; 28 : 487-91.

57. Johnson NC. Study of copper and zinc metabolism during pregnancy Proc Soc Exp Biol Med 1961; 108 : 518-19.

58. Martin Lagos F, Navarro - alarcon M, Terres - Martos C, Lopez Garcia de la serrana H, Perez-Valero V, Lopez - Martinez MC. Zinc and copper concentrations in serum from spanish women during pregnancy. Biol Trace Elem Res 1998; 61(1) : 61-70.

59. Anand S, Mittal R, Bhargava U, Vir SC. Copper status of Indian women of low socio-economic group and its effect on the outcome of pregnancy. Int J Vitam Nutr Res 1981; 51(4) : 410-5.

60. Fattah MM, Ibrahim FK, Ramadan MA, Sammour MB. Ceruloplasmin and copper level in maternal and cord blood and in the placenta in normal pregnancy and in preeclampsia. Acta Obstet Gynaecol Scand 1976; 55(5) : 383-5.

61. Borella P, Szilagyi A, Than G, Esaca I, Giardino A, Facchinetti F. Maternal plasma concentrations of magnesium, calcium, zinc and copper in normal and

pathological pregnancies. Sci Total Environ 1990; 99(1-2) : 67-76.

62. Zhao F. Ca, Mg, Cu and Zn contents of the maternal and umbilical cord serum in pregnancy induced hypertension (Article in Chinese) Zhonghua Fu Chan Ke Za Zhi. 1989; 24(4) : 212-4, 252.

63. Zehra Serdar, Esma Gur, Osman Develioglu. Serum Iron and copper status and oxidative stress in severe and mild preeclampsia. Cell biochemistry and function. May 2005.

64. Aksov H, Taysi S, Altinkayak K, Bakan E, Bakan N, Kumtepe Y. Antioxidant potential and transferin, ceruloplasmin, and lipid peroxidation levels in women with preeclampsia. J Investig Med 2003 Sep; 51(5): 284-7.

65. David B.Endres, Robert K.Rude, Mineral and bone metabolism, Tietz Textbook of clinical chemistry III Edition, Philadelphia, WB saunders 1999:1395-1457.

66. Hojo M, August P. Calcium metabolism in normal and hypertensive pregnancy. Semin Nephrol 1995; 15(6) : 504-11.

67. Lorrene D Ritchie, Ellen B Fung, Bernard P Halloran, Judith R Turnlund, Marta D Van Loan, Christopher E Cann and Janet C King. A longititudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. Am J Clin Nutr 1998; 67 : 693-701.

68. Gertner JM, Coustan DR, Kliger AS et al. Pregnancy as state of physiologic absorptive hypercalciuria. Am J Med 1986; 81:451-456.

69. Medical disorders in Obstetric Practice - Michael de Swiet. IV edition.

70. Lorrene D Ritchie and Janet C King. Dietary calcium and pregnancy - induced hypertension : is there a relation? Am J Clin Nutr 2000; 71 : 1371S-4S.

71. Pitkin RM., Gebhardt MP. Serum calcium concentrations in human pregnancy. Am J Obstet Gynaecol 1977; 127(7) : 775-8.

72. Reddy GS, Norman A.W. Regulation of vitamin D metabolism in normal human pregnancy. Obstet gynaecol 1983;88:170-173.

73. Varner MW, Crukshank DP, Pitkin RM. Calcium metabolism in the hypertensive mother, fetus and newborn infant. Am J Obstet Gynecol 1983; 147:762-5.

74. Huang HM, Leung PL, Sun DZ, Zhu MG. Hair and serum calcium, iron, copper and zinc levels during normal pregnancy at three trimesters. Biol Trace Elem Res 1999; 69(2) : 111-20.

75. Malas NO, Shurideh ZM, Does serum calcium in preeclampsia and normal pregnancy differ? Saudi Med J 2001; 22(10) : 868-71.

76. Jose Villar, Mario Merialdi, A.Metin Gulmezoglu, Edgardo Abalos, Guillermo Carroli, Regina Kulier and Mercedes de Ori. Nutritional Interventions during pregnancy for the prevention or treatment of maternal morbidity and preterm delivery. An overview of Randomized controlled Trials, American Society for Nutritional Sciences, 2003:1606S-1624S.

77. Jose villar and Jose M .Belizan. Same nutrient, different hypothesis : disparities in trials of calcium supplementation during pregnancy. Am J Clin Nutr 2000; 71 : 1375S-9S.

78. Jorge A. Prada, Richardus Ross, and Kenneth E. clark. Hypocalcemia and Pregnancy induced hypertension produced by maternal fasting. Hypertension 1992; 20:620-626.

79. Zemel MB, Zemel PC, Berry S. et al., Altered platelet calcium metabolism as an early predictor of increased peripheral vascular resistance and preeclampsia in urban black women. N Engl J Med 1990; 323 : 434-8.

80. Repke JT, Villar J. Pregnancy - induced hypertension and low birth weight : the role of calcium. Am J Clin Nutr 1991; 54 : 2376S - 41S.

81. Ajose A, Fasubaa B, Anetor JI, Adalakan DA, Makinde NO. Serum zinc and copper concentrations in Nigerian women with normal pregnancy. Niger Postgrad Med J 2001; 8(4) : 161-4.

82. Pathak Priyali, Kapil Umesh, Kapoor Suresh kumar et al. Prevalence of multiple micronutrient deficiencies amongst pregnant women in a rural area of Haryana. Symposium on micronutrient deficiency disorders 2004; 71:1007-1014.

83. David B.Milne, Trace elements, Tietz Textbook of clinical chemistry III Edition, Philadelphia, WB saunders 1999:1029-1055.