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Role of Micronutrients -"Selenium & Zinc" in Preeclampsia

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Abstract

Background: *Micronutrients are vitamins and minerals required in minute amounts for normal functioning, growth and development. Adequate maternal micronutrient status is especially vital during pregnancy and lactation. Micronutrient deficiencies are exacerbated in pregnancy, leading to potential adverse effects not only on the mother but also on fetus. Therefore, micronutrient status plays an important role in pregnancy and birth outcomes. Deficiencies of specific antioxidant activities associated with the micronutrients like Selenium and Zinc can result in poor pregnancy outcomes, including fetal growth restriction, preeclampsia and the associated increased risk of diseases in adulthood, including cardiovascular disease and type 2Diabetes.*

Aim*: To asses the status of Selenium & Zinc in preeclamptic women.*

Objective: *To prove Selenium & Zinc deficiencies as contributory factors for the onset of preeclampsia..*

Data Sources: *This is a prospective study done in the Department of Obstetrics and Gynaecology, Narayana Medical College & Hospital, Nellore for a period of 2yrs (October 2012 –October 2014).100 preeclamptic women, are recruited into the study (Study group). A similar number (100) of healthy pregnant women were taken as controls(Control group).*

Methodology: *Serum and tissue (Placenta) samples for SE and Zn are measured by atomic absorption spectrophotometry. The Statistical software namely SPSS 20.0 were used for the analysis.*

Results*: The mean serum & tissue SE and Zn levels are found to be significantly reduced in preeclamptic cases compared to controls.*

Conclusions*: Selenium and zinc supplementation either alone or in combination with a general multi-nutrient supplement may have a significant effect not only on the incidence of preeclampsia, but may also delay the onset and severity of the disease, ameliorating placental oxidative stress and buying valuable time for fetal development prior to delivery.*

Key Words: *Selenium-Zinc-Serum &Tissue Levels-Preeclampsia*

INTRODUCTION

Pregnancy-induced hypertension & preeclampsia (PE) are transient but potentially dangerous complications of pregnancy that affect approximately 5-10% of pregnancies worldwide. They account for 20-80% of the strikingly increased maternal mortality and 15% of preterm deliveries[1].Although many pathophysiologic factors have been implicated in their etiology, its specific etiology still remains obscure .Oxidative stress is generated during normal placental development. A number of micronutrients function as essential cofactors for or themselves acting as antioxidants. However, when supply of these anti oxidant micronutrients is limited or deficiencies exaggerated, oxidative stress occurs thus resulting in pregnancy disorders like preeclampsia. Selected micronutrient antioxidants like Selenium, via its incorporation into glutathione peroxidase enzyme and Zinc acting as co factor for superoxide dismutase reduces the oxidative stress and hence proved to be protective against preeclampsia[2].

AIMS & OBJECTIVES

To assess the status of Selenium & Zinc in Preeclamptic women

OBJECTIVE

To prove Selenium & Zinc deficiencies as contributory factors for the onset of preeclampsia.

DATA SOURCES

This is a prospective study done in the Department of Obstetrics and Gynaecology, Narayana Medical College & Hospital, Nellore for a period of 2yrs (October 2012 –October 2014).100 preeclamptic women, attending to the Obstetric department of Narayana Medical College & Hospital are recruited into the study (Study group). A similar number (100) of healthy pregnant women were taken as controls (Control group).

TYPE OF STUDY

Case control study.

INCLUSION CRITERIA

1) Diagnosed cases of preeclampsia
2) Age of patients between 18-39yrs
3) Gestational period >20 weeks.

EXCLUSION CRITERIA

Pregnant women with other comorbidities. like diabetes mellitus or gestational diabetes, chronic hypertension, renal, cardiovascular ,liver ,endocrine diseases and any other chronic illnesses, hydatidiform mole& malignancy, intake of vitamins, antioxidants during the current pregnancy are excluded. Exclusion was done by history, clinical examination and laboratory parameters.

PATIENT ANALYSIS

Detailed history (demography, complaints, period of gestation, past obstetric, medical and surgical) & clinical examination (complete general physical, systemic & obstetric).Apart from routine antenatal investigations, specific investigations like liver, renal function tests, coagulation profile, fundoscopy, 24 hr urinary protein were done in the study group..The biochemical tests pertaining to the present study done in common to both the

study and control groups are as follows: 1. Serum Selenium 2.Tissue Selenium .3.Serum Zinc 4.Tissue Zinc

METHODOLOGY

Blood sample collection

In fasting condition 5ml venous blood was drawn from each subject, transferred to a plain tube and serum was separated by centrifugation and stored at -20C to measure the analytes. Serum Selenium & Zinc are measured by atomic absorption spectrophotometry

TISSUE EXTRACTION

Maternal part of placental tissue of about 1g will be collected and washed with phosphate buffer [ph-4.0] and subjected to homogenization by using tissue homogenizer. The homogenate is subjected to centrifuge at 12000rpm to obtain supernatant. The supernatant is used for analysis.32.Tissue Selenium (µg/gm of protein) & tissue Zinc (µg/gm of protein) measured by atomic absorption spectrophotometry.

Principle

Five diluted serum samples are aspirated into the atomic absorption flame. The Zn/Se concentration is determined by comparing the signal from diluted serum with the signal from aqueous calibrations, which are prepared in a diluted glycerol matrix (5ml/dl) to stimulate the viscosity of diluted plasma. Same procedure employed with tissue supernatant also.

STATISTICAL METHODS

Continuous variables were expressed as means and standard deviations. Discrete variables were expressed as proportions. Significance is assessed at 5 % level of significance. Independent sample test has been used to find the significance of study parameters on continuous scale between two groups. Chi-square has been used to find the significance of study parameters on categorical scale between two or more groups.

STATISTICAL SOFTWARE

The Statistical software namely SPSS 20.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS

The present study is a hospital based prospective study conducted in the department of Obstetrics & Gynaecology, Narayana Medical College & Hospital. 100 preeclamptic women fulfilling the inclusion criteria were recruited into the study. Another 100 matched normal pregnant women were taken as controls and the levels of Selenium and Zinc in preeclamptic women were studied, taking the various parameters as follows:

Mean Serum Selenium levels - Study group Vs Controls

Normal serum Selenium levels in pregnancy are: 60-70(µg/l).Levels of ≤60 µg/l is taken as deficiency The mean serum Selenium of study group is found to be less (48.08 µg/l) compared to control group (71.96 µg/l).With a significant p value of <0.0001. 62% of preeclamptic women in the study group have levels of serum Selenium

≤50µg/l, whereas only 4% of them found to be in the normal range.

Mean Serum Selenium Vs Age & paritry

Low mean serum Selenium levels were found in the age group of 26 to 30 yrs. Compared to other age groups in the study population. Mean serum Selenium levels did not show any significant variations in relation to parity.

Mean serum Selenium – Mild Vs Severe Preeclampsia

30% of the study population were grouped under mild preeclampsia (≤160/110) & 70% as severe (>160/110). The mean serum Selenium levels in the latter group were observed to be lower (47.20 µg/l) than in former group (50.13 µg/l).

Mean Tissue Selenium - Study Group Vs Controls

The mean tissue Selenium levels in the study group were observed to be less (521.16 µg/gm of protein) when compared to that of control group (747.66 µg/gm of protein).As per the readings in the control group 600-700 µg/gm of protein is taken as normal and<600 µg/gm of protein is considered as deficiency.

Mean Tissue Selenium Vs Age

The mean tissue Selenium levels are found to be relatively less in 20-25 yrs age group when compared to others in the study population.

Mean Tissue Selenium Vs Parity

The lowest (504 µg/gm of protein) mean tissue Selenium levels were observed in third gravidas .The range in others is closely related.

Mean Tissue Selenium-Mild Vs Severe preeclampsia

Subjects of severe preeclampsia are having lower tissue Selenium levels, (520.69 µg/gm of protein) compared to the mild group (522.27 µg/gm of protein).

Mean Serum Zinc - Study Group Vs Controls

Normal serum Zinc levels in pregnancy are: 90-110µg/dl. Levels ≤ 90 µg/dl is taken as deficiency .The mean serum Zinc of study group was found to be less (71.82 µg/dl) compared to control group (99.02 µg/dl) with significant p value(<0.0001). 90% of the recruited study group were having serum Zn levels <80 µg/dl whereas rest of them were having values ranging between 81-90 µg/dl. None of them were found to be >90 µg/dl.

Mean Serum Zinc Vs Age

The mean Serum Zinc of the study group in various age groups had their levels within the range of 71-73 µg/dl .There appears to be not much variation among them.

Mean Serum Zinc Vs Parity

The mean serum Zinc values were found to be relatively lower in second (64.4 µg/dl) & third gravidas (69 µg/dl) compared to others.

Mean Serum Zinc-Mild Vs Severe preeclampsia

Subjects of severe preeclampsia had serum Zinc levels slightly lower (71.49 µg/dl) compared to the mild variety (72.6 µg/dl).

Mean Tissue Zinc-Study Group Vs Controls

The mean tissue Zinc of study group is found to be less than that of controls group (7.54 Vs 13.00 µg/gm of protein).54% of preeclamptic women had their tissue Zn levels <7 µg/gm of protein. Only 10% were observed to have >10.µg/gm of protein.

Mean Tissue Zinc Vs Age and parity

Mean tissue zinc levels were not found to be showing any gross variation with regard to age & parity groups.

Mean Tissue Zinc-Mild Vs Severe Preeclampsia

Subjects of severe preeclampsia had tissue Zinc levels relatively higher (7.63 Vs 7.33 µg/gm of protein) compared to the mild type.

Labor outcome-Term Vs Preterm

The percentage of preterm deliveries are more in the study group (20 %) when compared to controls (6%).

Mode of Delivery - Study group Vs Controls

The percentage of Cesarean sections were more in the study group when compared to controls(54% vs 38%). Specifically, the rate of emergency Cesarean sections were much higher than in controls (36% Vs 8%).

Neonatal outcome-APGAR score: Study group Vs Controls

Low APGAR scores were observed in 44% of study group compared to 10% in controls with a significant p value (<0.0001).

Maternal Serum levels (Se & Zn) Vs fetal Birth Weight (B.Wt)

Lower mean birth weights were observed (2.21 kg-2.34 kg) in both Selenium and zinc deficient mothers compared to those with higher levels .

Maternal Tissue Zinc Vs Fetal IUGR

As the mean Tissue Zinc levels are increasing, the percentage of IUGR babies is found to be decreasing .In those with < 8 µg/gm of protein, about 70% of babies showing growth restriction.

Maternal Serum & tissue Zinc Vs PROM

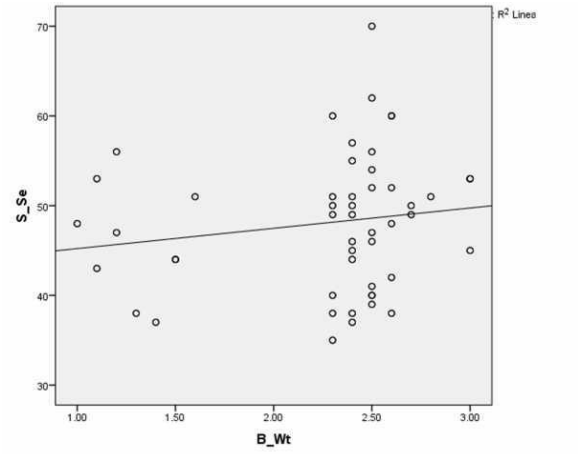
As the mean serum Zinc levels are increasing, the percentage of PROM is found to be decreasing .In women having levels less than 70µg/dl,

PROM occurred in 56% of cases compared to only 13% in those with >80 µg/dl. PROM was found in 81% of women with tissue zinc levels <8 µg/gm of protein. There were no cases of PROM in those with tissue Zinc levels >10µg/gm of protein.

Mat.Serum Se vs Fet.B.Wt

shows +ve correlation with no stat.signi.

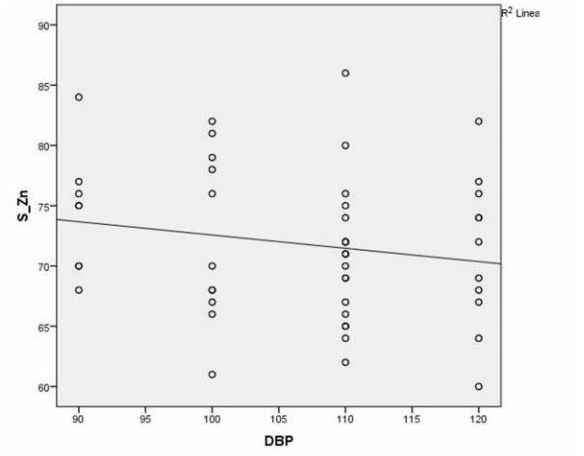
Fig1



Serum Zn vs degree of P.E.

shows -ve correlation with no stat.sig

Fig 2



DISCUSSION

Micronutrients have an important influence on the health of both mother and fetus. Deficiency of micronutrients during pregnancy may give rise to various complications like anaemia, preeclampsia, preterm labour and fetal growth restriction. PE is still one of the leading causes of maternal and fetal mortality in both developed and developing countries[3]. Despite extensive research, the etiology of preeclampsia is still one of the major unsolved mysteries in Obstetrics. There has been a great deal of interest in the use of antioxidant supplementation as a preventive stratergy against the uncontrolled production of reactive oxygen species in PE. It is speculated that pregnancy will progress uneventfully if adequate antioxidants exist to buffer ROS[4,5]. The role of Selenium & Zinc in the development and progression of preeclampsia is gaining favour in line with oxidative stress hypothesis.

The scarcity of studies concerning maternal trace elements status during pregnancy and the inconsistent findings from the published studies, led us to the present case-control, hospital based study- "The maternal serum and tissue levels of Selenium and Zinc and their role in preeclamptic women."

The study comprises of 100 preeclamptic women matched with 100 normal pregnant women. Numerous reports implicate Se deficiency in several reproductive and obstetric complications including male and female infertility, miscarriage, preeclampsia, fetal growth restriction, preterm labor, gestational diabetes, and obstetric cholestasis[6] The trace element Selenium is an essential component of antioxidant selenoproteins, Glutathione peroxidases, Thioredoxin reductases which remove the unwanted products by hydroperoxides & oxidised lipoproteins[7] thus preventing the oxidative stress. Therefore, decreased Selenium concentration reflects the deficiency that lead to oxidative stress. In the study of Mistry et al[8]& Atmar et al[9], the mean maternal Selenium levels are low in preeclampsia when compared to normal pregnant women. In our study also, the levels are found to be consistently low & are highly significant ($p<0.0001$). Levels of Se in maternal serum and plasma might be lower, partly, because of increased blood volume and therefore increase in hemodilution during pregnancy (Rayman, 2002)[10].In our study, 96 preeclamptic women were having Selenium levels of <60 µg/L (Normal >60 µg/L) whereas only 4 of them had normal levels. There are no reference studies available supporting the age & parity parameters in relation to Selenium till date. But, In our study, Serum Selenium vs Age: the relation of mean serum Selenium levels with regard to age has been estimated and were found that almost all women in study population had levels ranging from 44 - 50 µg/L. But, about 30 preeclamptic women below the age of 25 years were found to have considerably less values (35-37µg/L). This obviously reveals Selenium deficiency is more in younger preeclamptics. Serum Selenium vs Parity: As far as parity is concerned, the mean serum Selenium levels did not show significant variation between preeclamptic primigravidas & multigravidas. But, among the primigravidas, 10 members had much lower levels of Selenium(<40 µg/L) compared to multigravidas, supporting the fact that preeclampsia occurs mostly in

primigravidas. Our study has also shown that very low levels of Selenium are found mostly in primis compared to multis. Women having low Selenium levels in the initial trimesters itself reflect childhood or adolescent deficiency. They should be corrected preconceptionally to reduce the incidence of pregnancy induced hypertension. Our study population was divided into two groups based on the blood pressure recordings as mild (<160/110) PE & severe (≥160/110) PE. The mean serum Selenium levels in severe PE were significantly lower (47.20 µg/l) than in mild group (50.13 µg/l). Around half (35) of the preeclamptic women having severe PE had levels<45 µg/l which signifies that more the decrease in Selenium levels the more the severity of preeclampsia. Rayman et al (2003)[11] studied Median toe nail Selenium concentrations and found that lower Selenium status was significantly associated (P=0.029) with more severe expression of disease. Most of the cases of severe preeclampsia reported to us in later gestational ages with obvious placental insufficiency leading to increased oxidative stress. If this oxidative stress was buffered in the early gestational period itself, preeclampsia probably wouldn't have progressed to severe degree. Prophylactic supplementation of Selenium during the antenatal period was done by Tara et al.,(2010)[12] who conducted a RCT with 166 pregnant women from their first trimester until delivery were supplemented with 100µg Selenium or a placebo to investigate the risk of preeclampsia .Tara et al showed that in those with significantly increased plasma Se levels significantly decreased incidence of pre eclampsia was noted ,but the difference was not statistically significant. The "Selenium in Pregnancy Intervention Trial 2014" (SPRINT)[13] done in the UK, jointly by the University of Surrey and Oxford. This is a small RCT of Selenium supplementation (60 µg a day) to the pregnant women. It is not powered to demonstrate clinical benefit but will provide insight into the impact of Selenium supplements on laboratory measurements of circulating factors that are relevant to the development of preeclampsia. This study has also reported that adequate supplementation of Selenium has reduced incidence of preeclampsia. On the other hand, increased levels of plasma Se have been observed in patients with PE compared to controls (Mahomed et al., 1998). Also, median maternal leucocytic Se was 15% higher among pre-eclamptic women compared to normal pregnant (Mahomed et al 2000)[14]. Further, 18% increased levels of Se was found in study of Gromadzinska et al., 1998[15]among PE cases compared to their normal pregnant controls. At the same time, there was no observed significant difference as regard to Se levels between preeclamptic women and normal pregnant controls (Rayman et al.,1996)[16]. Interestingly, a recent publication has shown a genetic association between Selenoprotein S and the development of preeclampsia and has postulated a role for this selenoprotein in the exacerbated inflammatory response associated with development of the disease[17].

Placental Selenium Vs Preeclampsia

The placenta appears to be the principal source of free radical synthesis. A pivotal role of enhanced placental superoxide generation leading to oxidative stress (OS) is increasingly recognized

(Hubel,1999[18] and Raijmakers et al., 2004b)[19]. Placental OS has been shown to be a key feature in the pathogenesis of PE. This is the reason we have selected placenta to estimate the tissue Selenium levels in our study. There are no available studies in the literature that has examined the relation of placental Selenium with preeclampsia till date. In the present study tissue (placenta) levels of Selenium were observed and compared with levels of normal placenta taken as controls. As per the readings in the control group, 600-700 µg/gm of protein is arbitrarily taken as normal.<600 µg/gm of protein is considered as deficiency. The mean tissue Selenium levels in the study group were observed to be less (521.16µg/gm of protein) when compared to that of control group (747.66 µg/gm of protein) with a highly significant p value (<0.0001).Around 40 preeclamptic women in the study group had much lower levels of tissue Se (<500 µg/gm of protein).

In our study, Tissue Selenium vs Age

Mean tissue levels in different age groups were studied & observed lower mean levels (513.06 µg/gm of protein) in 20-25 years of age group. 32 women in this age group had much lower levels of tissue Selenium in the range of 440-480 µg/gm of protein .Surprisingly, very low serum Selenium levels were also found in the same age group and they were found to be in the severe preeclampsia category indicating severe Selenium deficiency in the younger mothers may increase the severity of preeclampsia.

Tissue Selenium Vs Parity

In relation to parity, lower mean tissue Selenium levels were found in multigravidas (504 µg/gm of protein) than in primigravidas(518.14 µg/gm of protein). 50% of these primigravidas had very low tissue Se levels (440-480 µg/gm of protein) who landed to the hospital with severe preeclampsia, four of them were admitted as impending eclampsia and two of them needed obstetric ICU management. This again strongly supports severe Selenium deficiency go hand in hand with severity of preeclampsia, especially in these young primigravidae failing to combat oxidative stress leading to preeclampsia.

Tissue Selenium –Severity of preeclampsia

Mean tissue Selenium levels were studied in relation to severity of preeclampsia and we observed that subjects of severe preeclampsia are exhibiting lower tissue Selenium levels (520.69 µg/gm of protein) compared to the mild group (522.27 µg/gm of protein) thus causing reduced activity of GSH-Pxs ,associated with increased generation of toxic lipid peroxides contributing to the endothelial dysfunction and hypertension of PE (Mistry et al.,2008)[8].Thus, severity seemed to increase with decrease in Selenium levels.

Zinc

Plays an important role in many biological functions including protein synthesis, cellular division & nucleic acid metabolism. Poor maternal Zinc status has been associated with fetal loss, congenital malformations, IUGR, reduced birth weight, prolonged labor & preterm deliveries. In studies of Brito et al[20]., Akhtar et al[21]& Ilhan et al[22]., the serum Zinc levels were low in preeclamptics, compared to normal pregnant women and the difference was statistically significant. Similar studies were

conducted by Bahordan et al[23].,Kolusari et al[24]. ,Lou et al[25].,& Atmar et al[9]., also observed similar results but the difference was not statistically significant. In par with above studies, the results of our study also showed that mean serum Zinc levels are low in study group (71.82±5.98 µg/dl) compared to controls(99.02±7.40 µg/dl) and this difference was statistically very highly significant.

Serum Zinc vs Age

90% of the recruited study group were having serum Zn levels <80 µg/dl, among which 38 members had much lower levels <70 µg/dl(normal>90 µg/dl). Zinc plays an important role in all ages starting from fetus for embryonic growth and proper neurodevelopment to varied functions in adults. Mean serum Zinc levels were studied in relation to age and observed that the levels did not show any gross variation among different age groups and were in range of 71-73 µg/dl. Among the 38 members having lower Zinc levels,32 of them were in 20-25 years age group & 6 in between 26- 30 years. Among these 32, 18 of them are included in severe preeclampsia.

Serum Zinc Vs Parity

Mean serum Zinc levels were studied with regard to parity and found that levels were low in multigravidas compared to primis .The lowest level recorded was 60µg/dl. Among the 38 women with lower levels, 28 were primigravidas & the rest were multigravidas, thus reinforcing the statement that preeclampsia is more common in primigravidae and Zn deficiency may be contributing to the menace.

Serum Zinc Vs Severity of preeclampsia

Subjects of severe preeclampsia had serum Zinc levels slightly lower(71.49 µg/dl) compared to the mild variety (72.6 µg/dl).Serum Zinc levels showed a negative correlation(r=-0.105) with severity of preeclampsia i.e with decreased concentrations of serum Zinc, the severity of preeclampsia is increasing & vice versa,but with statistically insignificant p value(p=0.297).On the other hand, not all researchers agree that maternal Zn decreased during pregnancy (Prema, 1980; Mahomed et al., 2000[26]; Borella et al.,2001[27] and Ajayi2002[28]). The median leucocytic concentration of Zinc is 31.0% higher in preeclamptics than controls (Mahomed et al., 2000). Also, Borella et al. 2001 reported that PE cases had a higher mean plasma concentration of Zn compared with controls, although this difference did not reach statistical significance (104.9±2.28 vs. 96.0±2.29 µg/dL, P> 0.05). Further, Ajayi (2002) in a study of Nigerian pregnant women showed that mean third trimester plasma Zinc concentrations were higher among women with PE compared with controls (142±3.71 vs. 101± 0.44µg/dl). In the study of Diaz et al[29] also the level of serum Zinc in preeclampsia was higher than that in normal pregnancy (110 vs. 99 µg/dl), but the difference was not significant.

Placental Zinc Vs Preeclampsia

As mentioned before, placenta is the main source of oxidative stress and reason for increased blood pressure in preeclampsia. This can be accepted by the fact that in most of the preeclamptic women the B.P. readings miraculously came down after delivery of the placenta. Studies relating tissue Zinc with preeclampsia are not available till date. In the present study tissue (placenta) levels of Zinc were observed and compared with levels of

normal placenta taken from controls. As per the readings in the control group, 10-15 µg/gm of protein is arbitrarily taken as normal. Less than10 µg/gm of protein is considered as deficiency. In our study the mean tissue Zinc levels in the study group were observed to be less (7.54 µg/gm of protein) when compared to that of control group (13µg/gm of protein) with a highly significant p value (<0.0001).

Tissue Zinc Vs Age & Parity

Mean tissue Zinc levels, on comparison with age did not show any gross variation among different age groups. Lower mean tissue Zinc was found in women with 26-30 years(7.00 µg/gm of protein) & ≤19 years (7.25µg/gm of protein).The mean tissue Zinc levels did not show any obvious variation with regard to parity. Low levels of Zinc if corrected in adolescent age groups itself with Zinc rich diets or supplements may help to prevent future maternal & fetal complications due to preeclampsia.

Tissue Zinc vs Severity of preeclampsia

Zinc deficiency has been related to with preeclampsia since the 1980s including adolescent pregnancies. Its deficiency, as a possible risk factor for preeclampsia, is questionable and the results of latest studies showed that lack of Zinc causes increased lipid level and peroxidation, which suggest the possibility of the role of Zinc deficiency in preeclampsia .In contrast, the mean tissue Zinc levels in our study were found to be relatively higher in severe preeclampsia (7.63 µg/gm of protein) compared to mild group in contrast to serum Zinc levels. The possible explanation for this is still like an enigma. But it can be explained by the active transport of Zn from the deficient mother via Placenta to the fetus(parasite) in a similar fashion as Iron from anaemic mother.

Labor outcome: Selenium & Zinc deficiency

Micronutrients (Selenium & zinc) are definitely expected to influence labour outcome apart from pregnancy complications.

Selenium & Zinc def.Vs Preterm birth

Dobrzynski et al. 2008, Nassiet al. 2009, Mentro et al. 2005 conducted studies to understand the Selenium influence on preterm deliveries and summarised that increased Se protects against preterm births. Similar to this, the results of our study showed that 20% of study group had preterm births when compared to only 6% in controls. This difference might be due to decreased antioxidant micronutrient levels and in turn preeclampsia. This is in correlation with the studies conducted by Rayman et al[30] 2011 to understand the maternal Selenium status in relation to preterm birth and concluded that having low serum Selenium was related to preterm birth and was independent of the mother having preeclampsia.

Zinc is essential for normal immune function. During pregnancy Zinc deficiency alters circulating levels of a number of hormones associated with the onset of labor. For example, lower levels of serum progesterone and prolactin concentrations in Zinc-deficient ewes was associated with preterm deliveries. Also, systemic and intra-uterine infections are a major cause of preterm birth. Several plausible explanations for the positive effect of Zinc on preterm births exist. Zinc supplementation may reduce the incidence or the severity of maternal infections, lowering the

risk of preterm birth. In a meta-analysis done by B.W.Chaffee et al[32]., 14% reduction in preterm birth with Zinc supplementation, was observed. Zinc is involved in more than 300 enzymes and acts as a stabilizer in molecular structure of the subcellular constituents and maintains membrane integrity. Increased generation of ROS as well as antioxidant deficiency may play an important role in the pathophysiology of PROM, which has been associated with enhancement of collagen degradation & subsequent damage to fetal membranes .In a study conducted by Sikorski et al. the mean maternal Zinc index in patients with PROM was significantly lower than that of the control women(p=0.0002). In the present study, occurence of PROM was found to be low with increasing levels of both serum & tissue levels of Zinc. 56% of cases with PROM had maternal serum Zinc levels <70 µg/dl, 31% between 71-80 µg/dl & only 4% had normal levels. Coming to tissue levels,81% of them had below 8 µg/gm of protein & 19% of them had between 9-10 µg/gm of protein.

Mode of delivery: Study group Vs Controls

Mistry et al (2008),conducted a study in which 44% of preeclamptics &15% of normal women delivered by EmLSCS. The results of our study are consistent with this, showing 36% in study group Vs 8% in control group. This increased rate of Cesarean section in cases is due to worsening maternal or fetal conditions like uncontrolled preeclampsia, antepartum haemorrhage, HELLP syndrome or fetal distress as a consequence of PE. We have also observed in our study an increased rate of instrumental deliveries (16% Vs 4%) in preeclampsia group, when compared to controls, mostly due to uterine inertia. Micronutrient deficiency may have role in the maternal and fetal performance during labour thus resulting in higher rates of Caesarean and instrumental deliveries.

Neonatal Outcome

APGAR Score

Study groups Vs controls :In a study conducted by Kolusari et al., the babies born to preeclamptic mothers had less APGAR score than controls with a stastical difference of p<0.005. In the present study, normal &low APGAR scores noted in the babies of study group were 56% & 44% respectively the corresponding figures for the control group babies were 90% Vs 10% with a statistically significant p value (p <0.0001).The predominant reason for low Apgar scores in the study group babies was found to be prematurity and low birth weight which in turn reflects on the need for termination of pregnancy in some preeclamptics keeping in view of the maternal safety.

Birth weight

Neonatal birth weight is one of the best markers of a favourable pregnancy outcome and determinant of neonatal prognosis .In our study Selenium levels & fetal B.Wt. are found to be positively correlated with lower mean B.Wt. babies being born to Se deficient mothers. Zinc is an essential element for function of many enzymes and growth hormones during pregnancy; therefore it can play an important role on fetal growth. For example, during pregnancy, placental alkaline phosphatase needs Zinc to induce DNA synthesis and cell proliferation[34]. Goldenberg *et* al. completed a randomised double-blind placebo-controlled trail

of Zinc supplementation (25mg per day) during pregnancy from 19th-week of gestation .Those given Zinc supplementation has led to significantly greater fetal birth weight and increased head circumference compared to the placebo group highlighting the importance of adequate Zinc supply during pregnancy for both maternal and fetal well being. Kirksey et al[35] revealed a significant correlation between maternal plasma Zinc concentration measured at mid pregnancy and birth weight. Negger et al.[36], reported that the prevalence of low birth weight infants was significantly higher (eight times) among women with serum Zinc concentrations in the lowest quartile in early pregnancy. In the present study, Zinc deficient women had less mean fetal birth weight (2.0-2.2 kg) when compared to controls (2.5-2.8kg). Mean fetal birth weights of women with low (<10 µg/gm of protein) tissue Zinc levels were 2.0 -2.2 kg ,thus reflecting the importance of zinc in fetal growth promotion.

IUGR

The most common definition of IUGR is the weight of the fetus below 10th percentile for gestational age or a birth weight two standard deviations below the mean for gestational age[37].IUGR has great significance due to it's untoward effects on fetal well being with long term sequelae.

Reports of Selenium concentrations with regards to fetal growth restriction are inconsistent. Strambi et al[38]., demonstrated that in 81 SGA (both term and preterm) subjects from Italy, infant plasma Selenium concentrations were significantly lower compared to those with adequate for-gestational-age (AGA) infants. Our study has shown that, as the mean serum Selinium & Zinc levels are increasing ,the percentage of IUGR babies is found to be decreasing .In women having Zn levels less than 70 µg/dl ,50% of babies were of IUGR when compared to only 10% in those with >80 µg/dl .Similar findings were observed with low levels of tissue Zinc. In those with < 8 µg/gm of protein, about 70% of babies had growth restriction. A recent meta-analysis evaluating the effects of antenatal multi – micronutrient supplementation on pregnancy outcomes has revealed a significant reduction in various complications of pregnancy

CONCLUSIONS

Rationale for multi-micronutrient supplementation

Multiple-micronutrient deficiencies often coexist and there is an increased interest in evaluating the benefit of multiple-micronutrient supplements in pregnancy. Considering that there may be multiple deficiencies in developing countries and it is difficult to evaluate the effects of all the potentially important micronutrients, as well as their possible interactions, have lead some to conclude that a multivitamin mineral supplement should be given during pregnancy (UNICEF 1999). Combining multiple micronutrients in a single delivery mechanism has been suggested as a cost-effective way to achieve multiple benefits.

The controversy: interactions and side-effects of over-dosage

Some authors have questioned the effectiveness of multi-micronutrient supplements due to possible interactions among nutrients resulting in their

impaired absorption. Studies have shown that high doses of Iron impair the absorption of Zinc and vice versa. Manganese affects Iron absorption in a way that indicates that the intestine cannot differentiate between Manganese and Iron. Similarly, high dose Zinc supplements (50 mg per day for 10 weeks) reduce indices for Iron and Copper status. Calcium was shown to have a depressing effect on Iron absorption. Clearly substantial evidence is required before the multi micronutrient supplementation programs are implemented on a global scale.

Selenium and zinc supplementation either alone or in combination with a general multi-nutrient supplement may have a significant effect not only on the incidence of preeclampsia, but may also delay the onset and severity of the disease, ameliorating placental oxidative stress and buying valuable time for fetal development prior to delivery.

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