Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.

Thesis submitted for the award of the degree of
Doctor of Philosophy
in
Community Medicine

By
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DECLARATION BY THE CANDIDATE

I declare that the thesis entitled **"Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study."** submitted by me for the degree of Doctor of Philosophy (PhD) is the record of work carried out by me under the guidance of **Dr. Rekha S Udgiri**, Professor of Community Medicine , BLDE (Deemed to be University)'s Shri B. M. Patil Medical college, Hospital and Research Centre, Vijayapura, Karnataka, and **Dr. Ashalata Mallapur**, Professor and HOD of OBG, S. Nijalingappa Medical College, Bagalkot, Karnataka, and has not formed the basis for the award of any degree, diploma, associateship, fellowship, titles in this university or other similar institution of higher learning.

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CERTIFICATE FROM THE HEAD OF THE INSTITUTION AND THE DEPARTMENT

This is to certify that the thesis entitled **"Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study."** submitted for the degree of Doctor of Philosophy (PhD) by is the record of research work carried out by **Dr. Manjula R** under the guidance and supervision of **Dr. Rekha Udgiri**, Professor of Community Medicine, BLDE (Deemed to be University)'s Shri B. M. Patil Medical college, Hospital and Research Centre, Vijayapura, Karnataka; and **Dr. Ashalata Mallapur**, Professor and HOD of OBG, S. Nijalingappa Medical College, Bagalkot, Karnataka; in partial fulfillment for the award of Doctor of Philosophy in the faculty of Medicine and that this work was carried out by her in the Department of Community Medicine.

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"Learn everything that is good from others but bring it in, and in your own way absorb it; do not become others."
- Swami Vivekananda

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LIST OF ABBREVIATIONS

Abbreviation	Full form
DNA	De-oxy Ribose Nucleic acid
Mt DNA CN	Mitochondrial DNA copy number
WHO	World health organization
Q-PCR	Quantitative Polymerase chain Reaction
BMI	Body mass index
Aod	Adjusted odd's Ratio
IOM	Institute of Medicine
GWG	Gestational Weight gain
BP	Blood Pressure
SGA	Small for Gestational age
Se	Selenium
Zn	Zinc
ROS	Reactive oxygen species
RDA	Recommended daily allowance
RAE	Retinal activity equivalents
FGR	Fetal growth restriction
NTD	Neural tube defects
ROS	Reactive oxygen species
IUGR	Intra-uterine growth restriction/Retardation
LBW	Low birth weight
RCT	Randomized Controlled trial
Fe	Iron
UNICEF	United Nations Children's Fund
FAO	Food and Agriculture organisation
BMJ	British Medical Journal
HIV	Human Immuno deficiency Virus

CPI-IW	Consumer Price Index Number for Industrial Workers
CLIA	Chemi-luminucence
ICP-MS	Inductive Couple-plasma MAss spectrometry
HPLC	High Performance Liquid Chromatography.
DDS	Dietary diversity score
ATP	Adenosine tri-phosphate
Ct number	Threshold cycle number

ABSTRACT

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.

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ABSTRACT

Background: Nutrient intake is important to the well-being of pregnant women and the fetus. Micronutrients are necessary for normal growth and development of the fetus and deficiencies have been found to be associated with intrauterine growth retardation and small for gestational age infants. The nutritive composition of a diet has important influence on mitochondrial health—nutrients provide the substrates to harvest energy in the form of adenosine triphosphate (ATP) to affect key cellular functions and processes. Micronutrients were known to stabilise the mitochondrial DNA which could be related to Birth weight. Hence this study was taken up with following objectives.

Objectives:

a. **Quantitative study:**

1. To estimate the micro nutrient levels such as serum Ferritin, vitamin B12, Vitamin A, Vitamin D, Calcium, Selenium and Zinc in apparently healthy term pregnant women(≥37 weeks) and to study the causes for nutritional anemia in this area.

2. To estimate the maternal mitochondrial DNA copy number in term pregnant women using Q-PCR technique.

3. To study the correlation between the different micronutrient levels with Dietary diversity and maternal mitochondrial DNA copy number among them.

4. To study the effects of different micronutrient levels and maternal mitochondrial DNA copy number on the birth weight of the baby.

b. Qualitative study:

1. To explore the cultural beliefs and practices related to diet and nutrition in pregnancy through focus group discussions among term pregnant women, Care givers like mothers and mother-in laws .

Methods: After obtaining ethical clearance, present study was conducted in 150 apparently normal term (>37 completed weeks) pregnant women at tertiary care centre. Informed consent was obtained. Data was collected using the pre-designed questionnaire. Collected 5ml of venous blood under aseptic precaution and they were stored in Central Research Lab at -20 Degree. After serum separation, Micronutrients estimations and mitochondrial DNA copy number was done using Q-PCR technique. Complete Blood Count(Using Penta ES 60 cell counter) Serum ferritin, serum Vitamin B12 and Vitamin D (SNIBE Maglumi 1000 autoanalyser, which works on the principle is Chemiluminucence method (CLIA). Zinc and Calcium was estimated using a Fully automated analyser BA-400 of Bio-system company. Selenium by inductive couple plasma mass spectrometry (ICP-MS). Vitamin A By chromatographic methods using HPLC method.

Results: In the present study Dietary diversity, Calorie intake, protein intake, Gestational weight gain was associated with Birth weight of the baby, Dietary Diversity and protein intake was found to be statistically significant($p<0.05$). There was statistically significant positive correlation between maternal serum zinc, serum calcium and serum folic acid with dietary diversity with correlation co-efficient of 0.183, 0.192 and 0.21 respectively There was a statistically significant negative correlation between Maternal mitochondrial DNA copy number and birth weight of the baby ($r=-0.25$, $p=0.002$). There was a statistically significant positive

correlation between maternal serum micronutrients such as ferritin (r=0.17, p=0.035), Vitamin D3(r=0.21,p=0.008), Calcium (r=0.16, p=0.04), and folic acid (r=0.23, p=0.004). Serum Ferritin, Vitamin D, Selenium, Zn, Calcium, Folate levels were significantly lower in mothers of low birth weight baby when compared with normal weight baby. Antioxidant micronutrients Se, Zn, Vitamin D was negatively correlated with Mt DNA CN. There is a reduced affordability for the protective food items which are rich in micronutrients. There is a high calorie malnutrition and hidden hunger noted in our study group. Dietary diversity is an important factor which determines the adequate micronutrient intake. It should be increased by incorporating the food items rich in micronutrients in pregnancy. There was a less compliance for IFA supplementation.

Conclusion: There is an association between micronutrients and Birth weight of the baby. Only improving Dietary diversity is not going to help in improving the micronutrient levels, but proper planning of menu with good representation of protective food could help. Since there is lot of prejudices in the food intake in pregnancy, nutrition education could help. Micronutrient Supplementation could have questionable benefit, since the compliance for tablet intake was very poor. Food fortification could be helpful in improving the micronutrient supplementation and good birth weight of the baby. Mitochondrial DNA copy number gave the objectivity for the present study at the molecular level, could have influenced by the micronutrients and in turn affect the birth weight of the baby.

Keywords: TERM PREGNANT, MICRONUTRIENTS, BIRTH WEIGHT, MITOCHONDRIAL DNA COPY NUMBER.

INTRODUCTION

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methowds Research Study.

CHAPTER 1.

INTRODUCTION

Nutrients intake is the important aspect for the health of pregnant women and the fetus. Insufficient nutrient/food intake may cause maternal anaemia, which increased the risk of bad pregnancy outcome, causing maternal morbidities and mortality, leading to intrauterine fetal growth retardation(IUGR) causing low birth weight (LBW). Birth weight determines the childs survival, is considered as an important health indicator for knowing the utilization of public health infrastructure, public health services and maternal health outcome. Low birth weight is known to contributes to 40-60 percent of infant mortality globally[1,2].

Micronutrients are necessary for normal growth and development of the fetus and its deficiencies had been associated with intrauterine growth retardation leads to small for gestational age (SGA). Micronutrients have many functions such as antioxidant process, interaction with intercellular signaling protein transcriptional regulation, cell proliferation etc[3]. Micronutrient deficiencies during pregnancy are a global public health problem, but the quantity of the burden and health consequences are not clear, because of infrequent and inadequate assessment. Women in low income and developing countries often enter pregnancy malnourished and the nutritional demands of pregnancy, would jeopardise micronutrient deficiencies with health consequences of herself and developing foetus. Unlike protein-energy undernourishment the health hazards of micronutrient deficiency are not always clinically visible; it is hence considered as hidden hunger, which is synonymously used for micronutrient deficiency. Population based studies in South-east Asia including India, Bangladesh and Nepal have reported deficiencies of Zinc (15-74%), Vitamin B12(19-74%), Vitamin E(α-tocopherol) (50-70%), Folate(6-26%) and Vitamin D Deficiency in (60%) in pregnant women[4].

Some studies done in the past assessed nutritional status based on only calories and protein intake in pregnant women. However recently there are few studies were done that indicates the dietary diversity score, which could be better indicator for assessing the nutritional status. The nutrition intake both in quality and quantity are very important during pregnancy. Balanced diet should include the food items from most food groups, thus increasing the diversity, which has better pregnancy outcome. Hence recent dietary guidelines on pregnancy nutrition has emphasized more on the importance of dietary diversity[5]. Dietary diversity means food consumptions from different groups of food guide pyramid**.** Diet diversity means a qualitative indicator of food consumed from the different food groups.The dietary diversity also reflects availability of variety of foods in household and hence it indicates for nutrient adequacy of the diet [6,7]. Pregnant women consuming food with Low dietary Diversity Score was associated with low birth weight[8].

In our country, weight measurements and nutrition assessments are not routinely done in the public health sectors, except for the estimation of hemoglobin. These malnourished pregnant mothers, if detected early, can improve maternal and new born health through nutrition supplementation in Anemia during pregnancy, which is a public health concern especially in developing countries. Nutrition anaemia is was observed to be correlated with adverse maternal and perinatal outcomes. Anemia is considered of a major public health importance if the prevalence rate is >40% according to WHO. The etiology for nutritional anemia in pregnancy are multi-factorial: these includes micro nutrient deficiencies such as iron, folate, Vitamin B12 and vitamin A deficiency. Parasitic infections like hook worm and Malaria infections are also contributing for anaemia. Certain chronic infections also contributes for Anaemia in pregnancy. In fact Anemia in pregnancy because of iron deficiency is

only in 50 percent of cases, rest all are because of deficiency of other micro nutrients. But the accepted practice was to supplement with IFA tablets during pregnancy till recently[9].

Fetal growth is totally dependent on maternal nutrition through the placenta. The small membrane permeable molecules such as oxygen (O2) and carbon dioxide (CO2) transport are influenced by umbilical gradient/concentration blood flow and placental structure. The larger nutrient molecules like amino acids, fatty acids and glucose are transported through placenta depends on the nutrient transporter proteins. The placental capacity of nutrient transport is influenced by various factors, that includes hormones, micronutrients and the placental function. Furthermore, placental oxidative stress placenta has been found to influence the nutrients transport by altering the gene expression of different nutrient transporter proteins (e.g., glucose and amino acid; 10).

Maternal leukocyte mitochondrial factors was found to be modulated by micronutrients components and optimize the mitochondrial function thereby improve the fetal outcome. Mitochondria have been reported to modulate the major stress response pathways and alterations in the leukocyte mitochondrial DNA copy number (mtDNA-CN) in adults. Maternal CN sensitive to energy demand and oxidative stress is needed to optimize mitochondrial function[11]. MtDNA-CN has been reported to correlate inversely with pregnancy outcomes [11]. Micronutrient deficiency has been shown to reduce the mitochondrial efficacy, leading to compensatory increases in mtDNA-CN. Optimum micronutrient levels in blood could reduce the oxidative stress and promote efficient mitochondrial function and protect against oxidative stress, both of which reduce mtDNA-CN[11-16].

Micronutrient deficiencies in pregnancy highly prevalent globally. This hidden hunger research in pregnancy has been the recent topic of interest. Micronutrient supplementation may be a cost-effective intervention to reduce the Low birth weight & adverse maternal outcomes. Antenatal micronutrients supplementation are not recommended as there are dangers associated with Vitamin A, D and B6. The child health and nutrition research initiative have taken up a initiative to enourage the research in this area of micronutrient deficiencies and its impact on the birth weight. [17]

Hence this study has been done to study the effects of Micronutrients on Mitochondrial DNA Copy number in turn its effects on birth weight among apparently healthy term pregnant women.

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OBJECTIVES

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.

CHAPTER 2.
OBJECTIVES

OBJECTIVES OF THESTUDY:

a. Quantitative study:

1. To estimate the micro nutrient levels such as serum Ferritin, vitamin B12, Vitamin A, Vitamin D, Calcium, Selenium and Zinc in apparently healthy term pregnant women(≥37 weeks) and to study the causes for nutritional anemia in this area.
2. To estimate the maternal mitochondrial DNA copy number in term pregnant women using Q-PCR technique.
3. To study the correlation between the different micronutrient levels with Dietary diversity and maternal mitochondrial DNA copy number among them.
4. To study the effects of different micronutrient levels and maternal mitochondrial DNA copy number on the birth weight of the baby.

b. Qualitative study:

1. To explore the cultural beliefs and practices related to diet and nutrition in pregnancy through focus group discussions among term pregnant women, Care givers like mothers and mother-in laws.

RESEARCH HYPOTHESIS:

- There could be an association between micro-nutrient levels and MtDNA-CN in apparently normal pregnant women, which in turn would affect the birth weight of the baby.
- Dietary diversity could be associated with the optimum micronutrient levels in pregnancy.

REVIEW OF LITERATURE

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.

CHAPTER 3
REVIEW OF LITERATURE

Nutrients intake is crucial for the health of pregnant women and developing fetus. Inadequate nutrients consumption during pregnancy will cause maternal anemia, which increases the risk for not only intrauterine growth retardation(IUGR) leads to low birth weight (LBW) but also maternal morbidities and mortalities[1]. Birth weight is a health indicator for assessing the status of public health infrastructure , health services availability and utilization, maternal health services and nutrition. Low birth weight causes upto 40-60 percent of infant mortality globally[1,2].

Micronutrients are necessary for normal growth and development of the fetus and deficiencies have been found to be associated with intrauterine growth retardation and SGA (small for gestational age) infants. Micronutrients have many functions antioxidant process, interaction with intercellular signalling protein transcriptional regulation, cell proliferation etc[3]. Micronutrient deficiencies during pregnancy due to inadequate intake are a major public health issue, but complete extent of their burden and health consequences are not clear, because the nutritional assessment is not done adequately always. Women from low income and developing countries often enter pregnancy already malnourished and the demands of gestation can increase micronutrient deficiencies with adverse pregnancy outcomes. Unlike protein-energy undernourishment the health effects of micronutrient deficiencies are not always visibly evident; it is therefore called as hidden hunger, which is synonymously used for micronutrient deficiency[3]. Population based studies in South-east Asia including India, Bangladesh and Nepal have reported deficiencies of Zinc (15-74%), Vitamin B12(19-74%), Vitamin E(α-tocopherol) (50-70%), Folate(6-26%) and Vitamin D Deficiencies in (60%) in pregnant women[4].

The studies done in the past assessed nutritional status based on only calories and protein intake among pregnant women. However recently there are few studies were done that indicates the use of dietary diversity score(DDS), which could be better marker of nutrition status assessment. The Nutrition intake interms of quality and quantity are very important. Healthy and balanced diets should include the food items from most food groups, thus increasing the diversity, which has better pregnancy outcome. Hence recent dietary guidelines emphasizes more on Dietary diversity (DD) in pregnancy nutrition [5]. The DD represents food consumptions from food pyramid guide and also each dietary group**.** The DD means a qualitative assessment of food consumption which are derived from the different food groups.The DD also reflects access to food groups variety and, hence a indirectly indicates for nutrient adequacy of the diet [6,7]. Pregnant women consuming food with Low DDS were found to be associated with low birth weight[8].

Maternal nutritional status can be assessed using routine anthropometric measurements such as weight and height, in turn calculation of body mass index, gestational weight gain, and mid-arm circumference. In few countries, it is compulsory to do these assessments, before giving nutritional counselling and dietary advices to the pregnant women. In our country, weight measurements and nutrition assessments are not routinely done in the public health sectors, except for the estimation of hemoglobin. These malnourished pregnant mothers, if detected early, can improve maternal and new born health through nutrition supplementation.

Anemia during pregnancy is public health concern especially in under developed countries; it is known to be associated with adverse pregnancy outcomes (maternal and neonatal adverse outcomes). Anaemia is considered as a severe public health importance if the prevalence rate is >40% according to WHO. The causes for

anaemia in pregnancy are due to multiple factors: which includes micronutrient deficiencies or iron, folate, Vitamin B12 and vitamin A deficiencies. Parasitic infections like hook worm infections and Malaria causes anaemia. Certain chronic infections also causes anaemia in pregnancy. Iron deficiency contributes to only 50% of cases of anaemia, rest of the anaemia cases are due to other micro nutrient deficiencies. In India, the policy was to supplement with mere IFA tablets during pregnancy till recently[9].

Dietary diversity: World health organisation (WHO) in 2017, advocates that micronutrients in pregnancy is important for growth of foetus. In India POSHAN ABHIYAN of NHM (March 2018) was emphasizes only on dietary diversity for achieving adequate intake of micronutrients. Few studies have correlated with dietary diversity and adequacy of Micronutrient intake[10]. The DD is the consuming of food derived from different food groups in a day, viz cereals and millets, pulses and legumes, roots and tubers, fruits , green leafy vegetables, other vegetables, milk products, Meat and egg, oils and fats, and the sugar and jaggery[11,12].

A study conducted by Kheirouri S, observed that DD was not associated with improved birth weight of the baby[13]. A Cohort study in Ghana by Osman SM, they concluded that the pregnant women, who consumed food with low diversity delivered low birth weight babies compared to those who consumed high diversity food[14].

A Systematic Review about nutrients intake, dietary diversity and nutritional status of pregnant women, was conducted by Ndung'u (2018)[15], they observed that higher dietary diversity score ensured the healthier diet and hence there were positive anthropometric outcomes. An another study also documented that there was a positive association between DDS in pregnant women and their gestational weight

gain. They also observed low BMI in pregnancy were associated with low dietary diversity score, nutrient intake and it significantly negatively influenced the pregnancy outcome [15].

A Study conducted in Ethiopia(2018), to study whether the undernutrition was determined due to Low dietary diversity score among pregnant women. This study stated that low dietary diversity score caused under-nutrition. They also found that the pregnant women who consumed food with low DDS were 2-4 times more likely to be malnourished, with AOR of 2.1 [16].

A study conducted in the Mumbai urban slums observed a statistically significant association between Dietary diversity score and the birth weight ($p<0.05$). [8].

A Cohort study in Norway (2014), found that any deviation in gestational weight gain (both more or less) compared to Institute of Medicine (IOM) recommendations were associated with adverse pregnancy outcomes. The Odds ratio for low birth weight increased when the weight gain during pregnancy (Gestational weight gain) were less than the recommendations [17].

A retrospective cohort study was conducted in China (2017), studied the influence of maternal body mass index in pre-pregnancy period and gestational weight gain on the birth weight. Compared with normal pre-BMI categories, pre-pregnancy underweight were associated with an increased odds ratio for low birth weight [18].

A study in Indonesia (2017), found that body mass index (BMI) in pre-pregnancy period, gestational weight gain(GWG) was found to be positively associated with the birth weight. Birth weight aMD -139, significantly low in women

with inadequate/less GWG, when compared to those with adequate/recommended GWG, while SGA aOR5.44 and for prematurity aOR is 3.55[19].

A study in China (2017), they studied the influence of gestational weight gain on birth weight. They found that neonatal birth weight is positively correlated with Gestational weight gain[20].

Micronutrients and Birth weight [21,22,23]

Micronutrients could influence in increasing the birth-weight of the baby by the following mechanisms.

a. By combating oxidative stress. (Se, Zn, Vitamin A, Vitamin D)
b. By one-carbon transfer for DNA synthesis and cell differentiation and organogenesis (Vitamin B12 and Folate)
c. By improving the oxygen and nutrient carrying capacity in blood.(Iron)
d. Mineralization of bones.(Calcium and Vitamin D)

Micronutrients are direct regulators of DNA stability and phenotypic adaptation by influencing the availability of methyl donors and mechanisms promoting DNA stability, thereby they serves as substrates, transcription factors and modifiers of gene expression, influencing the complex biological pathways involved in embryogenesis, as well as fetal growth and development.

Table 3.1: Micronutrients dietary sources and RDA in pregnancy[24]

Micronutrients	RDA	Dietary sources
Ferritin/Iron	100mg	Green leafy vegetables, Raisins, Jaggery, Millets, Legumes, Cashews, Red meat, Liver, Bean.
Vitamin B12	2.2mcg	Egg, Yogurt, Dairy products, Liver, Fish, Sea food, Pork
Vitamin A **(RAE-retinol activity equivalents) 1mcg Retinol=12 mcg of betacarotene**	770 mcg (RAE)	Meat, Fish, Dairy products, Eggs Green leafy vegetables, Orange and yellow fruits and vegetables.
Vitamin D	600IU	Dietary sources apart from Sunlight Egg yolk, Milk. Yogurt, Cheese, Fish , Diary products
Selenium	60mg	Sunflower seeds, Brown rice, Whole grains, Sea food, Shellfish, Salmon fish, Spinach, Cottage cheese, Turkey
Zinc	11mg	Cashews, Pumpkin Seeds, Whole grains, Lentils/ Legumes, Almonds, Egg, Meat.
Calcium	1300mg	Millets, Custard apple, Milk. Yogurt, Cheese, Fish, Diary products
Folate	0.5mg	Green leafy vegetables, legumes, nuts, liver

Ferritin

Ferritin is **a storage form of iron**. Red blood cells needs iron to form normally and carry oxygen and nutrients around the different parts of body. It is reduced in Iron deficiency anaemia. Hence its deficiency will leads to low birth weight. Iron supplementation is the policy, where every pregnant women is supplementation and found to be associated to good pregnancy outcome. A study by Goldenberg RL etal[25]. Studies have shown that IFA tablet supplementation in pregnancy in communities with increased prevalence of anaemia, there is an increase in birth weight following supplementation[26].

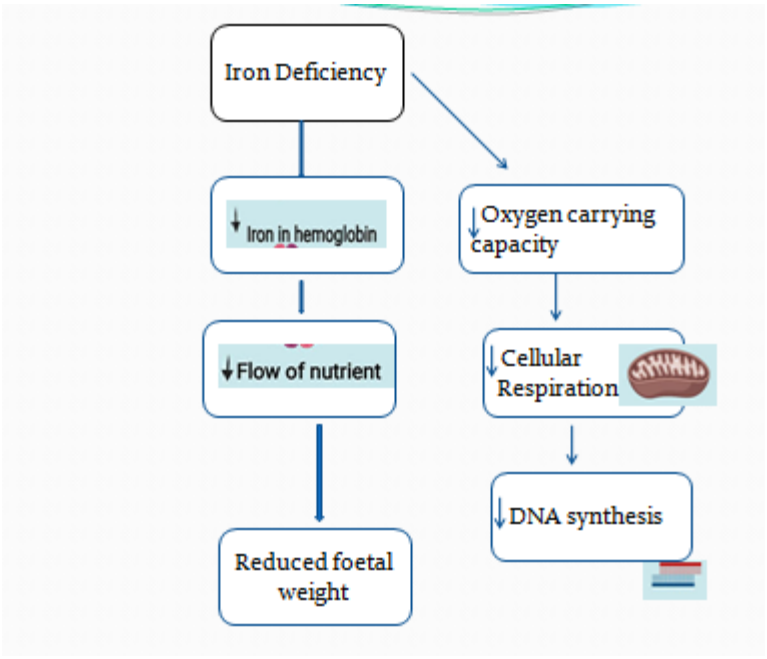

Figure 3.1: Mechanism of action of Iron[22].

A study in Kenya (2016), observed that 19 % of pregnant women were poorly nourished and about 16% were anaemic. There was positive correlation between the nutrients intake and nutritional status[27].

A cohort study in Madhya Pradesh(2016), found that calorie deficiency, protein deficiency and anaemia led to LBW babies. [28].

Vitamin B12 and Folic acid: These micronutrients is involved in the one-carbon transfer required for methylation of DNA, DNA synthesis and cell division, which is required for growth. Vitamin B12 and Folate is also required for heme synthesis, which will prevent anaemia in pregnancy. A cohort study conducted by Yuan X[29], observed adverse pregnancy outcome with Vitamin B12 and folate deficiency. Mishra, J and Yagnik CS, found that there was a positive correlation of maternal vitamin B12 and folic acid levels with birth weight of baby. In another study in Ireland found that, Dietary consumption of VitaminB12 and Folic acid were positively correlated with birth weight of the baby[30,31].

According to WHO, Nutritional anaemia is the most frequent nutritional deficiency disorder affecting more than a large number of pregnant women worldwide. During pregnancy, the need for erythropoiesis-related micronutrients are increased for feto-placental unit development [32,33]. Women entering the pregnancy with nutrition deficiency will jeopardize the situation, as there is an increased demand from growing foetus. These micronutrients, which are essential for erythropoiesis such as iron, folic acid, and vitamin B12 are known to cause nutritional anemia and its consequences.

Micronutrients like vitamin B12 and folic acid function as methyl donors in one-carbon metabolism which affects cell growth and differentiation by affecting DNA synthesis and epigenetic regulation. Hence, they are important regulators of fetal growth (34,35). Vitamin B12 deficiency is more prevalent in south India has been documented (36), more so in this part of region of North Karnataka, because of inadequate dietary intake and also the strict vegetarian diet style.

Although routine folic acid supplementation during per-conceptional period has been adapted for prevention of Neural tube defects (NTD), continuing supplementation of Folic acid beyond 12 weeks of pregnancy has not shown significant reduction of LBW and preterm term deliveries in systematic reviews [37,38].

Serum Vitamin A: A systematic review conducted by Thome-Lyman AL, observed that vitamin A has no significant effect on pregnancy outcome. Vitamin A had teratogenic effects, when increased the supplementation is 4 times that of RDA, Beta-caroteniods had no teratogenic effects [39]. It increases the risk of cleft-lip, cleft palate heart defects and hydrocephalus. Beta-carotene in food is observed to be safe [39].

Serum Vitamin D and Calcium: Vitamin D elicits a vaso-protective effect also through a decrease of oxidative stress increasing of anti-oxidative enzymes with effect on (NADH oxidase and ROS)[40].Vitamin D and calcium is essential for Bone mineralization which helps in increase in birth-weight of the baby. The RDA of Vitamin D fixed by the IOM is 600 IU/d. In case of vitamin D deficiency, supplementation of 1000 -2000 IU/d is observed to be safe. Few studies also observed that supplementation at the doses of 4000 IU/d was found to be safe during pregnancy in previous research. Vitamin D deficiency has been considered as a nutrient of public health importance in pregnancy[41]. About 30-96% of pregnant ladies had vitamin D levels of less than 50 nmol/L in previous research indicates how common this problem is [42-49]. There is a significant association between vitamin D deficiency and peripheral insulin resistance also[50], which could be reversed with just a single dose of the vitamin D injection[51]. Many studies also reported that higher long bone density was observed in fetuses of pregnant ladies with sufficient blood levels of vitamin D levels [52- 54]. The recommendations for supplementation is 1000 - 2000 mg/d. There are limited evidence to prove the effects of Vitamin D and maternal outcomes or fetal survival, birth weight or gestational period length [55], but studies which looks at bone health (and lung function and childhood asthma)[56],supports a optimum level of minimum 50 nmol/L in pregnant women, which implies there is a need for supplementation for every pregnant women[57-60], Women will lose 3- 5 percentage of the bone mass during lactating but soon they normalise within 6 months after stopping breast feeding. Calcium and/or vitamin D deficiency could cause porous and weak bones. A Cochrane Review has concluded that there is sufficiently enough proof to say that calcium supplementation will reduces the risks of hypertensive disorders of pregnancy [61].

Selenium: Selenium is an important trace element, which is a part of the body's redox reactions. Insufficient selenium consumption during pregnancy had been found to be associated with LBW and SGA[62]. Foetal growth and development dependents on nutrients that are transported from the maternal blood to the foetus through the placenta. The molecules like oxygen and carbon di-oxide are transported by umbilical blood flow and concentration gradient and the placental structure, where as the larger nutrients molecules such as glucose, amino acids and fatty acids are influenced on nutrient transporter receptor proteins[62,63]. The nutrient transporter receptor proteins in the placenta are influenced by factors such as, that includes hormones, micronutrient levels in blood and the placental functions [64]. Hence oxidative stress indicators in the placenta had been found to affect the transport of nutrients across the placenta by altering the gene expressions of nutrient transporters receptor proteins [65,66]. Few invitro studies has observed that selenium supplementation will protects placental structures and placental cells from oxidative stress by increasing the productions of Selenium-containing antioxidant enzymes, like glutathione and thioredoxin reductase [67]. Hence one of the accepted hypothesis, regarding low selenium could affect the foetal growth is by the selenium-dependent anti-oxidative defence system [65,68].

Selenium combine with the proteins forming Seleno-proteins, it forms the part of enzymes like glutathione per-oxidase, Thio redoxin reductase, which has the antioxidant properties, will stabilise the ROS, thus reducing the oxidative stress and thus improving the birth weight. The studies conducted by Mistry HD and Tsuzuki S also observed that, Reduced maternal selenium levels have been found to be associated with abortions (early pregnancy loss) and low birth weight. [69-73]

Thyroid hormones are required for regulation of nutrient transport through placenta. [74]. Hence, another hypothesis about selenium influencing fetal growth is by

optimizing the levels of thyroid hormones. In the animal experimental study, wherein mice intervened with diet deficient in selenium developed selenium deficiency. These mice had increased levels of both maternal and fetal plasma thyroid hormones triiodothyronine (T3) and tetraiodothyronine (T4)[71].

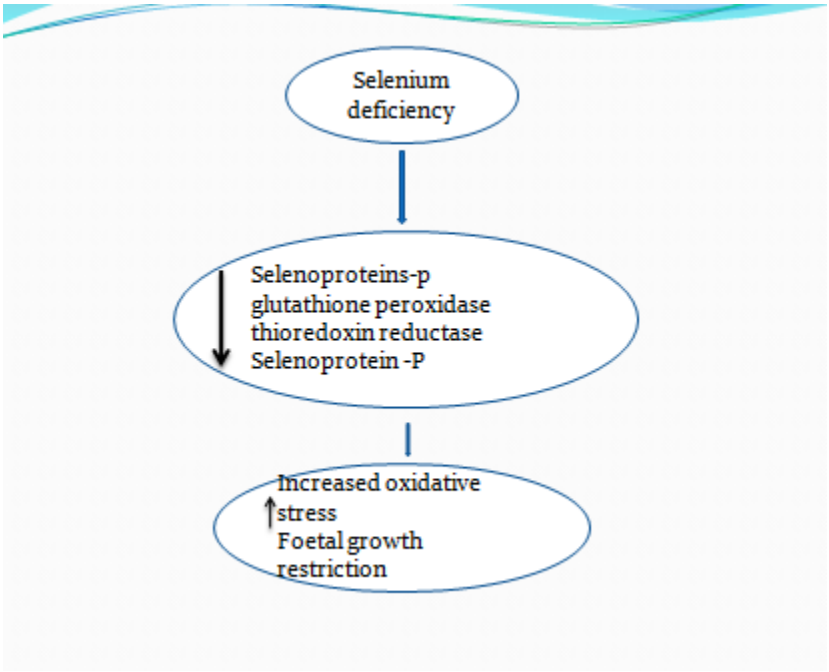

Figure 3.2: Mechanism of action of Selenium [22].

Zinc

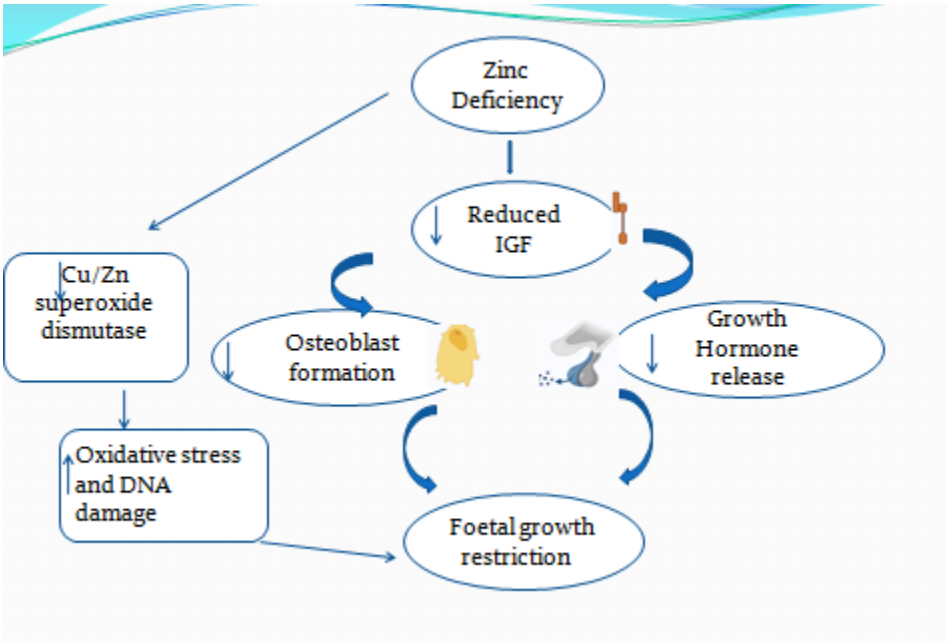

Figure 3.3: Mechansims of action of Zinc [22].

Zinc deficiency influences on embryonic and fetal development through reduction in cell proliferation, protein synthesis rates of tubulin polymerization and binding of hormones and transcription factors dependent on zinc-finger regions. Also zinc deficiency increase rates of cellular oxidative damage and rates of apoptosis. Many studies shown low plasma zinc concentration in the first and third trimesters of pregnancy increased risk of malformations and low birth weight. So zinc deficiency could be considered as a teratogenic risk, thus leading to adverse fetal development. [75,76,77]

A study conducted by King JC and Wang H found that Zinc deficiency have been associated with various effects on pregnancy includes intrauterine growth restriction (IUGR) and LBW[78,79]. An RCT conducted by Goldenberg found that the group who received Zinc supplementation had significantly greater birth weight compared to placebo[80].

- **Mitochondrial DNA copy number and maternal serum Micronutrients levels**: Mitochondrial DNA replication is regulated by D-loop, occurs in post-mitotic cells-independent of Nuclear DNA replication, and independent of the cell cycle. Hence we could observe the effect of micronutrients at gene level, giving objectivity. Mitochondrial genome is vulnerable to oxidative stress, because it lacks histones and nuclear DNA repair mechanisms. ROS damage mitochondrial DNA, causes dysfunctional respiratory chain and compensatory increase in Mt DNA copy number. Some studies have found that micronutrient supplements will stabilize the copy number. This number has least diagnostic/prognostic value when it is done at one point of time, can be utilized to see the effect of intervention of micronutrients **[**81,82,83]. Iron is essential for the synthesis of heme, a component of the mitochondrial electron transport chain. Iron deficiency can impair mitochondrial function and reduce mtDNA copy number. Selenium's Protective Effects on Mitochondrial DNA and Biogenesis. Selenium supplementation protects mitochondrial DNA from damage and

influences mitochondrial biogenesis. Folate plays a critical role in the prevention of uracil incorporation into DNA and hypomethylation of DNA. Folate deficiency causes expression of chromosomal fragile sites, chromosome breaks, excessive uracil in DNA, micronucleus formation, DNA hypomethylation and mitochondrial DNA deletions. Genomic instability in human cells is minimised. Vitamins B12, A, and D3 play a role in maintaining mtDNA copy number and integrity, and can affect mitochondrial biogenesis and membrane integrity. Zinc is a cofactors for enzymes involved in DNA repair and replication, and their deficiencies can lead to mtDNA damage and a decrease in mtDNA copy number. zinc deficiency also accelerates mitochondrial oxidative decay by inhibiting the pathway of heme biosynthesis in mitochondria, causing a deficit of heme-a. This results in oxidant leakage and accelerated mitochondrial decay, leading to DNA damage, neural decay and compensatory increase in mitochondrial DNA copy number.[84]

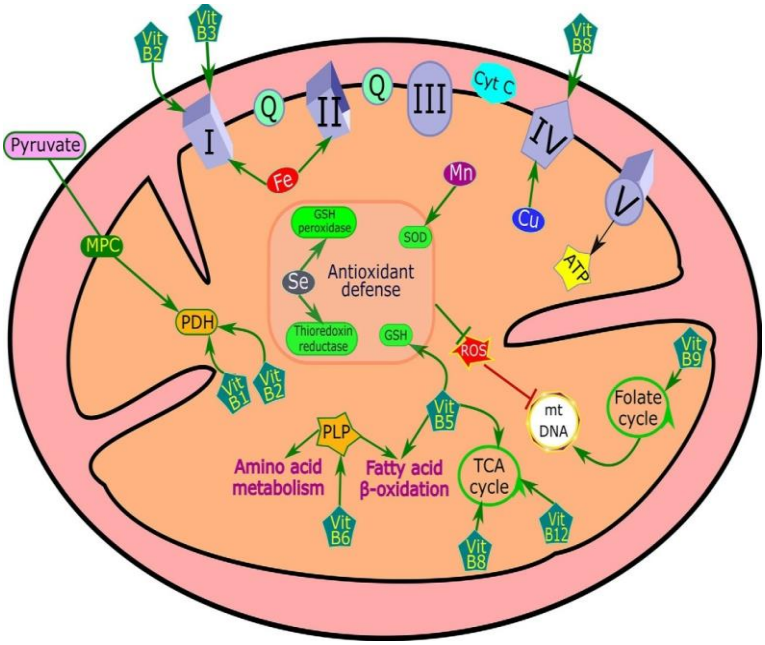

Figure 3.4: The effects of various dietary constituents that directly or indirectly serve as cofactors in mitochondria. Cofactor nutrients are essential for the activities of mitochondrial complexes I (Vit B2, Vit B3 and Fe), II (Fe) and IV (Vit B8 and Cu), antioxidant enzymes (Vit B5, Se and Mn), energy metabolism (Vit B1, B2, B5, B6, B8 and B12), and mtDNA synthesis (Vit B9). Arrows: *green*—beneficial or neutral effects, *red*—deleterious effects; arrowheads: sharp—activation, blunt—inhibition.[84]

Mitochondrial DNA copy number and Birth weight of the baby

Maternal leucocyte mitochondrial factors were observed to be modulated by micronutrients components and optimize the mitochondrial function thereby improve the fetal outcome. Mitochondria has been found to be involved to modulate the major stress response pathways and alterations in the mitochondrial DNA copy number (mtDNA-CN) in adults. Maternal mitochondrial ATP is very important for fetal growth and development, and an optimum mtDNA-CN sensitive to energy demand and oxidative stress are required to optimize mitochondrial function. MtDNA-CN has been correlated inversely with pregnancy outcomes. Micronutrient deficiency has been shown to reduce the mitochondrial efficacy, leads to compensatory increase in mtDNA-CN. Optimum micronutrient levels in blood was found to reduce the oxidative stress and supports efficient mitochondrial functions and thus protect against oxidative stress, both of which reduce mtDNA-CN [85-92]. Mitochondrial DNA replication is regulated by D-loop, occurs in post-mitotic cells-independent of Nuclear DNA replication, and independent of the cell cycle. Mitochondrial genome is vulnerable to oxidative stress, because it lacks histones and nuclear DNA repair mechanisms. ROS damage mitochondrial DNA, causes dysfunctional respiratory chain and compensatory increase in Mt DNA copy number. Some studies have found that micronutrient supplements will stabilize the copy number. This number has least diagnostic/prognostic value when it is done at one point of time, can be utilized to see the effect of intervention of micronutrients. [93,94,95] . Increased oxidative stress has been consistently shown to induce increases on the mitochondrial DNA (mtDNA) content and IUGR has been found to be associated to increased mtDNA copy number. Placenta being the primary source of oxidative stress, which is affected by micronutrient levels with antioxidant property. In our study, we observed there was

negatively correlated between mitochondrial DNA copy number and birth weight. A study by Richard Jones, found that the birth weight of the baby was affected by increased mitochondrial DNA similar to our study.[96]

A study in Indonesia (2019), found that the MtDNA-CN was stabilized by micronutrient supplementation in pregnant women. The MtDNA-CN was reduced more in Micronutrient supplementation group when compared to Iron and folicsupplementation group within 3 months of supplementation, which was found to be statistically significant[92].

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MATERIAL & METHODS

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.

CHAPTER 4.
MATERIALS AND METHODS

Study Design: Mixed Methods Research Study (Case series study + Focus Group Discussion).

Study Duration: Two years (March 2021- February 2023)

Source of Data: The present study was carried out in Outpatient department and Labor room of OBG Department at Tertiary Care centres, Bagalkot.

Sample Size: Sample size estimation was done using Medcalc software

At 95% confidence limits, and at 90% Power of the study,

$Z\alpha$= standard table value for 95% CI =1.84, $Z_{1-\beta}$ = Standard table value for 90% Power = 1.28

According to study conducted by Horan MK[3].

The correlation coefficient between the third trimester Vitamin D levels and birth weight of the baby=0.44

Formula used= ***Formula used:*** $N = ([Z_\alpha + Z_\beta]/C)^2 + 3$, **where** $C = 0.5 * \ln([1 + r]/[1 - r])$

Sample size estimated is 85

With design effect of 1.5= sample size calculated is 130

Taking 10% extra for sample loss, sample size estimated is 150.

Ethical Clearance:

Obtained Ethical clearance from IEC BLDE university (on **27/12/2019) (BLDE(DU)/IEC/409/2019-20**.

Obtained Ethical clearance from S N Medical college (on **05/02/2020) (No:SNMC/IECHSR/2019-20/A-68/1.1.**

Consent: Written Informed consent was obtained from all the participants before enrollment.

Inclusion criteria:

- Apparently Normal term (≥37 completed weeks) Pregnant Women who are admitted in the labour room for normal delivery or LSCS was included.
- Informed consent was obtained from the study participants.

Exclusion criteria:

- Multiple pregnancy like twins diagnosed anytime during pregnancy or after delivery. Chronic medical conditions such as hepatic, renal, cardiovascular diseases, obesity, hypertension including preeclampsia and diabetes mellitus includes gestational diabetes, known HIV and Hepatitis B infection
- Women with hyperemesis gravidarum with persistent severe nausea and vomiting beyond 12 weeks of pregnancy were excluded.

Methodology:

- A pretested questionnaire for obtaining basic demographic characteristics and dietary consumption by 24 hour recall method.
- Dietary diversity is a qualitative measure of food consumption that reflects household access to a variety of foods, and is also a proxy for nutrient adequacy of the diet of individuals.Dietary diversity is the consumption of different food groups in a day, viz cereals, pulses(Legumes,nuts and seeds), green leafy vegetables, roots and tubers, other vegetables, fruits, milk and milk

products, Meat,egg and sea foods, oils and fats, spices and condiments and the sugar and jaggery. [2] The food items consumed is considered if they have consumed minimum 50% of RDA, for calculating dietary diversity.

Balanced diet for 1 consumption unit =2110 Kcal, Women sedentary worker=0.8, Moderate Worker=0.9 ICMR 2020 guidelines [3,4]

Food groups	Foods to be consumed (g/day)	Pregnancy
Cereals including millets	275	+35
Pulses/ Flesh foods	80	+15
Milk/ curd (ml)	300	+100
Green Leafy Vegetable	100	
Other Vegetables	200	
Roots and Tubers	100	
Fruits	100	
Nuts & Seeds	30	+10
Fats & Oils	25	+5
Spices	10	

Socioeconomic classification according to modified B G prasad classification (2022)[5]

Table 4.1: Linking factors from the year 1960 to 2016[5]

Linking factors from the year 1960 to 2016	
Price index by old base 1960 for the original scale	100
Price index by new base 1982 for the year 1982	100
Linking factor between 1960 and 1982 series	4.93
Price index by new base 2001 for the year 2001	100
Linking factor between 1982 and 2001 series	4.63
Price index by new base 2016 for the year 2016	100
Linking factor between 2001 and 2016 series	2.88

Table 4.2: Calculation of new income value for the revised B. G. Prasad socioeconomic classification 2022.[5]

S. no.	Calculations
1	Calculating multiplying factor for May 2022 from the latest available CPI-IW for May 2022 (i.e., 129, available from ministry of labor and employment
2	Multiplying factor=current index value for May 2022 (=129)/ Base index value in 2016 (=100)=1.29[7]

The formula for calculating new income value for May 2022

New income value=Multiplying factor (1.29)×[Old value×linking factor between 1960 and 1982 series (4.93)×linking factor between 1982 and 2001 series (4.63)×linking factor between 2001 and 2016 series (2.88)].

New income value =1.196×15×4.93×4.63×2.88=1272.04176/1272

Table 4.3: Modified B. G. Prasad classification for May 2022.[5]

Social class	Per capita income (Rs) as per original classification in 1961	Per capita income (Rs) as per modified classification for May 2022
EI (Upper class)	≥100	≥8397
II (Upper middle class)	50-99	4156-8396
III (Middle class)	30-49	2460-4155
IV (Lower middle class)	15-29	1272-2456
V (Lower class)	<15	<1272

- Later physical examination includes general physical examination, Anthropometric measurements, vitals includes pulse rate, Blood pressure, Respiratory rate was measured.

- Collected 5ml of venous blood under aseptic precaution and they were stored in Central Research Lab at -20 Degree. After serum separation, Micronutrients estimations and mitochondrial DNA copy number was done using Q-PCR technique.
- Complete Blood Count(Using Penta ES 60 cell counter) Serum ferritin, serum Vitamin B12,folic acid and Vitamin D (SNIBE Maglumi 1000 autoanalyser, which works on the principle is Chemiluminucence method (CLIA).
- Zinc and Calcium was estimated using a Fully automated analyser BA-400 of Bio-system company.
- Selenium by inductive couple plasma mass spectrometry (ICP-MS). Vitamin A By chromatographic methods using HPLC method.
- Peripheral smear examination was done.
- **Maternal mitochondrial DNA copy number estimation[6,7]:**

Step1: DNA extraction: Genomic DNA was extracted from 1ml whole blood using the 51304 Q1Amp DNA mini kit (Batch No:172021026 QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The purity and quantification of DNA were estimated from the 260nm and 280nm absorbance ratio using the NanoDrop 2000 Spectrophotometer.

Step 2 : Quantification of mitochondrial DNA copy number: The mitochondrial DNA copy number was quantified by qPCR method using QuantStudio5 (quantitative PCR) Applied Biosystems (by Therma Fisher Scientific).

- To analyse MtDNA copy The forward and reverse primers of the mitochondrial gene (NADH-ubiquinone oxidoreductase chain 1)(ND1 gene) used to amplify a 153 bp product were 5'-AACATACCCATGGCCAACCT-3' and 5'-AGCGAAGGGTTGTAGTAGCCC-3', respectively.

- The forward and reverse primers of β-globin (used to amplify a 268 bp product) were 5'-GAAGAGCCAAGGACAGGTAC-3' and 5'-CAACTTCATCCACGTTCACC-3', respectively.
- The genomic DNA (50ng) was mixed with 10µl SYBR Green I Master Mix (Takara, USA) that contained 10pmol of forward and reverse primers in final volume of 20 µl.
- DNA samples were treated at 95∘C for 0.1 seconds, 58∘C for 6 seconds, and 72∘C for 18 seconds for 40 cycles.
- A total of 50 ng of DNA was used and the number of PCR cycles was defined as the threshold cycle (Ct).
- The following equation was used to quantify the mtDNA copy number relative to β-globin:relative copy number = $2\Delta Ct$ ($\Delta Ct = Ct\beta$-globin − Ct ND1)

Serum Ferritin estimation: The test was performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Maglumi 1000) using the procedure given in the kit.

PRINCIPLE OF THE TEST

Sandwich immunoluminometric assay: Use an anti-ferritin monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control are mixed thoroughly with FITC Label and nano magnetic microbeads in a cuvette incubated at 37°C, then cycle washing for 1 time. Then add ABEI Label, incubation and form a sandwich, then washing for the 2nd time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of ferritin present in samples. Sample material: serum.

Table 4.4: Procedure for Ferritin estimation.

40µl +100µl +20µl	Sample, calibrator FITC label Nano magnetic microbeads
10 min	Incubation
400µl	Cycle washing
+200µl	ABEI label
10 min	Incubation
400µl	Cycle washing
3s	Measurement

Serum Vitamin B12 estimation:

The test was performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Maglumi 1000) using the procedure given in the kit.

PRINCIPLE OF THE TEST

Competitive immunoluminometric assay: Use purified VB12 antigen to label ABEI, and use a VB12 binding-protein to label FITC. Sample, Calibrator or Control with ABEI Label, FITC Label and nano magnetic microbeads coated with sheep anti-FITC are mixed thoroughly and incubated at 37℃, forming antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for I time. Subsequently, the starter reagents are added and a flash

chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of VB12 present in samples. Sample material: serum

Table 4.5: Procedure for Vitamin B12 estimation.

100µl +100µl	Sample , Calibrator Displacing reagent
2min	incubation
+110µl +110µl +20µl	ABEI label FITC label Nano magnetic microbeads
15 min	Incubation
400µl	Cycle washing
3 s	Measurement

Serum 25-OH Vitamin D estimation

The test was performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Maglumi 1000) using the procedure given in the kit .

Principle of the test:

The 25-OH VITAMIN D assay is a competitive chemiluminescence immunoassay using FDA

previously cleared MAGLUMI 2000 instrument (k162698). The 25-OH Vitamin D assay is a two-incubation chemiluminescence immunoassay for the quantitative determination of total 25-OH vitamin D in human serum. In the first incubation, the 25-OH vitamin D is dissociated from its binding protein by the displacing reagent and binds to the 25-OH vitamin D antibody on the magnetic microbeads forming an antibody-antigen complex. Following a second incubation, the 25-OH Vitamin D labeled ABEI are added. The rest unbound material is removed during a wash cycle. Subsequently, the Starter 1+2 are added to initiate a flash chemiluminescent reaction. The resulting chemiluminescent reaction is measured as relative light units (RLUs), which is inversely proportional to the concentration of 25-OH Vitamin D present in the sample.

Table 4.6: Procedure for 25-OH Vitamin D estimation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
25-OH VD Controls (04108-04109)	15	-
Samples	-	15
25-OH VD Assay Diluent (04110)	120	120
Incubate at 37°C for 8 minutes		
25-OH VD Magnetic Particle Solution (04101)	20	20
Biotinylated 25-OH VD (04102)	25	25
Acridinium ester 25-OH VD antibody (04103)	50	50
Incubate at 37°C for 10 minutes		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	100-200	100-200
Trigger Solution B (P-595)	100-200	100-200

Folic Acid estimation:

The test was performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Maglumi 1000) using the procedure given in the kit.

PRINCIPLE OF THE TEST

Competitive immunoluminometric assay; Use FA-binding Protein to label ABEI, and use purified FA antigen to coat magnetic microbeads. Sample, Calibrator or Control with displacing Reagent are added first and incubate at 37 ℃ for 2 min. then add ABEI label and magnetic microbeads and incubated at 37 ℃ for 15 min, forming antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a

photomultiplier as RLU within 3 seconds and is proportional to the concentration of FA present in samples.

Table 4.7: Procedure for Folic acid estimation.

40µl +40µl	Sample , Calibrator Displacing reagent
2min	incubation
+100µl +20µl	ABEI label Nano magnetic microbeads
10 min	Incubation
400µl	Cycle washing
3 s	Measurement

Serum Calcium estimation: Calcium was estimated using a Fully automated analyser BA-400 of Bio-system company using the procedure given in the kit .

PRINCIPLE :

Calcium ion produces with methylthymol blue, in an alkaline medium, a blue color the intensity of which is in proportion to the calcium concentration. The presence of hydroxy 8-quinoline eliminate the interference due to the magnesium ions.

Table 4.8: Procedure for Calcium estimation.

Working reagent : Mix one volume of reagent 2 with one volume of reagent 3 befor use .			
Pipette into test tubes (FREE OF CALCIUM)			
	Blank ml	Standard ml	Sample ml
Sample	-	-	0.02
Standard (R1)	-	0.02	-
Working reagent	1.0	1.0	1.0
Mix well, wait 5 min. at room temperature . Read the absorbances of the sample (A_{Sample}) and the standard ($A_{Standard}$) against blank, at 585 nm. (575 - 590) . The color is stable for at least 60 min. Linearity up to 20 mg / dL .			

Serum Zinc Estimation: Serum Zinc was estimated using a Fully automated analyser BA-400 of Bio-system company using the procedure given in the kit.

Principle: Zinc in the sample reacts with 5-Br-PAPS in the alkaline medium forming a coloured complex that can be measured spectrophotometry.

Table 4.9: Procedure for Zinc estimation.

	Reagent blank	Standard	Specimen
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	____	50 µl	____
Specimen	____	____	50 µl
Mix and incubate for 10 min at 25 ^{0}C or 5 min at 37 ^{0}C. Measure the absorbance of the specimen ($A_{specimen}$) and the absorbance of standard ($A_{standard}$) against reagent blank.			

Qualitative data Collection:

- For the mixed method research adopted, convergent parallel, embedded and explanatory sequential methods were adopted using personal interviews during collection of personal details and Focus group discussions (FGDs).
- A total of 36 pregnant women and 32 Caregivers participated .
- A total of 5 FGDs for pregnant women and 5 FGDs for care takers **(mothers and mother-in laws)** were done.
- We ended this following achieving the data saturation and no new themes were developed.

Statistical Analysis:

Quantitative Data:

- Data was coded and then entered in the Microsoft excel and later analysed using SPSS software version 19.
- The normality of the data was checked using shapiro wilk test and z scores for both skewness and kurtosis. Later appropriate parametric and non-parametric tests were applied to the data.
- Percentages for qualitative data, and mean and standard deviation for quantitative data was used for representing the data.
- Chi-square test and students t test was applied for the independent parameters of the outcome variables.
- Mann-whitney U test was applied for the non-parametric data. Pearson's correlation co-efficient was calculated.

- Multivariate Logistical Regression analysis was done.
- $p<0.05$ was considered as statistically significant.

Qualitative Data:

The codes for the responses obtained from participants were developed using Python Version 3.1.1 and QDA Miner lite software are

a. Eight Themes developed based on the 58 codes derived from responses of the pregnant women.

b. Seven Themes developed based on the 63 codes derived from responses of the care-takers (mothers and mother-in laws) of pregnant women

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RESULTS

"Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study."

CHAPTER 5. RESULTS

Quantitative study:

In the present Research work, 150 apparently normal pregnant women participated. Maximum of 83.3% of study subjects were in the age group of 21-30 years. Maximum of 80% were from Urban area. 40% of them were studied beyond PUC. Maximum of 36.7% and 39.3% each were belonging to class II and class III socio-economic status respectively, according to Modified BG prasad classification. Maximum of 62% of them were primigravida. The birth weight of the baby ranged between 2.34 kg-3.56kg). In the present study 22% of the babies born were low birth weight (≤2.49kg) (Table 5.1).

Table 5.1: Baseline characteristics of study subjects.

Variables		Frequency	Percentage
Age	<= 20	7	4.7
	21 - 30	125	83.3
	31+	18	12.0
Address	Rural	25	16.7
	Urban	125	83.3
Educational status	Primary	11	7.3
	High school	20	13.4
	PUC	56	37.3
	Degree	54	36.0
	PG	4	2.7
	Professional	5	3.3
Socio-economic status (According to Modified B G prasad classification)	I(Upper class)	2	1.3
	II(Upper middle class)	55	36.7
	III (middle class)	59	39.3
	IV(Lower middle class)	32	21.3
	V(Lower class)	2	1.3
Gravida status	1	93	62.0
	2	32	21.3
	3	22	14.7
	4	3	2.0
Mode of delivery	Vaginal	98	65.3
	Caeserean section	52	34.7
Birth weight (in Kgs)	<= 2.49	**33**	**22.0**
	2.50+	117	78.0
	Total	150	100.0

Table 5.2: Association between Maternal Education, Socio-economic status and Gravida status with birth weight.

Variables		Baby birth weight (kg)		Total No	P*
		<= 2.49kg No (%)	2.50+ kg No (%)		
Maternal Education	Primary	6 (54.5%)	5(45.5%)	11	0.06
	High school	6 (30.0%)	14 (70.0%)	20	
	PUC	11 (19.6%)	45 (80.4%)	56	
	Degree	10 (18.5%)	44 (81.5%)	54	
	Post graduation	0 (0.0%)	4 (100.0%)	4	
	Professional	0 (0.0%)	5 (100.0%)	5	
Socio-economic status (according to Modified B G prasad classification)	I (Upper class)	0 (0.0%)	2(100.0%)	2	P=0.52
	II (Upper middle class)	11 (20.0%)	44 (80.0%)	55	
	III (middle class)	8 (13.6%)	51 (86.4%)	59	
	IV (Lower middle class)	12 (37.5%)	20(62.5%)	32	
	V (Lower class)	2 (100.0%)	0 (0.0%)	2	
Gravida	1	21 (22.6%)	72 (77.4%)	93	P=0.62
	2	6 (18.8%)	26 (81.2%)	32	
	3	3 (13.6%)	19 (86.4%)	22	
	4	3 (100.0%)	0 (0.0%)	3	
Total		33 (22.0%)	117 (78.0%)	150	

*** Fisher's Exact test**

In the present study, there was an association between education status, Socio-economic status, and gravida status with birth weight of the baby. But the association was not statistically significant. (p=0.06, 0.52, 0.62 respectively). As the education status and socioeconomic improved in mothers, the proportion of low birth weight reduced, and the risk of low birth weight is more in gravida 4.

Table 5.3: Association between Grades of Anaemia (According to WHO classification) with birth weight.

WHO classification of Anaemia in pregnancy	Baby birth weight (Kgs)		Total
	<= 2.49 Low birth weight	2.50+ Normal	
Normal (Hb> 11gm)	27	92	119
	22.7%	77.3%	100.0%
Mild Anaemia (9-10.9)	3	22	25
	12.0%	88.0%	100.0%
Moderate Anaemia (7-8.9)	3	3	6
	50.0%	50.0%	100.0%
Total	33	117	150
	22.0%	78.0%	100.0%

Chisquare=4.2 p=0.12

In the present study, 12% of the mothers with mild anaemia, and 50% of those who had moderate anaemia gave birth to Low birth weight babies. 22.7% of those who had normal haemoglobin gave birth to Low birth weight babies. The difference was not found to be statically significant (p=0.12).

Table 5.4: Association between the Maternal Peripheral blood smear findings at term with Birth weight of the baby.

Baby birth weight (in kgs)	Peripheral blood			Total
	Microcytic Hypochromic	Microcytic normochromic	Normocytic Normochromic	
<= 2.49	6	0	27	33
	18.2%	0.0%	81.8%	100.0%
2.50+	6	6	105	117
	5.1%	5.1%	89.7%	100.0%
Total	12	6	132	150
	8.0%	4.0%	88.0%	100.0%

Chisquare=7.3, p=0.02

we observed that the 18.2% of the mothers with microcytic hypochromic anaemia delivered Low birth weight babies, and 5.1% delivered Normal birth weight babies. 5.1% of Microcytic normochromic anaemia mothers had normal birth weight babies. 81.8% of Mothers with Normocytic normochromic mothers had Low birth weight babies. This difference was found to be statistically significant(p=0.02).

Table 5.5: Mean difference of Maternal micronutrients levels at term according to birth weight of the baby.

Micronutrients	LBW(33) (<= 2.49 kg)		NBW(117) >2.50 kg		t	p
	Mean	SD	Mean	SD		
Ferritin µg/dL (Ref V: 15 µg/dL)	13.7	12.8	14.5	10.8	0.20	0.83
Vitamin B12 pg/ml (Ref V: 526 pg/ml)	225.7	128.4	245.5	152.5	0.74536	0.457236
Vitamin A ng/ml (Ref V: 300ng/ml)	254.6	92.1	268.1	148.9	0.63	0.52
Vitamin D3 ng/ml (Ref V: 30ng/ml)	21.9	8.7	23.7	15.8	0.63	0.52
Selenium µg/l (Ref V: 150µgm/l)	157.4	44.1	161.4	66.5	0.31771	0.751161
Zinc µ gm/dL (Ref V: 200µ gm/dL)	101.5	55.2	108.5	41.4	0.721587	0.471
Calcium mg/dl (Ref V: 9.5mg/dl)	7.8	1.02	8.4	0.9	**3.05427**	**0.002676**
Folic acid ng/ml (Ref V: 20ng/ml)	0.6	0.1	4.7	1.9	**3.154194**	**0.001959**

(Ref V: Reference value)

Mean maternal serum Ferritin, Vitamin B12, Vitamin A, Vitamin D3, Selenium, zinc reduced in mothers who gave birth to Low birth weight babies, compared to mothers who gave birth to normal birth weight babies, but the difference was not statistically significant. The mean calcium(mg/dl) levels in mothers of low birth weight babies was 7.8±1.02 and in mothers who gave birth to normal weight babies was 8.4±0.9 which was found to be statistically significant (p=0.0026). The mean folic acid (ng/ml) levels in mothers who gave birth to low birth weight babies was 0.6±0.1 and mothers who gave birth to normal birth weight babies was 4.7±1.9, which was found to be statistically significant (p=0.001).

Table 5.6: Association between the Maternal serum Micronutrients at term with Low birth weight of the baby.

Micronutrients	Cut-off	Low birth weight No(%)	Chi-square	p
Serum Ferritin µg/dl	<=15.0	25(75.7%)	**4.1**	**0.001**
	15.1+	8(24.3%)		
S. Vitamin B12 pg/ml	<526	31(93.9%)	**7.1**	**0.001**
	527+	2(6.1%)		
S. Vitamin A ng/ml	<= 300.0	18(54.5%)	0.73	0.45
	300.1+	15(45.5%)		
S. Vitamin D3 ng/mL	<= 30.0	27(81.8%)	**5.16**	**0.001**
	30.1+	6(18.2%)		
Serum selenium µg/l	<=150	21(63.6%)	**2.21**	**0.01**
	>151	12(36.4%)		
Serum Zinc µ gm/dL	<= 200.0	31(72.7%%)	**7.1**	**0.001**
	200.1+	2(27.3%)		
Serum Calcium mg/dl	<= 9.5	33(100%)	**8.12**	**0.001**
	9.6+	0(0)		
Serum Folic acid ng/ml	<20	30(90.9%)	**6.64**	**0.001**
	20.1+	3(9.1%)		

Maternal serum ferritin levels of low birth weight babies were below the cutoff for optimum 15µg/dl in 75.7%, which was found to be statistically significant($p<0.001$), 93.9% of the them had vitamin B12 level below the cutoff for optimum 526pg/ml ($p<0.001$), 54.5% of them had Vitamin A level below the cutoff for optimum of 300ng/ml ($p=0.45$, not significant), 81.8% of them had vitamin D levels below the cutoff of optimum of 30ng/ml ($p<0.001$), 63.6 % of them had selenium levels below the cutoff of optimum 150µg/l ($p<0.01$), 72.7% of them had zinc levels below the cutoff of optimum 200µg/dl ($p<0.001$), 100% of them had serum calcium levels below the cutoff of optimum 9.5 mg/dl($p<0.001$) and 90.9% of them had serum folic acid levels below the cutoff of optimum 20ng/ml p(<0.001).

Table 5.7: Mean difference of Maternal Dietary Diversity, Dietary calorie consumption, dietary protein intake and Gestational weight gain according to birth weight of the baby.

Baby Birth weight		N	Mean	Std. Deviation	t	p
Maternal Dietary Diversity	Normal birth weight	117	5.44	0.957	**7.58**	**0.001**
	LBW cases	33	3.97	.910		
Maternal Dietary calorie consumption	Normal birth weight	117	1556	220	0.90	0.36
	LBW cases	33	1520	119		
Maternal Dietary Protein intake	Normal birth weight	117	48.5	11.0	**2.06**	**0.04**
	LBW cases	33	44.1	10.0		
Maternal Gestational weight gain	Normal birth weight	117	9.4	3.8	1.33	0.18
	LBW cases	33	8.5	1.2		

Maternal dietary diversity score at term with Low birth weight babies and normal birth weight babies were 3.97±0.91 and 5.44±0.95 respectively, this difference was found to be statistically significant (p=0.001). The maternal dietary protein intake at term with Low birth weight babies and normal birth weight babies were 44.1±10gm and 48.5±11 gm respectively, this difference was found to be statistically significant (p=0.04). Mean dietary calorie consumption and gestational weight gain was not statistically significant.

Table 5.8: Association between the Maternal Dietary Diversity score (DDS) at term and Birth weight of the Baby.

Maternal DDS	Low birth-weight (<2.49 kg)	Normal birth-weight (>2.50 kg)	Total
Low DDS (≤3)	5	3	8
	62.5%	37.5%	100.0%
Moderate DDS(4-5)	19	93	112
	16.9%	83.1%	100.0%
High DDS(≥6)	9	21	30
	30%	70%	100.0%

Chisquare=10.4, p=0.005

In the present study, 62.5% of mothers who gave birth to low birth weight had low dietary diversity score (<=3), 16.9% of them had moderate dietary diversity score (4-5) and rest had high DDS, where as 37.5% of mothers with normal birth weight had low DDS(<=3), 83.1% of the Moderate dietary diversity score and rest had high dietary diversity score. this difference was found to be statistically significant (p=0.005).

Table 5.9: Correlation between the Maternal Dietary diversity and Micronutrient levels at term.

Maternal Micronutrients at term		Maternal Dietary Diversity at term
Serum Ferritin µg/dL	r	.086
	p	.299
S. Vitamin B12 pg/mL	r	.125
	p	.129
S. Vitamin A ng/mL	r	-.139
	p	.092
S. Vitamin D3 ng/mL	r	-.030
	p	.720
Selenium µg/L	r	.155
	p	.062
S. Zinc µ g/dL	r	.183*
	p	.035
S. Calcium mg/dl	r	.192*
	p	0.01
S. Folic acid ng/ml	r	.21*
	p	0.008

r=pearson's correlation co-efficient.

There was correlation between the maternal serum micronutrient levels with Maternal dietary diversity score at term. There was statistically significant positive correlation between maternal serum zinc, serum calcium and serum folic acid with dietary diversity with correlation co-efficient of 0.183, 0.192 and 0.21 respectively. The correlation between serum ferritin, vitamin b12, vitamin A, vitamin D3, selenium was not statistically significant.

Table 5.10: Correlation between the maternal micronutrients levels at term and Mitochondrial DNA copy number.

Micronutrients	Mitochondrial DNA CN (ct mean)	
	Pearson Correlation(r)	p
Serum Ferritin µg/dl	.039	.640
S.Vitamin B12 pg/ml	-.079	.339
S.Vitamin A ng/ml	-.077	.348
S. Vitamin D3 ng/mL	-.246**	**.003**
Selenium µg/l	-.306**	**.000**
S. Zinc µ gm/dL	-.183*	**.034**
S. Calcium mg/dl	.090	.273
S. Folic acid ng/ml	.105	.208

*Significant, ** Highly significant

There was correlation between the maternal serum micronutrient levels with Maternal Mitochondrial DNA copy number at term. There was statistically significant negative correlation between maternal serum vitamin D3, Selenium and Zinc with correlation coefficient of -0.246, -0.306, and -0.183 respectively. The correlation between serum ferritin, Vitamin B12, Vitamin A, calcium and folic acid was not statistically significant.

Table 5.11: Correlationbetween the maternal serum micronutrients and Mt DNA CN at term and the birth weight of the baby.

Micronutrients and Mitochondrial DNA CN	Baby birth weight	
	Pearson Correlation(r)	p
Mitochondrial DNA CN (Ct Mean)	**-.250****	**.002**
Serum Ferritin µg/dl	**.173***	**.035**
S. Vitamin B12 pg/ml	.023	.783
S. Vitamin A ng/ml	-.084	.304
S. Vitamin D3ng/mL	**.217****	**.008**
Serum selenium µg/l	.133	.107
Serum Zinc µ gm/dL	.049	.572
Serum Calcium mg/dL	**.166***	**.042**
Serum folic acid ng/ml	**.235****	**.004**

*Significant, ** Highly significant

There was a statistically significant negative correlation between Maternal mitochondrial DNA copy number and birth weight of the baby (r=-0.25, p=0.002). There was a statistically significant positive correlation between maternal serum micronutrients such as ferritin (r=0.17, p=0.035), Vitamin D3(r=0.21,p=0.008), Calcium (r=0.16, p=0.04), and folic acid (r=0.23, p=0.004).

Table 5.12: Comparison of Maternal mitochondrial DNA copy number with normal birth weight and Low birth weight using Mann-whitney U test.

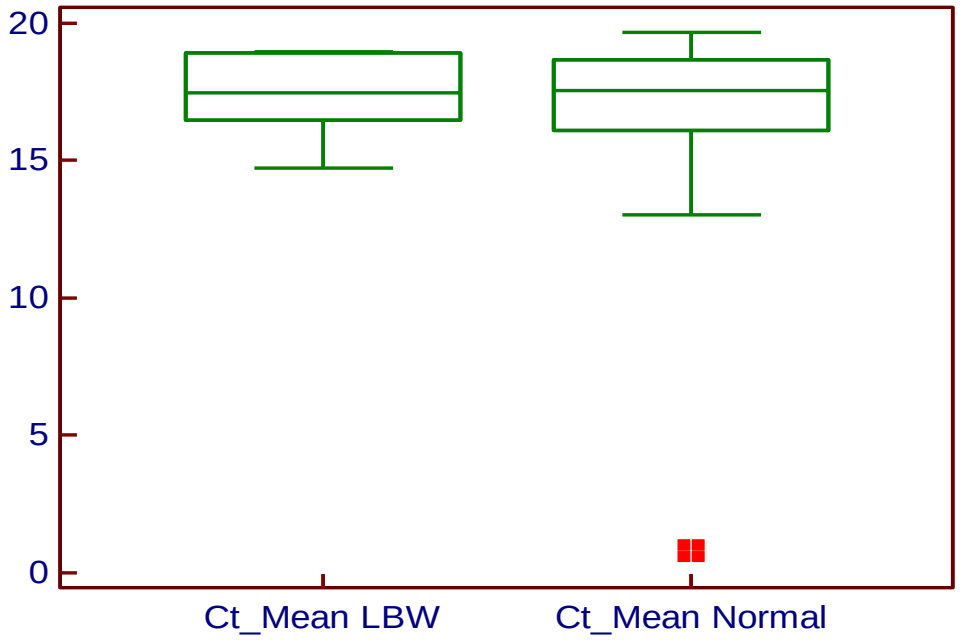
	Ct LBW	**Ct Normal weight**
Sample size	**33**	**117**
Lowest value	**14.74**	**0.59**
Highest value	**18.96**	**19.67**
Median	**17.48**	**17.56**
95% CI for the median	**16.5900 to 18.8000**	**17.0300 to 17.8100**
Interquartile range	**16.4600 to 18.9300**	**16.0950 to 18.6800**

Mann-Whitney test (independent samples)

Average rank of first group	80.5441
Average rank of second group	74.6795
Mann-Whitney U	1834.5
Test statistic Z (corrected for ties)	0.689
Two-tailed probability	P = 0.4911

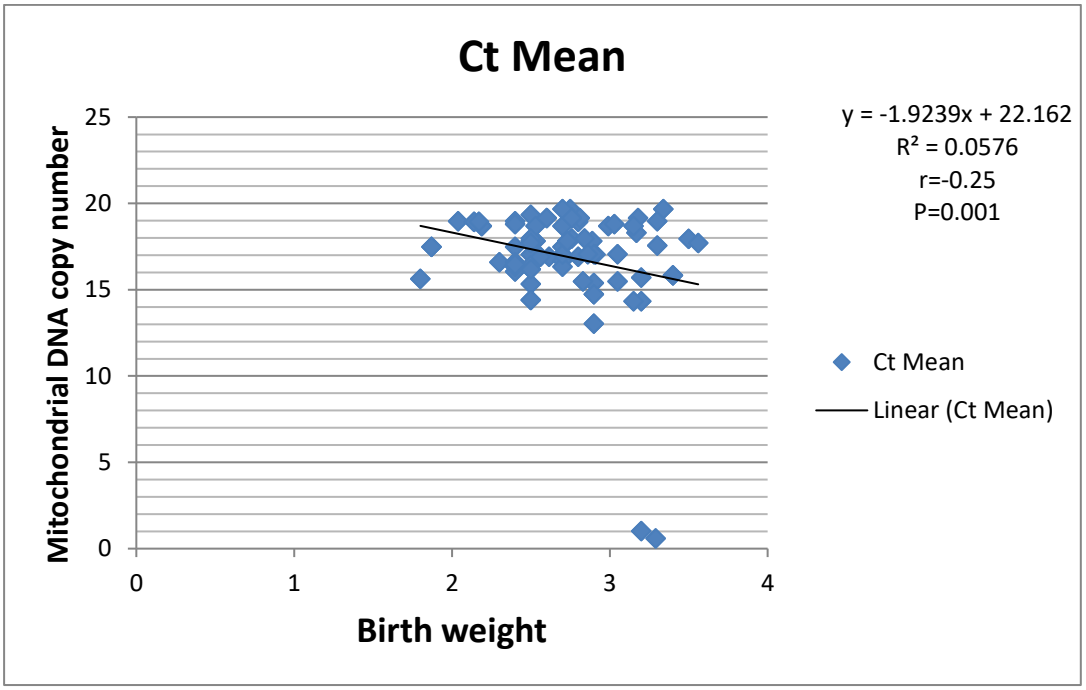
Median (IQR) in Low birth weight 17.48(16.46-18.9) and Normal weight 17.56(16.09-18.6). The difference was not statistically significant (p=0.68).

Figure 5.1: Box and whisker plot diagram showing the Median values of CT-Mean values of Maternal Mitochondrial DNA copy number in Low birth weight babies and Normal Birth weight babies.



Box and whisker plot diagram showing Median (IQR) values of Delta CT values of Mitochondrial DNA copy number in low-birth-weight babies were 3.07(1.7-5.74) and in normal birth weight babies was 3.71 (0.83-4.4). The difference in median values was not statistically significant .

Figure 5.2: Correlation between Maternal Mitochondrial copy number and Birth weight of the baby.



In this scatter diagram, it was observed that there was a negative correlation between the Mitochondrial DNA copy number and birth weight of the baby, it was found to be statistically significant.(r=0.25, p=0.001).

Table 5.13: Multivariate Logistic regression analysis of Micronutrients on birthweight of the baby.

		Univariate analysis			Multivariate analysis
Micronutrients (reference Value)	β	Crude Odds Ratio (95%CI)	p	Adjusted odds ratio (95%CI)	p
Ferritin µg/dL (15 µg/dL)	2.1	1.12(0.45-2.75)	0.09	1.14(0.43-2.68)	0.08
Vitamin B12 pg/ml(526 pg/ml)	1.9	1.44(0.26-7.8)	0.08	1.46(0.24-7.99)	0.074
Vitamin A ng/ml(300ng/ml)	-1.3	0.51(0.23-1.13)	0.16	0.49(0.22-1.13)	0.23
Vitamin D3 ng/ml (30ng/ml)	2.6	1.88(1.71-4.9)	0.01	1.90(1.82-5.0)	0.001
Selenium µg/l(150µgm/l)	2.3	1.58(0.68-3.54)	0.07	1.6(0.56-3.66)	0.05
Zinc µ gm/dL(200µ gm/dL)	2.56	1.8(1.2-2.4)	0.008	1.9(1.2-2.4)	0.01
Calcium mg/dl(9.5mg/dl)	1.3	3.11(0.38-5.22)	0.13	2.98(0.89-5.8)	0.06
Folic acid ng/ml(20ng/ml)	2.5	1.9(0.8-2.1)	0.10	2.1(1.1-2.2)	0.04

Maternal age, education, occupation and socioeconomic status was adjusted. Vitamin D3, Selenium,zinc and folic acid was associated with birth weight of the baby with adjusted odds ratio(95% CI) was 1.9(1.82-5.0), 1.6 (0.56-3.66), 1.9 (1.2-2.4) and 2.1(1.1-2.2) respectively.

Results: Qualitative Data

Table 5.14 : Coding and development of themes for responses of Pregnant women.

1. **Dietary Beliefs and Practices:** This theme encompasses beliefs about the impact of various types of food on the health of the mother and the baby, including restrictions and recommendations. Subthemes includes beliefs about specific foods (e.g., Papaya, Pineapple, Leafy Vegetables, Fruits), meal frequency and portion size, and preferences for freshly prepared meals.
2. **Role of Supplements**: This theme includes beliefs about the use of supplements like iron and folic acid tablets during pregnancy, the perceived benefits and side effects, and adherence challenges. Subthemes might include beliefs about the impact of these tablets on the baby's size and delivery, and the perceived trade-off between food and tablets.
3. **Impact of Diet on Baby's Health Post Birth**: This theme captures beliefs about the influence of the mother's diet on the health of the newborn, including potential respiratory problems, jaundice, and cough.
4. **Dietary restriction due to poverty**: This theme covers the issues related to scarcity of different food items due to lack of money.
5. **Cultural and Religious Dietary Restrictions**: This theme covers dietary restrictions based on cultural and religious beliefs, such as avoiding non-vegetarian food or certain fruits and vegetables.
6. **Diet and Physical Health of the Mother**: This theme revolves around the beliefs about the effect of diet on the mother's physical health and strength, especially concerning delivery and post-delivery recovery. Subthemes could include beliefs about the strengthening effects of specific foods and the perceived impact of diet on blood circulation and bone strength.
7. **Guidance from Health Workers**: This theme involves advice received from health workers (ASHA and ANM), indicating their influence on dietary practices during pregnancy.
8. **Diet Adaptation Due to Physical Discomfort**: This theme covers changes in diet due to physical discomforts like vomiting, nausea, indigestion, and increased hunger.

Table 5.15: Coding and development of themes for responses of Care givers .

1. **Food Intake and Recommendations**: This theme can encompass the specifics about food types, their benefits, and how they should be consumed. This includes the codes about 'Food Intake Quantity', 'Freshly Prepared Food', 'Food Recommendations', 'Food vs Supplements', 'Food Sources', and 'Food Intake'.
2. **Food Restrictions**: This theme can cover all the codes pertaining to foods that should be avoided during pregnancy. Codes like 'Food Restrictions', 'Miscarriage Concerns' and 'Newborn Health' would fall under this.
3. **Supplement Use**: This theme relates to beliefs and practices surrounding the consumption of tablets and supplements. 'Vitamin/Mineral Supplements', 'Delivery Concerns', and 'Baby Weight' would come under this theme.
4. **Dietary restriction due to poverty**: This theme covers the issues related to scarcity of different food items due to lack of money.
5. **Perceived Health Impacts**: This theme encompasses beliefs about the impact of different foods and practices on the health of the mother and baby. Codes here might include 'Impact on Baby', 'Health Benefits', 'Maternal Strength', 'Baby Health', 'Baby Activity', 'Maternal Health', and 'Baby Size'.
6. **Effects on Delivery**: Codes related to beliefs about how diet and practices during pregnancy affect delivery could be grouped under this theme. These might include 'Delivery Concerns', 'Baby Size', and 'Maternal Strength'.
7. **Specific Food Benefits and Risks**: This theme could combine codes related to the believed benefits or risks of specific food items, such as 'Dairy Intake', 'Protein Intake', 'Fruits', and 'Fluid Intake'.

DISCUSSION

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.

CHAPTER 6. DISCUSSION

Nutrients intake is very crucial for the health of pregnant women and the fetus. Inadequate nutrients consumption may cause nutritional anemia, which increases the risk of adverse pregnancy outcomes(Maternal morbidities and Low birth weight)[1]. Birth weight is a predictor for survival and it indicates the availability of public health facilities and utilization and maternal health.

In the present study, 150 apparently healthy term pregnant women participated. The proportion of Low birth weight baby was 33(22%).

Maternal Dietary Diversity and the micronutrients and birth weight of baby: Dietary diversity was found to be associated with the birth weight. Dietary diversity was correlated positively with the micronutrients levels in blood, but not statistically significant. Similar to study by Kheirouri S[2,3]. This could be because of lack of representation from protective foods in their diet, which was confirmed by the Focus group discussion. Another study conducted Teng Y etal[4], observed that Dietary diversity with different categories, including meat based, sea food based were observed to be associated with increased birth weight. The study conducted in the Mumbai urban slums found the significant association between DDS and birth weight ($p<0.05$)[5].

A cohort in Ghana by Osman SM, observed DD of pregnant women and its association with pregnancy outcomes. They found that the pregnant women consumed diet with low DD. [6].

A Systematic Review about nutrients intake, dietary diversity and the nutritional status of pregnant women, was conducted by Ndung'u (2018)[7], they

observed that higher dietary diversity score ensured the healthier diet and hence there were positive anthropometric outcomes. An another study also documented that there was a positive association between dietary diversity score in pregnant women and their nutritional status. They also observed low BMI in pregnancy were associated with low dietary diversity score, nutrient intake and it significantly negatively influenced the pregnancy outcome [7].

A Study in Ethiopia(2018), to study whether the undernutrition was determined due to Low DDS among pregnant women. This study stated that low dietary diversity score caused under-nutrition in them. They found that the pregnant women who consumed diet with low DDS were 2-4 times more often malnourished(AOR = 2.1) [8].Though we did not studied food security, socio-economic status was used as a proxy-indicator for the same.

Maternal serum Selenium and birth weight: In the present study, Serum selenium was associated with increased birth weight. It was negatively correlated with the mitochondrial DNA CN. Selenium combined with the proteins forming Seleno-proteins, it forms the part of enzymes such as glutathione per-oxidase, Thio redoxin reductase, which has the antioxidant properties, will quench the ROS, thus reducing the oxidative stress and improving the birth weight [9,10]. In the present study, the maternal serum Selenium levels with low-birth weight babies was 157±44.09 and with normal birth weight babies was 161.42±66.48. We found that 63% of low birth weight babies had the Serum selenium cutoff of 150µg/l and odd's ratio of 1.58(0.68-3.54). This observation could be due to moderate consumption of food rich in Selenium such as Spinach (11mcg/cup of cooked and blanched), Brown rice(19mcg/cup), and jowar(20mcg/100gm), while the RDA for Selenium is 60mg/day. Similarly studies conducted by Mistry HD and Tsuzuki S found that,

reduced maternal selenium levels have been shown to be associated with abortions (early pregnancy loss) and low birth weight[11,12].

The nutrient transporter receptor abilityof the placenta is affected by various factors includes micronutrient levels, hormones and placental function [13]. Furthermore, placental oxidative stress markers had been found to affect nutrients transport, by changing the expression of genes of nutrient transporters protein receptors [14,15]. In invitro studies has shown that selenium supplementation protects placenta from oxidative stress factors by increasing expressions of Selenium-containing antioxidants viz. glutathione and thio-redoxin reductase [16]. Thus one of the accepted hypothesis regarding low selenium could influence the birth weight is through the selenium-dependent anti-oxidative defence system [14,17-19].

Another selenium-dependent proteins includes iodothyronine deiodinase that influences the metabolism of thyroid hormones [20]. Thyroid hormones are also essential for regulating placental nutrient transport [21)]. Hence, another accepted hypothesis that selenium could influence fetal growth is by influencing the thyroid hormones. In the animal study, the mice were fed with a food deficient in selenium developed selenium deficiency. These mice also clinical symptoms of hyperthyriodism [increased triiodothyronine (T3) and tetraiodothyronine (T4)] in both maternal and fetal plasma [18].

Maternal serum Zinc and birth weight:In the present study, Serum Zinc was found to be associated with the birth weight. And Zinc also positively correlated with DD, and correlated negatively with Mt DNACN. About 72.7% of maternal serum of babies with LBW was below the cutoff of <200 µgm/dL. In the present study, the consumption of rich sources of food for zinc was less, but they consumed poor

sources of zinc, which is consumed as staple viz. grains/millets(2.63-3.5mg/100gm), and lentils(4.78mg/100gm) as dal, rice (0.8mg/100gm)[22,23,24]. The RDA for pregnant women is 11mg. An RCT conducted by Goldenberg found that the group who received Zinc supplementation had significantly greater birth weight compared to placebo[22].

Zinc is an important component of many anti-oxidative metallo-enzymes participating in protein and carbohydrate metabolism, nucleic acid synthesis, and antioxidant functions through the Cu/Zn superoxide dismutase [25]. Changes in zinc homeostasis has been related with various effects on pregnancy includes intrauterine growth restriction (IUGR) and LBW (26,27). Similar to our study, a study conducted by King JC and Wang H found that Zinc deficiency have been associated with various effects on pregnancy includes intrauterine growth restriction (IUGR) and LBW[26,27].

The mechanism for the association of maternal serum concentration and increased risk of LBW could be explained due to the dual function of zinc, as an anti-oxidant as well as a pro-oxidant. The placenta is equipped with antioxidants inclusive of selenium-dependent enzymes of glutathione dismutase, thioredoxin reductases, seleno-protein-P and Cu/Zn superoxide dismutase which require the optimum levels of Selenium and Zinc. Free radical ions damage mitochondria and NADPH oxidases to produce Reactive oxygen species (ROS) [28].

Zinc deficiency influences on embryonic and fetal development through reduction in cell proliferation, protein synthesis rates of tubulin polymerization and binding of hormones and transcription factors dependent on zinc-finger regions. Also zinc deficiency increase rates of cellular oxidative damage and rates of apoptosis.

Many studies shown low plasma zinc concentration in the first and third trimesters of pregnancy increased risk of malformations and low birth weight. So zinc deficiency can be considered as a teratogenic risk, thus leading to adverse fetal development. [29,30,31]

Maternal serum Vitamin B12 and Folate and the birth weight: In this study, we observed that vitamin B12 and Folate were associated with birth weight of the baby. These micronutrients are involved in the one-carbon transfer required for methylation of DNA, DNA synthesis and cell division, which is required for growth. Vitamin B12 and Folate is also required for heme synthesis, which will prevent anaemia in pregnancy. A cohort study conducted by Yuan X[32], observed adverse pregnancy outcome with Vitamin B12 and folate deficiency. In our study, we observed that the lack of compliance of Iron and folic acid tablets. This was confirmed by observing that 90 % mothers with LBW had serum levels <20ng/ml. Serum vitamin B12 of mothers of Low birth weight babies, 93.6% had <526pg/ml. Consumption of Vitamin B12 and folic acid rich food was very less in this area, due to many misconceptions about diet and supplements. Similar to our study, Mishra, J and Yagnik CS, found that maternal vitamin B12 and folic acid levels with birth weight were correlated positively. In another study conducted in Ireland, Dietary consumption of VitaminB12 and Folic acid were positively correlated with birth weight of the baby[33,34].

According to WHO, Nutritional anaemia is the most frequent nutritional deficiency disorder affecting maximum number of pregnant women worldwide [35]. During pregnancy, the requirements for erythropoiesis-related micronutrients are increased for feto-placental unit development [36,37]. Women entering the pregnancy with nutrition deficiency will jeopardize the situation, as there is an increased demand

from growing foetus. These micronutrients, which are required for erythropoiesis such as iron, folic acid, and vitamin B12 are known to cause nutritional anemia and its consequences. Micronutrients such as vitamin B12 and folic acid function as methyl donors in one-carbon metabolism which affects cell growth and differentiation by affecting DNA synthesis and epigenetic regulation. Hence, they are important regulators of fetal growth [38,39]. Vitamin B12 deficiency is more prevalent in south India has been documented [40], more so in this part of region of North Karnataka, because of inadequacy in dietary intake and the strict vegetarian diet style.

Although routine folic acid supplementation during peri-conceptional period has been adapted for prevention of Neural tube defects (NTD), continuing supplementation of Folic acid after 12 weeks of pregnancy has not shown significant reduction of Low birth weight in systematic reviews [41,42]

A study conducted in a tertiary care center in India, found that Hemoglobin levels were not associated with birthweight, [43] which is similar to observation of our study. In our study, there were no significant statistical association between the type of anemia and Birth weight of the baby with Odds ratio (95% CI): 2.01(0.71-5.64).

Maternal serum Vitamin A and the birth weight: In our study Vitamin A was associated with birth weight. And vitamin A levels were also found to be correlated with MtDNACN, but it was not significant statistically. Similar to our study, A systematic review conducted by Thome-Lyman AL, observed that vitamin A has no influence on pregnancy outcome. Vitamin A had teratogenic effects, when increased the supplementation is 4 times that of RDA, Beta-caroteniods had no teratogenic effects[44].

Maternal serum Vitamin D and Calcium and the birth weight: Vitamin D and Calcium was associated with the birth weight. But only Vitamin D was correlated with Mt DNACN. Vitamin D elicits a vaso-protective effect also through a decrease of oxidative stress increasing of anti-oxidative enzymes with effect on (NADH oxidase and ROS)[45]. Vitamin D and calcium is required for Bone mineralization which helps in increase in birth-weight of the baby. It has been considered as a nutrient of public health concern [46]. Many studies have shown a prevalence of vitaminD levels of less than 50 nmol/L in 30- 96% of pregnant women [47-53]. An RCT conducted in multi-sites observed that vitamin D supplements during pregnancy had a beneficial effects[54]. There is a relationship between vitamin D deficiency and resistance to insulin [55], which was corrected with a single dose of vitamin D injection[56]. Few studies also reported that higher long bone density in fetuses of pregnant women with adequate levels of serum vitamin D[57,58,59]. The recommendation is 1000 to 2000 mg/d of supplementation. There are no clear evidence showing influence of Vitamin D on maternal outcomes or fetal survival, birth weight, or gestational length [60], but studies on foetal bone calcification and health [61], supports at a level of at least 50 nmol/L, which indicates a need for Vitamin D supplementation for pregnant women[62-65]. Women lose 3- 5% of their bone mass during lactation which they rapidly regain it within 6 months following weaning. Calcium and/or vitamin D deficiency leads to porous and weak bones. A Cochrane Review has concluded and provided sufficient proof to show that Vitamin D and calcium supplementation significantly lowers the risk of preeclampsia and other hypertensive disease of pregnancy, especially [66].

Maternal serum Ferritin and birth weight: In our study, serum ferritin was associated with the birth weight. Ferritin is a storage form of iron. Red blood cells

need iron to form normally and carry oxygen and nutrients around the different parts of body. It is reduced in Iron deficiency anaemia. Hence its deficiency will leads to LBW. Iron supplementation is the policy, where every pregnant women is supplementation and found to be related to good pregnancy outcome. The observation was similar to observation done by Goldenberg RL etal[67]. Studies have shown that IFA supplementation in pregnancy in populations with increased prevalence of anaemia, there is an increase in birth weight[68].

Mitochondrial DNA copy number and maternal serum Micronutrients levels: In the present study, Mitochondrial DNA copy number is negatively correlated with Birth weight, Se, Zinc, Vitamin D3. Mitochondrial DNA replication is regulated by D-loop, occurs in post-mitotic cells-independent of Nuclear DNA replication, and independent of the cell cycle. Hence we could observe the effect of micronutrients at gene level, giving objectivity. Mitochondrial genome is vulnerable to oxidative stress, because it lacks histones and nuclear DNA repair mechanisms. ROS damage mitochondrial DNA, causes dysfunctional respiratory chain and compensatory increase in Mt DNA copy number. Some studies have found that micronutrient supplements will stabilize the copy number. This number has least diagnostic/prognostic value when it is done at one point of time, can be utilized to see the effect of intervention of micronutrients**[**69,70,71]. Iron is essential for the synthesis of heme, which is a mitochondrial electron transport chain component. Iron deficiency can impair mitochondrial function and reduce mtDNA copy number. Selenium's Protective Effects on Mitochondrial DNA and Biogenesis has been studied. Selenium supplementation protects mitochondrial DNA from damage and influences mitochondrial biogenesis. Folate plays a critical role in the prevention of uracil incorporation into DNA and hypomethylation of DNA. Folate deficiency

causes expression of chromosomal fragile sites, chromosome breaks, increased uracil, micronucleus formation, DNA hypomethylation and mitochondrial DNA deletions. Genomic instability is minimised. Vitamins B12, A, and D3 has a role in maintaining mtDNA copy number and integrity, and can affect mitochondrial biogenesis and membrane integrity. Zinc is a cofactors for enzymes involved in DNA repair and replication, and their deficiencies could lead to mtDNA injury and a decrease in mtDNA copy number. zinc deficiency also accelerates mitochondrial oxidative decay by inhibiting the pathway of heme biosynthesis in mitochondria, causing a deficit of heme-a. This results in oxidant leakage and accelerated mitochondrial decay, leading to DNA damage, neural decay and compensatory increase in mtDNA CN.[72]

Mitochondrial DNA copy number and Birth weight of the baby: High oxidative stress has been consistently shown to induce have an impact on the mtDNA content, and its increase is linked with IUGR. Placenta being the primary source of oxidative stress, which is affected by micronutrient levels with antioxidant property. In our study, there was a statistically significant negative correlation between mitochondrial DNA copy number and the birth weight. A study by Richard Jones, found that the birth weight was affected by increased mitochondrial DNA similar to our study.[73]

Qualitative study:

Qualitative study:

Narratives of FGD of pregnant women along with theme.

Dietary Beliefs and Practices: A 26 year old multipara women, participant 1, had the belief that large quantity of food for dinner is avioded" should eat less quantity of food in the night, as the food will compress on the baby and cause abortions. "papaya and pineapple should not be eaten in pregnancy"

Participant 3"Food should be prepared fresh everytime, before eating. its helpful for digestion of baby in stomach, food which is not freshly prepared will cause indigestion of baby, and cause pain abdomen to it.

Impact of Diet on Baby's Health Post Birth: Participant 6 mentioned that, " Fried food should be restricted, as it will cause breathing difficulty in the new born'....."drinking sugarcane juice will prevent jaundice in new born..

Diet and Physical Health of the Mother: only roti and chapatis should eat. rice should be less. as it doesnot provide energy, for pushing during delivery. roti with ghee is good to eat, it helps in strengthening of bones and muscles of mother. Excess dal will cause bloating .

Dietary restriction due to poverty: ……We get very less money from work, can't afford for all fruits and all..very rarely we get fruits.

Role of Supplements: Participant 2 told about Iron and folic acid tablets, Iron and folic acid tablets should not be taken , because my mother-in law told, these tablets make the baby big, causing difficulty in normal delivery. Participant 3 mentioned, I took only 50 tablets, out of prescribed 100 tablets, as i dont need so many tablets, i eat more food in the place of tablets...trading off the tablets for food intake. tablets iron

and folic acid was taken for few days, later discontinued because of boredom, it was causing increase in the size of belly.

Cultural and Religious Dietary Restrictions: pregnant ladies did not eat egg and non-vegetarian food, as it is religiously not allowed.

Guidance from Health Workers: jaggery and fried ground nut was given, as ASHA told. it increases blood. and ANM advised to eat more food and more sprouts and green leafy vegetables.

Diet Adaptation Due to Physical Discomfort: only freshly prepared food to be eaten. if it is not fresh, causes pain abdomen. bittergaurd and other bitter vegetables should not be eaten. it causes vomiting of baby (inside uterus) stopped eating few favorite food like palav and idli, because it was causing nausea.

Narratives of FGD of pregnant women attenders along with theme

Food Intake and Recommendations: food is given more, to make strong, with withstand the delivery.....give more jaggery, it prevents jaundice in new born.

drumstick is good, it increases blood.

Supplement Use: tablets, we never took, its not good. its given, only when she is not eating properly. taking lot of tablets, makes delivery difficult, as the baby grows in size. if baby born is big, it will not be active. small babies are good and active. taking iron and folic acid is not good, it increases baby weight and causes bp in mother. either eat good food or take tablets, not both.

Perceived Health Impacts: rice is not good, it does not make her strong to withstand delivery pains. milk and curd is given more, it makes the back(Pelvic bone) of

pregnant mother strong and increase milk secretion after delivery. milk is given with all food items. it makes the baby healthy and strong. wheat is restricted, as it causes heat.

Food Restrictions: fresh coconut is not given. papaya and pineapple is not given. papaya, pumpkin, and pineapple is not given, it causes abortion. papaya, noodles should not be given. causes abortion.

Effects on Delivery: dry fruits given, it makes the lower back strong, to bear the delivery pain. taking tablets make baby big, and delivery will be difficult.

Specific Food Benefits and Risks: milk and curd is given more, it makes the back of pregnant mother strong and increase milk secretion after delivery. Milk and curd should be diluted. Milk is OK, but curds shouldnot be given, its sour and causes vomiting in the New born.

In our study, we observed that pregnant women and their care-givers have got lot of prejudices about the pregnancy nutrition. Dietary diversity is very poor, since when the pregnancy is known to them, they will stop giving many food items, like citrus fruits, papaya, Jamoon fruit, coconut, etc. Even pulses will be reduced in intake, thinking that it will cause bloating and gas in the new born. Most of the time Dal water will be used for preparation of Sambar. Even vegetables are resticted. Care givers (Mothers and Mother-in laws) would advice regarding food inatake, encouraged to eat Roties and Chapaties more, and less of Rice. During third trimester, they discouraged the intake of more (Quantity) of food.

Food restriction was followed, thinking that full stomach will compress on the growing foetus, and reducing food intake during night. We observed certain good practices also, like giving green leafy vegetables, but it was restricted to first few days

in a week. There were restrictions for the locally available green leafy vegetables, thinking that it will cause meconium stained liquor, which is green in colour, and due to consumption of green leafy vegetables especially in the last trimester. Giving freshly prepared food every time, was practiced by most of them. Eating small and frequent amount of food, thinking that large amount of food will cause pressure on the uterus and baby. Milk and curds were given, thinking that it is good for baby and helps in good secretion of milk after delivery, but it was always diluted. Fruits (banana and watermelon) were occasionally consumed. Mangoes, oranges and citrus fruits were rarely consumed, thinking that it will cause rhinitis in new born. Vegetables consumption was very less, and there was lot of restrictions in the intake .

Compliance for the nutrient supplements were very poor.IFA tablets consumption was irregular. Few were restricting intake due to side effects, like pain abdomen, constipation etc. Lot of misconception were noted here, like nutrients supplements intake would cause increase in the bith weight of the baby, which will make the natural /Normal delivery difficult. Care givers would discourage them regarding this, giving their life experiences as examples. They discouraged them to take tablets, as it would increase the size of the baby, making delivery difficult. Even they went on believing that, its the consipiracy from health department to make convert normal deliveries to Caeserean section. Care givers gave their own experiences of giving birth to 5 to 6 healthy children, without taking any nutrient supplements.

Affordability for the food items which provides micronutrients is a big issue, since they are costly. Food items provided in Public distribution system was an advantage, it would meet a lot of calorie requirement, hence there is a chance of High calorie malnutrition and increased chances of hidden hunger(micronutrient deficiency).

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SUMMARY & CONCLUSION

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.

CHAPTER 7.

SUMMARY AND CONCLUSION

In the present study,

- In the present study Dietary diversity, Calorie intake, protein intake, Gestational weight gain was associated with Birth weight of the baby, Dietary Diversity and protein intake was found to be statistically significant.
- Serum Ferritin, Calcium. Vitamin D and Folate was significantly positive correlation with the birth weight of the baby, and Mitochondrial DNA CN was significantly negative correlated with birth weight.
- Serum Ferritin, Vitamin D, Selenium, Zn, Calcium, Folate levels were significantly lower in mothers of low birth weight baby when compared with normal weight baby.
- Antioxidant micronutrients Se, Zn, Vitamin D was negatively correlated with Mt DNA CN.
- There is a reduced affordability for the protective food items which are rich in micronutrients.
- There is a high calorie malnutrition and hidden hunger noted in our study group.
- Dietary diversity is an important factor which determines the adequate micronutrient intake. It should be increased by incorporating the food items rich in micronutrients in pregnancy.
- There is less compliance for IFA supplementation.

Public health initiatives for micronutrient deficiency

- Hidden hunger has been realised and Poshan Abhiyan has started distributing the fortified rice in 90 districts in 2022.
- Government of India was resolved to distribute fortified rice(Iron, folic acid and vitamin B12) for rest of the districts, through central government schemes by 2024 to address the issue of malnutrition among the poor.
- Bio-fortification of wheat, rice, oil and salt with iron, zinc, and vitamins will go a long way in addressing hidden hunger – or micro-nutrient malnutrition -- among the people, especially pregnant women, and help take forward the government's Poshan Abhiyaan mission.
- Fortified rice is made as per the standards fixed by the food safety regulator FSSAI, which has prescribed blending rice with three micronutrients - Iron, Folic Acid and Vitamin B12.

Recommendation:

- The compliance for IFA is very poor, can't expect compliance for any other supplements.
- Hence there is a need for fortification of food items which is consumed regularly by all with micronutrients.
- Though dietary diversity is given importance in national programme now, only the food charts with different groups are used for giving health education.
- Preparing Dietary charts/menus with good dietary diversity, with good representation of protective foods, which is acceptable to different religious group should be formed for giving health education.

Limitations of the study:

- This is a study where micronutrient estimation was done at one point of time, though mitochondrial DNA copy number gives an idea about the micronutrient levels in the past 10-20 days, gives the objectivity.

Future perspective:

- Since it is realised that micronutrient supplementation is important for normal development of foetus, triple fortified rice has been introduced recently 2022-23 in public distribution system. Further studies can be taken up to study the effect of this fortified rice for foeto-maternal outcome of pregnancy using experimental study. Further experimental studies/RCT may be conducted to study the impact of fortified rice/micronutrient supplementation on the micronutrient levels and birth weight, which will establish the causal association between micronutrient deficiency and birth weight.

ANNEXURES

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study

CHAPTER 8. ANNEXURES

Informed consent form CONSENT FORM I
INFORMATION FOR PARTICIPANTS OF THE STUDY

Title of the project: Effects of Micronutrient levels on the maternal mitochondrial DNA copy number in term pregnant women and in turn its effect on birth weight of the baby- A Mixed Methods Research Study.

1. Name, Designation, Address, Phone No. and Email ID of the Investigator:

Dr. Manjula R
Professor,
Department of Community Medicine,
S.Nijalingappa Medical College, Navanagar, Bagalkot-587102.
Cell No.: 9481981702
E-mail: drmanjulakashinakunti@gmail.com

2. Name of Guide with designation, Department, Phone No. and Email ID:

Dr. Rekha S Udgiri
Professor,
Department of Community Medicine,
Shri. B. M. Patil Medical College,
BLDE University,
VIJAYAPURA,
Cell No: 9448217015
Email: drrekhaudgiri@gmail.com

Name of Co-guide with designation, Department, Phone No. and Email ID:

Dr. AshalataMallapur
Professor and HOD
Department of OBG
S. Nijalingappa Medical College,
Bagalkot.
Phone: 9945699986
Email: drashalatamallapur@gmail.com

3.Purpose/ Objectives of this project /study:

4. a. Quantitative study:

1. To estimate the micro nutrient levels such as serum Ferritin, vitamin B12, Vitamin A, Vitamin D, Calcium, Selenium and Zinc in apparently healthy term pregnant women(≥37 weeks) and to study the causes for nutritional anemia in this area.
2. To estimate the maternal mitochondrial DNA copy number in term pregnant women using Q-PCR technique.
3. To study the correlation between the different micronutrient levels with Dietary diversity and maternal mitochondrial DNA copy number among them.
4. To study the effects of different micronutrient levels and maternal mitochondrial DNA copy number on the birth weight of the baby.

b. Qualitative study:

1. To explore the cultural beliefs and practices related to diet and nutrition in pregnancy through focus group discussions among term pregnant women, Care givers like mothers and mother-in laws .

1. **Procedure/Methods of the study:** A pretested questionnaire for obtaining basic demographic characteristics, dietary consumption by 24 hour recall method, Questionnaire related physical activity during pregnancy. Later physical examination includes general physical examination, vitals includes pulse rate, Blood pressure, Respiratory rate will be measured. An anthropometric measurement includes Height, weight, and Mid-arm circumference using standard operating procedures. About 5 ml of venous blood will be collected by venipuncture and following investigations will be done Complete Blood Count(Using Penta ES 60 cell counter),, Serum ferritin, serum Vitamin B12, serum Vitamin A, Vitamin D and Zinc (SnibeMaglumi 1000 autoanalyser), and peripheral smear examination will be done. Maternal mitochondrial DNA copy number will be estimated on packed cell samples using the real-time qPCR assay.
2. **Expected duration of the subject participation:** 30-45minutes

3. **Expected benefits from the research to the participant:** The results of the present study will help us to understand the importance of micronutrients in the pregnancy and its beneficial effects on the growth of the foetus. It is going to give

us very important information on the specific micronutrients and its effect on the growing foetus.

4. **Any risks expected from the study to the participant**: More than minimal risk is involved.

5. **Maintenance of confidentiality of records:** The study records will be kept confidential. Your personal identity will not be revealed in any publication or release of results. Study record will be kept indefinitely for analysis.

6. **Provision of free treatment for research related injury:** Although the study procedure itself carries more than minimal risk, treatment of any unforeseeable event will be provided free of cost by the Institute to you.

7. **Compensation of the participants for disability or death resulting from such injury:** Compensation for any unforeseeable research-related injury or death resulting from such injury will be duly given to you through hospital insurance policy number 68040236170200000011.

8. **Freedom to withdraw from the study at any time during the study period without the loss of benefits that the participant would otherwise be entitled:**

   It is entirely your decision to participate in the study. If you want to discontinue from the study, you are free to leave without stating any reason. Your withdrawal would in no way result in SNMC withholding goodwill or normal medical care**.**

9. **Possible current and future uses of the biological material and of the data to be generated from the research and if the material is likely to be used for secondary purposes or would be shared with others, this should be mentioned**

   All the data and materials obtained from you will be used only for research purposes. It will not be used for secondary purposes nor will it be shared with others**.**

10. **Name, Designation, Address, Phone No. and Email ID of the Investigator:**

    Dr. Manjula R
    Associate Professor,
    Department of Community Medicine,
    S.Nijalingappa Medical College, Navanagar, Bagalkot-587102.
    Cell No.: 9481981702
    E-mail: drmanjulakashinakunti@gmail.com

10. Name of Guide with designation, Department, Phone No. and Email ID:

Dr. Rekha S Udgiri
Professor,
Department of Community Medicine,
Shri. B. M. Patil Medical College,
BLDE University,
VIJAYAPURA,
Cell No: 9448217015
Email: drrekhaudgiri@gmail.com

11. Name of Co-guide with designation, Department, Phone No. and Email ID:

Dr. AshalataMallapur

Professor and HOD

Department of OBG
S. Nijalingappa Medical College,
Bagalkot.
Phone: 9945699986
Email: drashalatamallapur@gmail.com

12. Contact details of Chairman of the IEC for appeal against violation of rights

Dr. S.L. Hoti,
Director Grade Scientist (Scientist G),
ICMR-National Institute of Traditional Medicine
Belgagavi- 590010
Phone No. 0831-2477477
Fax. 0831-2475479

CONSENT FORM II
PARTICIPANT CONSENT FORM

Participant's name:

Address:

PhoneNo.:

Email ID:

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number in term pregnant women and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.

The details of the study have been provided to me in writing and explained to me in my own language. I confirm that I have understood the above study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided. Such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the above study.

Signature of the Participant: _____ Date: _____

Signature of the Witness: _____ Date: _____

Effects of Micronutrient levels on the maternal mitochondrial DNA copy number in term pregnant women and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.

PROFORMA FOR COLLECTION OF DATA

Name:	**Mother Card No:**
Age:	**Husband's age:**
Address:	**Husband's education:**
Education:	**Husband's occupation:**
Occupation:	**Type of marriage:**
Duration of marriage:	**Age at marriage:**
Family composition:	**Type of family:**
Per-capita income:	**Obstetric score:**

Obstetric history:
Details of present pregnancy:
1st Trimester:
IInd Trimester:
IIIrd Trimester:
Diet history by 24 hour recal :Morning Breakfast:……..... Mid morning:..... Afternoon:..... Lunch:.....Evening Snacks:.....Night:.....
Dietary Diversity score:

Do you consume food that is provided in Anganawadi: under Matrupoorna scheme: Y/ N

Did you receive Iron and folic acid tablets: yes/No. If Yes how many??

General Physical Examination:
Pallor :…Icterus:…Clubbing:… Cyanosis:… Lymphadenopathy:…Edema:.....

Vitals:
BP Pulse:…….. Respiratory Rate :…… Temperature:…….

Systemic Examination:
RS :
CVS :
Per abdomen:
CNS:
Investigations:
CBC
Serum Ferritin
Serum Vitamin B12
Vitamin A
Vitamin D
Serum Zinc.
Serum Selenium

Serum Calcium
Peripheral blood smear
Maternal Mitochondrial DNA Copy Number using Q-PCR.
Focus Group Guide

The purpose of this focus group (Pregnant women) is:

- To explore the participants' experiences and understanding of Dietary practices during pregnancy through a group discussion.

These questions are a guide only; we would explore topics as they arise.

Thank you all for agreeing to participate today.

We're going to start by talking about a Dietary practices in pregnancy?	*What do you understand about this ?*
What impact might Nutrition have on a woman's pregnancy and her baby?	*How might it affect the mother?* *How might it affect the baby?*
What concerns do you have about dietary intake during pregnancy?	*What do you understand about this?* *Can you tell me any more about this?*
Do you restrict to eat any food item or items during pregnancy?	*Can you tell me more about this?* *List all the food items you restrict?*
Do you consume any food item or food items more in pregnancy?	*Can you tell me more about this?* *List all the food items you restrict?*
Did you change your dietary pattern after knowing that you are pregnant?	*What do you understand about this?* *Can you tell me any more about this?*
Did you had any specific likings for food items during pregnancy?	*Can you tell me more about this?* *List all the food items you like?*
Did you had any specific likings for non-food items during pregnancy?	*Can you tell me more about this?* *List all the non-food items you like?*
Did you consume all the tablets given during pregnancy?	*How might it affect the mother?* *How might it affect the baby?*
Did you choose to not consume/partially Consume the number of tablets during pregnancy?	*How might it affect the mother?* *How might it affect the baby?*
Do you think, it is important to take all the tablets given during pregnancy?	*How might it affect the mother?* *How might it affect the baby?*

The purpose of this focus group (Pregnant women care taker (Mother or Mother-in-law) is:

- To explore the participants' experiences and understanding of Dietary practices during pregnancy through a group discussion.

These questions are a guide only; we would explore topics as they arise.

Let's going to start by talking about the Dietary practices in pregnancy?	*What do you understand about this ?*
What impact might Nutrition have on a woman's pregnancy and her baby?	*How might it affect the mother?* *How might it affect the baby?*
What concerns do you have about dietary intake during pregnancy?	*What do you understand about this?* *Can you tell me any more about this?*
Do you restrict to eat any food item or items during pregnancy?	*Can you tell me more about this?* *List all the food items you restrict?*
Do you see any importance to change the dietary pattern after knowing that women are pregnant?	*What do you understand about this?* *Can you tell me any more about this?*
Do you consume any food item or food items more in pregnancy?	*Can you tell me more about this?* *List all the food items you restrict?*
Do you know about any specific likings for food items during pregnancy?	*Can you tell me more about this?* *Can You list them?*
Do you know about any specific likings for non-food items(PICA) during pregnancy?	*Can you tell me more about this?* *List all the non-food items you know?*
Is it important to consume all the tablets given during pregnancy?	*How might it affect the mother?* *How might it affect the baby?*
Is it correct to choose to not consume/partially Consume the number of tablets during pregnancy?	*How might it affect the mother?* *How might it affect the baby?*
Do you think, it is important to take all the tablets given during pregnancy?	*How might it affect the mother?* *How might it affect the baby?*

BLDE (DEEMED TO BE UNIVERSITY)

Annexure -I

BLDE (DEEMED TO BE UNIVERSITY)

Annexure -I

PLAGIARISM VERIFICATION CERTIFICATE

1. Name of the Student: **Dr. Manjula R** Reg No: **18PhD003**
2. Title of the Thesis: **"Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study"**
3. Department: **Community Medicine**
4. Name of the Guide & Designation: **Dr. Rekha S Udigiri,** Professor Community Medicine, Shri. B. M. Patil Medical College, BLDE (Deemed to be University), Vijayapura
5. Name of the Co Guide & Designation: **Dr. Ashalata A. Mallapur,** Professor and HOD, Department of OBG, S. Nijalingappa Medical College, Navanagar, Bagalkot.

The above thesis was verified for similarity detection. The report is as follows:

Software used:**URKUND** / DrillBit Date: 11·01·2024.

Similarity Index (8% **): (**Eight Percent**) Total word Count:** 7866

The report is attached for the review by the Student and Guide. The plagiarism report of the above thesis has been reviewed by the undersigned. The similarity index is below accepted norms. The similarity index is above accepted norms, because of following reasons:

The thesis may be considered for submission to the University. The software report is attached.

Signature of the Guide Name & Designation	Signature of Co-Guide Name & Designation	Signature of Student Name & Designation

Verified by (Signature)
Name & Designation
Dr. Prasanna Kumara BM
BLDE (Deemed to be University)
Shri B M Patil Medical College
Vijayapura - 586103

2

Ethical clearance certificate

BLDE

(DEEMED TO BE UNIVERSITY)

Declared as Deemed to be University u/s 3 of UGC Act, 1956

The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 409/2019-20 27th December, 2019

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The ethical Committee of this University met on 27th December, 2019 at 11.00 a.m. scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

Title: *Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study.*

Name of the Principal Investigator: Dr.Manjula R., Ph.D. Scholar (18PHD 003), Community Medicine.

Dr. Santoshkumar Jeevanagi
Chair person
IEC, BLDE (DU),
VIJAYAPURA

Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA

MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303 , Website: www.bldedu.ac.in, E-mail:office@bldedu.ac.in
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B.V.V. Sangha's
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(Recognized by Medical Council of India and Affiliated to Rajiv Gandhi University of Health Sciences, Karnataka)
SNMC-INSTITUTIONAL ETHICS COMMITTEE ON HUMAN SUBJECTS RESEARCH
☎08354-235340 Fax: 08354-235360 Website: www.snmcbgk.in Email: iechsrsnmcbgk@gmail.com

Office of the Institutional Ethice Committee

Ref. No. : **Date:**

File No: SNMC/IECHSR/2019-20/A-68/1.1 Date: 05/02/2020

To:

Dr Manjula R

Associate Prof of Community Medicine

SNMC, Bagalkot

Topic of Protocol: Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study

Subject: Approval for conducting the above mentioned study & related documents by IEC.

Dear Dr Manjula

The Ethics Committee (EC) meeting of SNMC was held on 25-01-2020 from 10.30 AM onwards in the Hall of Medical Education Department of S. Nijalingappa Medical College & Hanagal Shri Kumareshwar Hospital & Research Centre, Bagalkot.

Following members of the committee were present:

1. Dr. S. L Hoti, Scientist-G,Director grade scientist ICMR-NITN, Belgaum.	**Chairman**
2. Dr.Yasmeen Maniyar , Professor & HOD of Pharmacology , SNMC, Bagalkot.	**Member**
3. Dr Anita Herur Professor of Physiology,SNMC,Bagalkot	**Member**
4. Dr Ashalata Mallapur Prof &HoD OBG, SNMC,Bagalkot	**Member**
5. Dr Chandrashekar V M Professor of Pharmacology HSK Pharmacy college	**Member**
6. Dr. Chandrashekharayya S. Hiremath, Professor of ENT, SNMC, Bagalkot	**Member**
7. Dr Manjula R Associate professor of Community Medicine	**Member**
8. Mr. Vittal Kamble , Near Vallabhbai chowk, Bagalkot.	**Member**
9 Mr.Jagdeesh, Budihal, advocate Navanagar , Bagalkot.	**Member**
10. Mr. D. G. Bannur, Holebasaveshwar Nilaya, 10th Cross, Vidyagiri, Bagalkot.	**Member**
11. Dr.Vijayamahantesh SN Professor of Forensic Medicine, SNMC, Bagalkot.	**Member Secretary**

B.V.V. Sangha's
S. Nijalingappa Medical College & Hanagal Shri Kumareshwar Hospital & Research Centre
Navanagar, Bagalkot-587102, Karnataka State, India.
(Recognized by Medical Council of India and Affiliated to Rajiv Gandhi University of Health Sciences, Karnataka)
SNMC-INSTITUTIONAL ETHICS COMMITTEE ON HUMAN SUBJECTS RESEARCH
☎08354-235340 Fax: 08354-235360 Website: www.snmcbgk.in Email: iechsrsnmcbgk@gmail.com

Office of the Institutional Ethice Committee

Ref. No. : Date:

The Ethical Committee of SNMC reviewed the following documents:

1. **Research Protocol** entitled Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study submitted by Dr Manjula R Associate Prof of Community Medicine S N Medical college, Bagalkot
2. **Information sheet for participants of the study (Consent Form –I) and (Consent Form –I) of the protocol** Effects of Micronutrient levels on the maternal mitochondrial DNA copy number (mtDNA-CN) in apparently healthy term pregnant women (≥37 weeks) and in turn its effect on birth weight of the baby-A Mixed Methods Research Study

NOTE: It is to be noted that neither PI nor any of the proposed study team members were present during the decision-making procedures of the Ethics Committee, and members who are independent of the Investigator, have voted/ provided opinion on the trial.

Dr Manjula R being PI abstained from voting.

Discussion points:
After reviewing the documents submitted by the Principal Investigator, the Committee has decided to grant approval for conducting the above mentioned study.

You are requested to report to the Ethics Committee the Following:
1. Progress of the study at the end of 4 months.
2. Provide a report to the Ethics Committee on completion of the study.

The Ethics Committee of SNMC follows procedures that are in compliance with the requirements of ICH (International Conference on Harmonization) related to GCP (Good Clinical Practice), schedule Y and all other applicable Indian regulations.

If you have any Questions concerning the above, please feel free to contact the undersigned.

Thanks & Regards,

(Dr. Vijayamahantesh SN)
Member Secretary
Member Secretary,
IEC
S. N. Medical College
BAGALKOT

p 2/2

Paper presentation certificates

REVA UNIVERSITY
Bengaluru, India

Certificate of Appreciation

School of Allied Health Sciences

This is to certify that

Dr./Ms./Mr. Manjula R

of BLDE Medical college, Bagalkot

has presented Oral/~~Poster~~ presentation titled Effect of maternal blood ..... birth weight of the baby

in the International Conference on **"Recent Advances in Allied Health and Biological Sciences Research"** held on 18th and 19th November 2022.

Convener
School of Allied Health Sciences

Registrar
REVA University

Vice Chancellor
REVA University

AMRITA VISHWA VIDYAPEETHAM Institute of Medical Sciences

AMRITACON - 2020

AMRITA INTERNATIONAL PUBLIC HEALTH e - CONFERENCE 2020

Amalgamation of Medicine, Research and Innovative Technology for Action

HEALTHY AGEING : KNOWLEDGE TO ACTION

Certificate of Appreciation

Awarded to

Manjula R.

for presenting **Scientific Paper** / ~~**Scientific Poster**~~ titled

Effect of Dietary Diversity on the Nutritional status in pregnant women and in turn its effect on birth weight of the baby

during the AMRITA International Public Health e-Conference 2020 held on 4th and 5th December 2020

at Amrita Institute of Medical Sciences and Research Center, Kochi, Kerala.

Dr. Prem Nair	Dr. Vishal Marwaha	Dr. Beena K.V.	Dr. George Paul	Dr. Aswathy S.
Medical Director	Principal School of Medicine	Addl. Medical Superintendent	Organizing Chairperson	Organizing Secretary

AMRITA VISHWA VIDYAPEETHAM Institute of Medical Sciences

AMRITACON - 2020

AMRITA INTERNATIONAL PUBLIC HEALTH e - CONFERENCE 2020

Amalgamation of Medicine, Research and Innovative Technology for Action

HEALTHY AGEING : KNOWLEDGE TO ACTION

Certificate of Appreciation

Awarded to

Manjula R

for presenting ~~**Scientific Paper**~~ / **Scientific Poster** titled

A study of correlation between pre-pregnancy weight , gestational weight gain and physical activity to the birth weight of the baby

A Hospital based Crosssectional study

during the AMRITA International Public Health e-Conference 2020 held on 4th and 5th December 2020

at Amrita Institute of Medical Sciences and Research Center, Kochi, Kerala.

Dr. Prem Nair	Dr. Vishal Marwaha	Dr. Beena K.V.	Dr. George Paul	Dr. Aswathy S.
Medical Director	Principal School of Medicine	Addl. Medical Superintendent	Organizing Chairperson	Organizing Secretary

PUBLICATIONS

Paper 1: Manjula R. Rekha U, Ashalatha M, S V. Kashinakunti. A study of Correlation between pre- pregnancy weight, Gestational weight gain, erythropoiesis-related micronutrient levels in term pregnant women and the birth weight of the Baby. Biomedicine 2022 ;42(6):1356-60.

Paper 2: Manjula R, Rekha Udgiri, Ashalatha Mallapur, Sangappa V. Kashinakunti, Kavita Hiremath. Effects of Maternal blood selenium and Zinc levels on Mitochondrial DNA copy number at term and in-turn their effect on the birth weight of the Baby. Biomedicine 2023;43(1):323-328.

Paper 3: Manjula R, Rekha U, Ashalatha M. Effect of Dietary Diversity on the Nutritional Status in Pregnant Women and in turn its effect on Birth Weight of the Baby. Journal of Medical Sciences and Health (JMSH). Jan-April 2023;9(1):50-56

Biomedicine: 2022; 42(6): 1356-1360 **November - December 2022**

Original article

A study of correlation between pre-pregnancy weight, gestational weight gain, erythropoiesis-related micronutrient levels in term pregnant women and the birth weight of the baby

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[1]Department of Community Medicine, BLDE Medical College, BLDE deemed to be University, Bijapur, Karnataka, India

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(Received: September 2022 Revised: December 2022 Accepted: December 2022)

Corresponding author: **Manjula R.** Email: drmanjulakashinakunti@gmail.com

ABSTRACT

Introduction and Aim: Adequate Nutrients intake is very crucial for the health of pregnant women and the fetus. Inadequate intake will cause maternal anemia, increase the likelihood for the Antepartum and postpartum maternal complications, fetal growth restriction resulting in low birth weight (LBW) babies. Micronutrients which are involved in erythropoiesis may affect the birth weight of the newborn. The objective was to study the correlation between the pre-pregnancy weight, gestational weight gain, erythropoiesis-related micronutrient levels in apparently normal term pregnant women and birth weight of the baby.

Materials and Methods: An Institutional ethical clearance was obtained. A total of 168 term pregnant women were selected for the present study. They were apparently normal term pregnant women who were willing to participate and who gave consent were included. Maximum of 86.9% of study subjects belonged to the age group of 21-30 years. The present hospital based cross sectional study was conducted in the department of OBG of tertiary care centres of North Karnataka between December 2019 to February 2020. Sample size was estimated to be 158.

Results: In this study, we found that there is a statistically significant positive correlation between the maternal weight in first trimester, maternal weight at term, gestational weight gain, maternal serum ferritin, Serum total Iron binding capacity, Serum Vitamin B12, and Serum folic acid. The association was found between the type of anemia and birth weight of the baby with Odds ratio (95% CI): 2.01(0.71-5.64), p=0.08, though it is not statistically significant.

Conclusion: Hence there is an association between pre-pregnancy weight, gestational weight gain and erythropoiesis-related micronutrients and birth weight of the baby. Hence it is very important to provide nutrition education to pregnant women along with iron and folic acid supplementation, other micronutrients which affect the pregnancy outcome.

Keywords: Erythropoiesis-related micronutrients; gestational weight gain; birth weight.

INTRODUCTION

Adequate Nutrients intake is very crucial for the health of pregnant women and the foetus. Inadequate intake will cause the maternal anemia, increases the likelihood for the Antepartum and postpartum maternal complications, fetal growth restriction resulting in low birth weight (LBW) babies. (1). The birth weight is an important indicator of the status and services of public health, maternal health, and nutrition. Globally low birth weight (LBW) is an important health indicator and a risk factor, which contributes to 40-60% of infant mortality (1). Adequate maternal nutrition is crucial in determining the birth weight of newborn (2).

Micronutrients are crucial for normal intrauterine growth and development of the foetus and its deficiencies due to maternal inadequate intake have been found to be associated with intrauterine growth retardation, resulting in small gestational age (SGA) infants. Micronutrients have many functions; antioxidant process, interaction with intercellular signaling protein transcriptional regulation, cell proliferation etc., (3). Micronutrient deficiencies in pregnancy is an important global public health problem, yet the full extent of their burden and implications on health are not clear, because it is not routinely estimated during pregnancy. Women in low and middle-income countries will become malnourished and the extra requirement of nutrients can intensify micronutrient deficiencies with adverse

health consequences for both mother and the baby. Unlike protein-energy malnourishment, the signs and symptoms of micronutrient deficiency are not always acutely visible; it is therefore considered as hidden hunger, which is synonymously used for micronutrient deficiency (4).

Anemia during pregnancy is an important public health concern and it is linked with adverse maternal and perinatal outcomes. According to WHO, anemia is considered of a very high public health significance, if its prevalence rate is more than 40%. The causes for anemia during pregnancy in developing countries are multifactorial: these include micronutrient deficiencies such as iron, folate, vitamin A and Vitamin B12 deficiency, Malaria and hookworm infections and other chronic infections. In fact, nutritional Anemia in pregnancy due to iron deficiency is only 50%, rest all are caused due to other micronutrient deficiencies. But the policy according to the National programme Reproductive, maternal, neonatal, child health and adolescent health (RMNCH) is to supplement with iron and folic acid (IFA) during pregnancy (5). This would lead to inadequate treatment of anemia, and leads to adverse pregnancy outcomes.

Nutritional anaemia is the most frequent nutritional deficiency disorder affecting more than a large number of pregnant women worldwide (6). During pregnancy, the requirements for erythropoiesis-related micronutrients are increased for feto-placental unit development (7,8). Women entering the pregnancy with nutrition deficiency will jeopardize the situation, as there is an increased demand from growing foetus. These micronutrients, which are required for erythropoiesis such as iron, folic acid, and vitamin B12 are known to cause nutritional anemia and its consequences.

Hence the study was undertaken to study the correlation between the pre-pregnancy weight, gestational weight gain, erythropoiesis-related micronutrient levels in apparently normal term pregnant women and birth weight of the baby.

MATERIALS AND METHODS

This study was conducted in the outpatient department and Labor room of OBG Department at Tertiary Care centre of North Karnataka, India. An Institutional ethical clearance (BLDE(DU)/IEC/409/2019-20 dated 27th December 2019) was obtained. We included apparently healthy term pregnant women of ≥37 weeks. Those pregnant women who give consent to participate in the study. Multiple pregnancies like twins are diagnosed anytime during pregnancy or after delivery. Chronic medical conditions such as hepatic, renal, cardiovascular diseases, women who are known HIV, hepatitis B infection, hypertension including preeclampsia and diabetes mellitus including gestational diabetes were excluded. Babies born with severe congenital anomalies were excluded for analysis.

Sample size estimation was done using Openepi software version 2.3.1. At 95% confidence limits, and at 80% Power of the study, Zα is the standard table value for 95% CI =1.96, $Z_{1-\beta}$ is the Standard table value for 80% Power = 0.84 According to the study conducted by Wadhwani (9). The correlation coefficient between the third trimester Vitamin B-12 levels and birth weight of the baby=0.22. Formula used= $N = ([Z_\alpha + Z_\beta]/C)^2 + 3$, where C= 0.5*ln ([1 + r]/ [1 − r]) Sample size estimated is 159, which is rounded off to 165.

After obtaining ethical clearance, the present study was conducted in the outpatient department(OPD) and labor room of OBG Department of Tertiary care Centre of North Karnataka. Informed consent was obtained from the study subjects. All apparently healthy term pregnant women(≥37 weeks) who were coming to OPD for antenatal care and those who were admitted in labor rooms for safe confinement, inclusion and exclusion criteria were included in the study. The pregnant women who were willing to participate by giving the informed consent for the present study were included for the study.

A pretested questionnaire for obtaining basic demographic characteristics, dietary consumption by 24-hour recall method, questionnaire related physical activity during pregnancy. Later physical examination includes general physical examination, vitals include pulse rate, blood pressure, respiratory rate was measured. An anthropometric measurement includes Height and weight, using standard operating procedures. Weight and Height were measured using UNICEF SECA weighing scale and UNICEF SECA microtoise respectively, following the standard protocol. Investigations done during (≥37 weeks): About 5 ml of venous blood was collected by venipuncture and following investigations will be done Complete Blood Count (Using Penta ES 60 cell counter) and Peripheral smear examination was done Serum ferritin, Serum iron, serum total iron binding capacity, serum transferrin, serum vitamin B12, Serum folic acid estimation was done using Autolumo 1000, fully automated analyzer which works based on the principle of Chemiluminescence (CLIA) method. Low birth weight (LBW) is defined as the birth weight of a newborn is less than 2500 gm. IUGR/SGA was diagnosed if the birth weight is below the 10th centile for gestational age at delivery.

Statistical analysis

Data was analyzed statistically using SPSS package 'IBM SPSS Statistics for Windows, version 19 (IBM Corp., Armonk, N.Y., USA). Data was expressed as percentages and mean ±standard deviation (SD) for

qualitative and quantitative data respectively. Later the data was statistically analyzed using statistical tests such as chi-square test, odds ratio (95% CI) and student unpaired t-test. Pearson's correlation coefficient was calculated for the variables. The p value(<0.05) was considered as significant.

RESULTS

Table 1: Socio-demographic and baseline characteristics of the study population

Variables	Value	No.	%
Age	<= 20	6	3.6
	21 - 30	146	86.9
	31+	16	9.5
Gravida	1	84	50.0
	2	47	28.0
	3	31	18.5
	4	4	2.4
	5	2	1.2
Socio-economic status (modified BG Prasad classification)	1	6	3.6
	2	38	22.6
	3	81	48.2
	4	42	25.0
	5	1	.6
Birth weight of baby	<= 2.4900(LBW)	46	27.3
	2.4901+(Normal)	122	72.7
	Total	168	100

In this study, a total of 168 apparently normal term pregnant women participated in the present study.
In this study, a total of 168 apparently normal term pregnant women participated in the present study. Maximum of 86.9% of study subjects were in the age group of 21-30 years. About 50% of the women were primigravids. About 70% of them belonged to class 2 and class 3 socio-economic status according to Modified B G Prasad classification. In the present study, 27.3% of them gave birth to low-birth-weight babies (Table 1).

In this study, we found that maternal weight during the first trimester and at term, gestational weight gain, Folic acid was found to be statistically significant between normal birthweight and low birth weight (Table 2). In the present study, there is a statistically significant positive correlation between the maternal weight in 1st trimester, maternal weight at term, gestational weight gain, serum ferritin, total iron binding capacity, vitamin B12, and folic acid (Table 3; Fig. 1). There was no statistically significant association between the type of anemia and Birth weight of the baby with Odds ratio (95% CI): 2.01(0.71-5.64), p=0.08 (Table 4).

Table 2: Effect of maternal weight and erythropoiesis-related micronutrient levels on birth weight of the baby

Baby birth weight (Binned)		N	Mean	Std. Deviation	t	p
Age	<= 2.4900(LBW)	46	25.39	3.543	-.415	.679
	2.4901+(Normal)	122	25.64	3.514		
Gestational age in weeks	<= 2.4900(LBW)	46	38.367391	.9791276	-1.559	.121
	2.4901+(Normal)	122	38.627273	.9555975		
Maternal weight in 1st trimester	<= 2.4900(LBW)	46	49.409	7.6148	-1.825	.070
	2.4901+(Normal)	122	52.025	8.5073		
Maternal weight at term	<= 2.4900(LBW)	46	57.96	7.857	-3.00	.003
	2.4901+(Normal)	122	62.26	8.449		
Gestational weight gain	<= 2.4900(LBW)	46	8.5273	5.00845	1.85	.050
	2.4901+(Normal)	122	9.9744	3.79760		
Hemoglobin g%	<= 2.4900(LBW)	46	10.995652	1.1236758	-.773	.078
	2.4901+(Normal)	122	11.380992	1.3005328		
Serum ferritin mg/dl	<= 2.4900(LBW)	46	13.738421	12.8862089	-.208	.836
	2.4901+(Normal)	122	14.567656	15.8831159		
Serum Iron µg/dl	<= 2.4900(LBW)	46	118.777778	126.6235112	1.675	.098
	2.4901+(Normal)	122	84.800000	54.2391211		
Total Iron Binding Capacity µg/dl	<= 2.4900(LBW)	46	420.610714	97.0929038	-.804	.424
	2.4901+(Normal)	122	445.508596	105.3296436		
Transferrin %	<= 2.4900(LBW)	46	19.572143	13.5834918	.972	.335
	2.4901+(Normal)	122	16.444364	9.9525174		
Vitamin B12 pg/ml	<= 2.4900(LBW)	46	247.50	166.235	.432	.667
	2.4901+(Normal)	122	229.51	131.688		
Folic acid ng/ml	<= 2.4900(LBW)	46	3.058	7.0963	3.154	.002
	2.4901+(Normal)	122	.728	1.8786		

Table 3: Correlation between maternal weight and erythropoiesis-related micronutrient levels and the birth weight of the baby

Variables		**Baby birth weight**
Maternal weight in 1st trimester	Pearson Correlation(r)	.209*
	Sig. (p)	.010
Gestational weight gain	Pearson Correlation(r)	.315**
	Sig. (p)	.001
Maternal weight at term	Pearson Correlation(r)	.356**
	Sig. (p)	.001
Hb%	Pearson Correlation(r)	.080
	Sig. (p)	.329
Serum ferritin µg/dl	Pearson Correlation(r)	-.173*
	Sig. (p)	.035
Serum Iron µg/dl	Pearson Correlation(r)	.068
	Sig. (p)	.407
TIBC µg/dl	Pearson Correlation(r)	-.224**
	Sig. (p)	.006
Transferrin %	Pearson Correlation(r)	-.058
	Sig. (p)	.482
Vitamin B12 pg/ml	Pearson Correlation(r)	.223
	Sig. (p)	.006
Folic acid ng/ml	Pearson Correlation(r)	.235**
	Sig. (p)	.004

Table 4: Relationship between type of anemia and birthweight of the baby

Baby birth weight	**Peripheral blood**		**Total**
	Microcytic Hypochromic	**Normocytic Normochromic**	
<= 2.4900(LBW)	7	39	46
	15.2%	84.8%	100.0%
2.4901+(N0rmal)	10	112	122
	8.3%	91.7%	100.0%

Chi square=1.81, p=0.08; Odds ratio (95% CI): 2.01(0.71-5.64)

DISCUSSION

Adequate nutrient intake is very crucial for the health of pregnant women and the foetus. Inadequate intake will cause maternal anemia, increase the likelihood for the Antepartum and postpartum maternal complications, fetal growth restriction resulting in low birth weight (LBW) babies. Globally LBW newborn babies contribute to 40-60% of infant mortality rate (1). Maternal nutrition is a very important factor in determining the birth weight of newborns (2). A total of 168 apparently normal term pregnant women participated in the present study.

In this study, we found that maternal weight during the first trimester and at term, gestational weight gain, Folic acid was found to be statistically significant between normal birthweight and low birth weight (Table 2). There is a statistically significant positive correlation between the maternal weight in first trimester (pre-pregnancy weight), maternal weight at term, gestational weight gain, Serum ferritin, Total Iron binding capacity (TIBC), Vitamin B12, and folic acid (Table 3). A study conducted in China, to study the effects of pre-pregnancy body mass index and gestational weight gain on neonatal birth weight found that neonatal birth weight is positively affected by both maternal pre-BMI and gestational weight gain (10) which is like the present study. Pre-Pregnancy weight and gestational weight gain was significantly correlated with the birthweight of the baby, like our observation (11,12).

Micronutrients such as vitamin B12 and folic acid function as methyl donors in one-carbon metabolism which affects cell growth and differentiation by affecting DNA synthesis and epigenetic regulation. Hence, they are important regulators of fetal growth (9,13). Vitamin B12 deficiency is more prevalent in south India has been documented (14), more so in this part of region of North Karnataka, because of inadequacy in dietary intake and the strict vegetarian diet style.

Like our study, there was a positive correlation of maternal vitamin B12 and folic acid levels with birth weight of the baby (9, 15, 16). In another study conducted in Ireland, Dietary consumption of Vitamin

B12 and Folic acid were positively correlated with birth weight of the baby (3). Although routine folic acid supplementation during per-conceptional period has been adapted for prevention of Neural tube defects (NTD), continuing supplementation of Folic acid beyond 12 weeks of pregnancy has not shown significant reduction of Low birth weight and preterm term deliveries in systematic reviews (17,18).

A study conducted in a tertiary care center in India, found that Hemoglobin levels were not associated with the birthweight of the baby (19). which is like our study. In the present study, there was no statistically significant association between the type of anemia and Birth weight of the baby with Odds ratio (95% CI): 2.01(0.71-5.64).

Iron supplementation and its impact on reduction of maternal anemia and Iron deficiency to 70% has been studied in the previous studies. The impact on pregnancy outcomes in terms of maternal and neonatal outcomes is less clear, with no statistically significant results, which is like our study (20).

CONCLUSION

Hence there is clear evidence that there is an association between pre-pregnancy weight, gestational weight gain and erythropoiesis-related micronutrients and birth weight of the baby. Hence it is very important to provide nutrition education to pregnant women along with iron and folic acid supplementation, other micronutrients which affect the pregnancy outcome. Nutrition education should include the facts about the importance of healthy weight during periconceptional period and gestational weight gain. Apart from the calories and protein intake, it is important to incorporate diverse food groups. Healthy diets with the most diverse foods, and balance in eating food provides micronutrients required for healthy mother and baby. Hence it reduces the prevalence of low birthweight.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

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Research article

Effects of maternal blood selenium and zinc levels on mitochondrial DNA copy number at term and in-turn their effect on the birthweight of the baby

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ABSTRACT

Introduction and Aim: The micronutrients such as selenium and zinc have the antioxidant property. They are cofactors of antioxidant enzymes, regulate the inflammatory response by counterbalancing the oxidative stress. Reduced maternal selenium and zinc levels have been shown to be associated with early pregnancy loss and low birthweight. The aim is to study the effects of maternal blood selenium and zinc levels on mitochondrial DNA copy number at term and in-turn their effect on the birth weight of the baby.

Methods: An Institutional ethical clearance was obtained and the present hospital based cross sectional study was conducted in the OBG department of a medical college of North Karnataka between December 2019 to February 2022.

Results: A total of 150 term pregnant women participated in the study. There was a slightly increased serum level of zinc and selenium found in the term pregnant mothers who gave birth to normal birth weight babies when compared to low-birth-weight babies, though this difference was not statistically significant. Median (IQR) values of Delta CT values of Mitochondrial DNA copy number in low-birth-weight babies were 3.07(1.7-5.74) and in normal birth weight babies was 3.71 (0.83-4.4). The difference in median values was not statistically significant (p=0.57). We observed a positive correlation between the maternal zinc, selenium, and mitochondrial DNA copy number with birth weight of the baby. Though the correlation between Delta CT means and birthweight of the baby is found to be statistically significant.

Conclusion: In the present study, apparently healthy pregnant women participated. The serum selenium and zinc levels were found to be within normal limits according to lab reference values. There was an increase in Mitochondrial DNA copy number in the present study and it was positively correlated with the birthweight of the baby.

Keywords: Mitochondrial DNA copy number; maternal selenium; maternal zinc; birthweight.

INTRODUCTION

Nutrient intake is very crucial for the well-being of pregnant women and the fetus. Birth weight is an important indicator of the status of public health, maternal health, and nutrition. Globally low birth weight is an important risk factor contributing to infant mortality (40-60%; 1). Maternal nutrition is an important determinant for the birth weight of neonate (2).

In nutrition, Micronutrients are considered as an important factor for normal growth and development of the fetus. Micronutrient deficiencies have been seen to be associated with intrauterine growth retardation (IUGR) and small for gestational age (SGA) infants. Micronutrients have many functions: antioxidant process, interaction with intercellular signaling protein transcriptional regulation, cell proliferation etc., (3). The protein-energy undernourishment is clinically evident and acutely visible, whereas the health impacts of micronutrient deficiency are not always acutely visible; Hence this micronutrient deficiency is also synonymously called as hidden hunger (4).

Micronutrients such as selenium and zinc have antioxidant property. They are cofactors of antioxidant enzymes, regulate the inflammatory response by counterbalancing the oxidative stress (5). Selenium is a trace element which is bound and present in the seleno-proteins, which includes glutathione peroxidase, thioredoxin reductases and seleno-protein-P (5). Selenium is also an important part of the key antioxidative enzyme glutathione peroxidase and iodothyronine deiodinases D1, D2 and D3, at their active sites (6). Glutathione peroxidases have an impact on redox status and has a key role in regulating oxidative stress, while the iodothyronine deiodinases have key roles thyroid homeostasis by regulating circulating and intracellular levels of thyroid hormones (T3 and T4; 7) Reduced maternal selenium levels have been shown to be associated with abortions (early pregnancy loss) and low birth weight (LBW; 8,9).

Zinc is an important component of many anti-oxidative metallo-enzymes participating in protein and carbohydrate metabolism, nucleic acid synthesis, and antioxidant functions through the Cu/Zn superoxide dismutase (10). Changes in zinc homeostasis have been associated with various effects on pregnancy includes intrauterine growth restriction (IUGR) and LBW (11,12).

Mitochondria are intra-cellular organelles which are membrane-enclosed, contributing to the functions of pyruvate and fatty acid oxidation, nitrogen metabolism, heme biosynthesis and apoptosis regulation (13). This organelle functions either through transcription factors or through retrograde regulation in mitochondria itself. Transcription factors are influenced by environmental factors, nutrition or exercise. The stimulation of mitochondrial DNA (Mt DNA) biogenesis has been explained with oxidative stress conditions. Nutrition factors such as micronutrients which have antioxidant properties could regulate the mitochondrial DNA copy number (14).

METHODOLOGY

The present study was carried out in the outpatient department and Labor room of OBG Department at Tertiary Care Centre of North Karnataka, India. An Institutional ethical clearance (BLDE(DU)/IEC/409 /2019-20 dated 27th December 2019) was obtained. We included Apparently Healthy term Pregnant women of ≥37 weeks. Those pregnant women who give consent to participate in the study. Multiple pregnancies like twins have diagnosed anytime during pregnancy or after delivery. Chronic medical conditions such as hepatic, renal, cardiovascular diseases, women who are known to have HIV, hepatitis B infection, hypertension including preeclampsia and diabetes mellitus including gestational diabetes were excluded. Babies born with severe congenital anomalies were excluded during the time of analysis.

Sample size estimation was done using Openepi software version 2.3.1. At 95% confidence limits, and at 80% Power of the study, Zα= standard table value for 95% CI =1.96, $Z_{1-\beta}$ = Standard table value for 80% Power = 0.84 According to study conducted by Lili *et al.,* (15). The correlation coefficient between the Maternal zinc levels and birth weight of the baby=0.5. Formula used= $N = ([Z_\alpha + Z_\beta]/C)^2 + 3$, where C= 0.5*ln ([1 +*r*]/ [1 −*r*]) Sample size estimated is 120, which is rounded off to 150.

After obtaining ethical clearance, this study was conducted in the outpatient department (OPD) and Labor rooms of OBG Department of Tertiary care Centre of North Karnataka. Informed consents were obtained from the study subjects. All apparently healthy term pregnant women (≥37 weeks) who are coming to OPD for Antenatal care and those who are admitted in labor room for safe confinement, inclusion and exclusion criteria was considered for the study. The pregnant women who are willing to participate by giving the informed consent for the present study were selected for the study.

A pretested questionnaire for obtaining basic demographic characteristics, investigations done during (≥37 weeks): About 5 ml of venous blood was collected by venipuncture and following investigations will be done Whole blood is taken in EDTA bulb was stored at -20°C, and later selenium was analyzed inductively coupled plasma mass spectrometry (ICP-MS).

Zinc was estimated using Autolumo 1000, a fully automated analyzer which works based on the principle of Chemiluminescence (CLIA) method.

Quantification of Mitochondrial DNA Copy Numbers in Peripheral Blood. MtDNA in peripheral leukocytes was extracted from 1 mL of whole blood using the QIAamp Tissue Kit 250 (Qiagen Inc., Valencia, CA, USA). The relative mtDNA copy number was quantified by a real-time polymerase chain reaction (QPCR) and corrected by simultaneous measurement of the nuclear DNA (*β*-globin) using the method given by Wong and Cortopassi (16) and Liu *et al., (*17) Reactions were performed using a Lightcycler-Faststart DNA Master SYBR Green I kit. The forward and reverse primers of *β*-globin (used to amplify a 268 bp product) were 5'GAAGAGCCAAGGACAGGTAC- 3' and 5'-CAACTTCATCCACGTTCACC-3', respectively. The forward and reverse primers of the mitochondrial gene (ND1 gene) used to amplify a 153 bp product were 5'-AACATACCCATGGCCAACCT-3' and 5'-AGCGAAGGG-TTGTAGTAGCCC-3' respectively. The genomic DNA (50ng) was mixed with 10 µl SYBR Green I Master Mix that contained 10 pmol of forward and reverse primers in a final volume of 20µl. After denaturation at 95°C for 30 seconds, DNA samples were treated at 95°C for 0.1 seconds, 58°C for 6 seconds, and 72°C for 18 seconds for 40 cycles. A total of 50 ng of DNA was used and the number of PCR cycles to reach this amount of DNA was defined as the threshold cycle (Ct). The following equation was used to quantify the mtDNA copy number relative to *β*-globin: relative copy number = 2ΔCt (ΔCt = Ct*β*-globin − Ct ND1; 18).

Low birth weight is determined by the birth weight of less than 2500 g. IUGR/SGA as birth weight below the 10th centile for gestational age at delivery.

Statistical analysis

Data was analyzed statistically using SPSS package 'IBM SPSS Statistics for Windows, version 19 (IBM Corp., Armonk, N.Y., USA)'. Data was expressed as percentages and mean ±SD for qualitative and quantitative data respectively. Later it was

statistically analyzed statistical tests such as Chi-square test, odds ratio (95% CI), student unpaired t-test, Mann-Whitney U test and Pearson's correlation coefficient. The p value at 0.05 was considered as statistically significant.

RESULTS

In the present study 150 apparently normal pregnant women participated. In the present study, a total of 150 pregnant women participated in the study. Maximum of 83.3% were in the age group of 21-30 years. About 16.7 % were from rural areas. 6.7% were illiterate, whereas 54% of them had studied up to a degree. About 62% of them were *primigravida*. 65.3% of them delivered vaginally. The birth weight of 22% of neonates were below 2.49 kg (Table 1). There was an increased serum level of zinc and selenium was found in the term pregnant mothers who gave birth normal birth weight when compared to low birthweight babies, though this difference was not statistically significant (Table 2). Median (IQR) values of Delta CT values of Mitochondrial DNA copy number in low-birth-weight babies were 3.07(1.7-5.74) and in normal birth weight babies was 3.71 (0.83-4.4). The difference in median values was not statistically significant (p=0.57; Table 3 and Fig. 1).

Table 1: Demographic characteristics of the study subjects.

Variables		**Number**	**Percentage**
Age	<= 20	7	4.7
	21 - 30	125	83.3
	31+	18	12.0
Address	Rural	25	16.7
	Urban	125	83.3
Educational status	Illiterate	10	6.7
	Primary	11	7.3
	High school	10	6.7
	PUC	56	37.3
	Degree	54	36.0
	Post-graduation	4	2.7
	Professional	5	3.3
Gravida	1	93	62.0
	2	32	21.3
	3	22	14.7
	4	3	2.0
Mode of delivery	Vaginal	98	65.3
	Caesarean section	52	34.7
Birth weight of baby in kg	<= 2.49	33	22.0
	2.50+	117	78.0
	Total	150	100.0

Table 2: Comparison of maternal zinc and selenium levels with low birth weight and normal birth weight

Baby birth weight (Binned)		**Mean**	**Std. Deviation**	**t**	**p**
Zinc µg/dL	<= 2.49	101.552	55.2840	0.72	0.42
	2.50+	108.562	41.4530		
Selenium µg/l	<= 2.49	157.414	44.0907	0.31	0.75
	2.50+	161.427	66.4856		

Table 3: Comparison of maternal mitochondrial DNA copy number with normal birth weight and Low birth weight using Mann Whitney U test.

	Low birth-weight	**Normal birth-weight**
Sample size	33	117
Lowest value	-8.8500	-7.0100
Highest value	-0.1300	10.9800
Median	-3.0700	-3.7100
95% CI for the median	-4.5042 to -1.7658	-3.8400 to -3.0926
Interquartile range	-5.7400 to -1.7000	-4.4000 to -0.8300

Average rank of first group	71.7273
Average rank of second group	76.5641
Mann-Whitney U	1806.00
Test statistic Z (corrected for ties)	0.565
Two-tailed probability	P = 0.5720

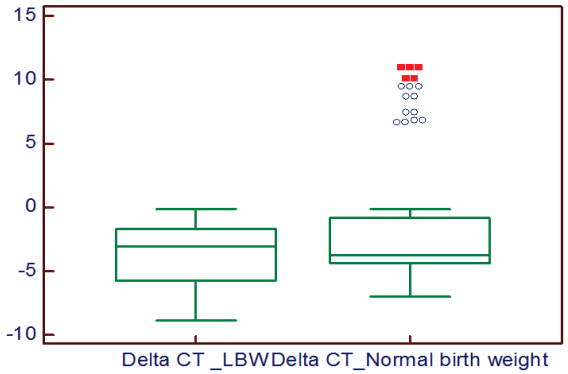

Fig. 1: Box and whisker plot diagram showing the Median values of Delta CT values of mitochondrial DNA copy number in low-birth-weight babies and Normal Birth weight babies

There was a positive correlation between the maternal zinc, selenium, and mitochondrial DNA copy number with birth weight of the baby. Though the correlation between Delta Ct means, and birth weight of the baby is found to be statistically significant (Table 4; Fig. 2).

Table 4: Correlation between the maternal zinc, selenium, and mitochondrial DNA copy number with birthweight of the baby

Variable	Baby birth weight	
	Pearson Correlation (r)	p
Zinc µg/dL	.049	.572
Selenium µg/l	.133	.107
CT	.025	.764
Delta Ct Mean	.250**	.002

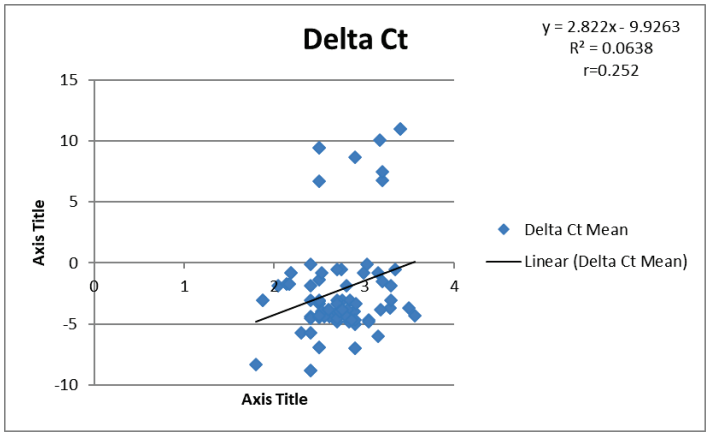

Fig. 2: Correlation between birth weight of the baby and the mitochondrial DNA copy number (Delta Ct values).

In the present study, almost 98.6 % of the women had zinc levels less than the recommended levels for pregnancy. Among them 81.9% of women gave birth to a normal birth weight baby. And this association was found to be statistically significant. Serum selenium was found to be <150 µg/l in 64.5% of the low-birth-weight babies. Odd ratio (95% CI) was 1.58(0.68-3.54), though it was not statistically significant (Table 5).

Table 5: Association between the maternal zinc and selenium levels with birth weight of the baby

Variables			Outcome		Chi square	p	Odds Ratio (95% CI)
			Normal	LBW cases			
Zinc µg/dL	<= 200.0	Count	109	24	4.05*	0.02	0.11(0.9-1.27)
		% within Zinc µ gm/dL (Binned)	82.0%	92.3%			
	200.1+	Count	0	2			
		% within Zinc µ gm/dL (Binned)	0.0%	7.7%			
Selenium	<150	Count	63	20	1.13	0.28	1.58(0.68-3.54)

µg/l		% within outcome	53.8%	64.5%			
	>151	Count	54	11			
		% within outcome	46.2%	35.5%			

*Yates corrected Chi square

DISCUSSION

In the present student, 150 term pregnant women participated. About 22% of the newborns were weighed<2.49 kg. Birth weight of the baby is determined by so many factors, in that maternal nutrition is foremost important. Dietary intake of balanced food and other micronutrients is very important for the birth weight.

Fetal growth is totally dependent on maternal nutrition through the placenta. The transport of small membrane permeable molecules such as oxygen (O_2) and carbon dioxide (CO_2) are influenced mainly by umbilical gradient blood flow and placental structure, whereas the larger nutrient molecules such as amino acids, fatty acids and glucose transport through placenta are dependent on nutrient transport proteins. The nutrient transport capacity of the placenta is influenced by numerous factors, including hormones, nutrient levels, and placental function. Furthermore, oxidative stress in the placenta has been shown to influence the transport of nutrients through altering the gene expression of different nutrient transporters (e.g., glucose and amino acid; 19).

In the present study, it was found that serum levels of Selenium were more among mothers who gave birth to normal weight babies, when compared to the low-birth-weight babies. In vitro studies have shown that selenium supplementation protects placental cells from oxidative stress through increased expression of selenium-containing antioxidants, such as glutathione and thioredoxin reductase. Hence, one of the leading hypotheses regarding how selenium may affect fetal growth is through the selenium-dependent anti-oxidative defense system (19,20). Other selenium-dependent proteins are the iodothyronine deiodinase (DIOs) that are involved in thyroid hormone metabolism (21). Thyroid hormones are essential in regulating placental nutrient transport, for example, hyperthyroidism is known to reduce circulating glucose in fetal tissues (22). Hence, another hypothesis on how selenium may influence fetal growth is through regulating the levels of thyroid hormones. In the animal study, wherein mice fed a diet low in selenium developed selenium deficiency. These mice had increased levels of both maternal and fetal plasma levels of the thyroid hormones triiodothyronine (T3) and tetraiodothyronine (T4; 19).

Mitochondria are both the culprit and victim of oxidative stress, being the major intracellular source and important target of oxidative stress. Investigators have observed that cells affected by oxidative stress synthesize more copies of their mtDNA and increase their mitochondrial density or abundance for compensating the damage, and to meet increased respiratory demand required for clearance of reactive oxidative species (23). Conversely, oxidative stress resulting from increased mitochondrial abundance may contribute to oxidative damage to mitochondria and other intracellular constituents including nuclear DNA, RNA proteins and lipids. Their preliminary investigation showed that placental mtDNA copy number is statistically significantly and positively associated with increased oxidative stress. But in the present study, MtDNA copy number was positively associated with the birthweight of the baby. This could be because of the optimum serum levels of selenium levels and zinc levels in pregnant women, who are apparently normal. The serum selenium levels were little less than the lab reference value, could be due to the hemodilution in the last trimester of pregnancy. The mechanism for the association of maternal serum concentration and increased risk of low birth weight could be explained due to the dual function of zinc, as an anti-oxidant as well as a pro-oxidant. The placenta is equipped with antioxidants inclusive of selenium-dependent enzymes of glutathione dismutase, thioredoxin reductases, seleno-protein-P and Cu/Zn superoxide dismutase which require the optimum levels of Selenium and Zinc. Free radical ions damage mitochondria and NADPH oxidases to produce Reactive oxygen species (ROS; 23).

Reactive oxygen species (ROS) increase has been documented in IUGR pregnancies. And ROS may have a role in altering the mtDNA copy number. It has been proposed that metabolic stress in mammalian cells, altering the expression of nuclear oxidative phosphorylation genes and mitochondrial biogenesis (24,25).

The increase of mtDNA copy numbers could be either due to the result of the feedback response that compensates for defective mitochondria bearing impaired respiratory chain or mutated mtDNA, caused by higher levels of ROS or due to the physiological increase in mitochondrial DNA copy numbers required for the fetal growth. Probably a vicious cycle results, when impaired mitochondria produce elevated levels of ROS, which further activate the nuclear response for the expression of oxidative phosphorylation genes (25).

There was an increase in Mitochondrial DNA copy number in the present study and it was positively

correlated with the birthweight of the baby. Though the selenium and Zinc levels were within the normal range in most of them, and not associated with the birthweight of the baby. This increase in mitochondrial DNA could have been raised to meet the increased demand by the developing fetus.

CONCLUSION

In the present study, apparently healthy pregnant women participated. The serum selenium and zinc levels were found to be within normal limits according to lab reference values. There was an increase in Mitochondrial DNA copy number in the present study and it was positively correlated with the birthweight of the baby. Hence further studies are required to study the actual interplay of micronutrients and MtDNA levels.

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CONFLICT OF INTEREST

There is no conflict of interest.

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ORIGINAL ARTICLE

Effect of Dietary Diversity on the Nutritional Status in Pregnant Women and in Turn its Effect on Birth Weight of the Baby

Manjula R[1,2], Rekha Udgiri[3], Ashalatha Mallapur[4], Shailaja Patil[5]

ABSTRACT

Background: Nutrient intake is important to the well-being of pregnant women and the fetus. Most of the previous studies points nutrition status based on energy and protein intake. However there are few studies indicate the use of dietary diversity as a marker of assessing the nutrition status. Healthy diets include the most diverse foods, and balance in eating food provides maternal and fetal health and reduces the prevalence of Low birthweight. **Objectives:** The objectives of the present study are to find out the association between Dietary diversity score with the nutrition status of the Pregnant Women (Gestational weight gain and nutritional anaemia.) And to find out the effects of gestational weight gain and nutritional anemia on the birth weight of the baby. **Methods:** An Institutional ethical clearance was obtained and the present Hospital based study was carried out in the OBG department of tertiary care centre of North Karnataka between December 2019 to February 2020. Sample size was estimated to be 120. **Results:** In the present study, a total of 120 women participated in the study. The Mean age of the study participants was 25.2±3.4, and the study found mean DDS was 7.04±1.58, with the scores ranging from 3 to 12. Association between the Dietary diversity score (DDS) and birthweight of the baby was found to be statistically significant (p=0.03) There is a statistically significant difference in DDS mean score between anaemic and normal pregnant women (p=0.007). There was a statistically significant positive correlation between Gestational weight gain and birth weight of the baby (p=0.03). There were positive correlation between the DDS and the Birthweight and Gestational weight gain, though it was statistically non-significant. **Conclusion:** Nutrition education for pregnant women should include the facts about the different food groups, it advantages will help to include diverse food items in their diet.

KEY WORDS: Dietary diversity score, Gestational weight gain, Birth weight.

Introduction

Nutrient intake is important to the well-being of pregnant women and the fetus. Inadequate nutrient intake can lead to maternal anemia, increasing the risk for other maternal morbidities and mortality, fetal growth retardation and low birth weight. Birth weight is an important indicator of the status of public health, maternal health and nutrition. Globally low birth weight contributes to 40-60% of infant mortality.[1] Maternal nutrition is crucial in determining the birth weight of neonates.[2]

Most of the previous studies point to nutrition status based on energy and protein intake. However, there are few studies indicate the use of dietary diversity as a marker of assessing nutrition status.

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The quality and quantity of nutrition are very important during pregnancy. Healthy diets include the most diverse foods, and balance in eating food provides maternal and fetal health and reduces the prevalence of low birthweight, hence dietary guidelines in pregnancy emphasized the importance of dietary diversity.[3] Dietary diversity represents various food consumptions among different groups of the food guide pyramid as well as each dietary group. Diet diversity is a qualitative measure of food consumption that reflects household access to a variety of foods and, is also a proxy for nutrient adequacy of the diet of individuals.[4,5] Pregnant with Low dietary Diversity Score was associated with low birth weight.[6]

Household food security represents the knowledge, availability, access and psychologically comfortable situation of a family towards the food they need for healthy living on a regular basis. The Household Food Insecurity Access Scale (HFIAS), developed by USAID measures insufficient quality and quantity of food or market. HFIAS captures a mix of sufficiency and psychological factors.[7]

Anemia during pregnancy is a public health problem, especially in developing countries and it is associated with maternal and perinatal adverse outcomes. According to WHO, anemia is considered of a severe public health significance if its rate is >40%. The causes of anemia during pregnancy in developing countries are multifactorial: these includes micronutrient deficiencies or iron, folate, vitamin A and Vitamin B12 deficiency, Malaria and hookworm infections and other chronic infections. In fact, nutritional Anemia in pregnancy due to iron deficiency is only 50%, rest are caused due to other micronutrient deficiencies. But the policy is to supplement only with iron and folic acid during pregnancy.[8]

Excessive Physical activity in pregnancy is associated with the low birth weight of the baby.[6] The concerns surrounding excessive physical activity during pregnancy included fears that it could weaken the blood supply to the developing fetus, which could potentially raise the risk of miscarriage, or that it may curb the level of nutrients the fetus receives, therefore reducing birth weight. Undernourished women along with excessive physical activity will jeopardize the nutrition status of a pregnant woman and in turn its consequences.

The dietary intake of pregnant women is a key determinant of nutritional status and of nutrient depletion during pregnancy, which is a risk factor for reduced fetal growth resulting in low birth weight. A number of studies in developed countries have linked Dietary diversity score (DDS) to nutrient intake. Dietary diversity (DD)during pregnancy impacts on the birthweight of the baby, and data on DD of pregnant women in this area is absent. The Dietary Diversity score is a simple and inexpensive tool for assessing diet quality and the recent focus on nutritional epidemiology has shifted from examining the effect of single nutrients to assessing overall diet quality. There is a pitfall in the documentation of nutritional status assessment in the ANC register.

Considering the importance of dietary diversity and nutrition during pregnancy and its outcome, the present study is being taken up with the following objectives.

The objectives of the present study are to find out the association between Dietary diversity score with the nutrition status of the Pregnant Women (Gestational weight gain and nutritional anaemia.) And to find out the effects of gestational weight gain and nutritional anemia on the birth weight of the baby.

Materials and methods

An Institutional ethical clearance (BLDE(DU)/IEC/409/2019-20 dated 27th December 2019) was obtained and the present Hospital based study was carried out in the OBG department of tertiary care center of North Karnataka between December 2019 to February 2020. Sample size estimation was done using Openepi software version 2.3.1. At 95% confidence limits, and at 80% Power of the study, $Z\alpha$= standard table value for 95% CI =1.96, $Z_{1-\beta}$ = Standard table value for 80% Power = 0.84 Considering the reference of study conducted by Willy K.[9] Mean±SD of DDS among Anaemic pregnant women was 6.30±1.38 & Mean±SD of DDS among normal pregnant women was 6.95±1.45 Formula used was $n= 2(Z\alpha+ Z_{1-\beta})^2 \sigma^2 / d^2$ For Studying and finding the mean difference of 1, Sample size estimated was 120.

Apparently Normal term (≥37 completed weeks) Pregnant Women who are admitted to the labor room for normal delivery or LSCS was included. Informed consent was obtained from the study participants. Multiple pregnancies like twins diagnosed anytime during pregnancy or after delivery. Chronic medical

conditions such as hepatic, renal, cardiovascular diseases, hypertension including preeclampsia and diabetes mellitus including gestational diabetes, known HIV, Hepatitis B infection, and women with hyperemesis gravidarum with persistent severe nausea and vomiting beyond 12 weeks of pregnancy were excluded. Babies born with severe congenital anomalies were excluded for the analysis.

A pretested questionnaire for obtaining basic demographic characteristics and dietary consumption by 24-hour recall method. Later physical examination includes a general physical examination, vitals including pulse rate, Blood pressure, Respiratory rate was measured. An anthropometric measurement includes Height and weight, using standard operating procedures. Weight was measured using UNICEF SECA weighing scale with a least count of 100 g. Height was recorded using UNICEF SECA microtoise with a least count of 0.1 cm. A complete Blood Count (Using Penta ES 60 cell counter) was done.

Dietary intake assessment and Dietary Diversity score calculation

A detailed dietary history by a 24-hour recall method was obtained by interview technique on a pretested proforma. Protein and calorie intake of cooked food in each case will be estimated by simple household measures like bowl/Katori, cup and spoon.

Diet diversity score

It is measure of food consumption in terms of such as cereals, pulses, roots and tubers, dark green leafy vegetables, other vegetables, vitamin A rich fruits, other fruits, organ meat, flesh meats, eggs, fish, nuts and seeds, milk and milk products, oils and fats, sweets, spices and condiments than an individual has consumed in 24 hours inclusive of the diet diversity within the various food groups. The score ranged from 0-12 wherein a score less than 3 will be considered as low diet diversity, 4-5 will be considered as medium diet diversity and >6 high diet diversity scores.

Gestational age is calculated based on the LMP and ultrasound findings.

Gestational Weight gain: Maternal weight at delivery minus the reported weight at conception divided by gestational age in weeks.

Statistical analysis

Data was coded and then entered in Microsoft excel and later analysed using SPSS software version 19. The normality of the data will be checked using shapiro wilk test and z scores for both skewness and kurtosis. Percentages for qualitative data, and mean and standard deviation for quantitative data will be used for representing the data. The chi-square test and students t-test will be applied for the independent parameters of the outcome variables. Pearson's correlation was done. $P<0.05$ was considered as statistically significant.

Results

In the present study, a total of 120 women participated in the study. out of 12 food groups (Table 1). The Mean age of the study participants was 25.2±3.4, and the study found mean DDS was 7.04±1.58, with the scores ranging from 3 to 12. Based on the categories developed, 58% of respondents were in high diversity category(≥6 food groups), 38% with medium diet(4-5 food groups) and 4% were having low diversity food (≤3 food groups) (Table 1). There was no association between DDS and Anaemia ($p>0.05$) (Table 2).

Association between the DDS and birthweight of the baby was found to be statistically significant ($p=0.03$) (Table 3). There was a reduced weight gain in all the weight categories when compared to IAM recommendations, except in pre-gestational obese women, there is a significant weight gain in them. ($p<0.01$)(Table 4). There is a statistically significant difference in DDS mean score between anaemic and normal pregnant women ($p=0.007$) (Table 2).

There was a statistically significant positive correlation between Gestational weight gain and birth weight of the baby ($p=0.03$). There was negative correlation between DDS and Anaemia, though it was not statistically significant. There were positive correlation between the DDS and the Birthweight and Gestational weight gain, though it was statistically non-significant. (Figure 1).

Discussion

Nutrient intake is important to the well-being of pregnant women and the fetus. Inadequate nutrient intake can lead to maternal anemia, increasing the risk for other maternal morbidities and mortality, fetal growth retardation and low birth weight. Birth weight is an important indicator of status of public health, maternal health and nutrition.

Table 1: Baseline characteristics of the pregnant women

Variable	No	%
Age(years)		
≤ 20	12	10
21-30	98	81.7
≥31	10	8.3
Mean age 25.2±3.4		
Parity		
1	50	41.6
2	34	28.4
≥3	36	30
Education		
Illiterate	4	3.3
Primary	36	30
High School	46	38.3
Pre-University	24	20
Degree	7	5.8
Post-Graduation	3	2.6
Occupation		
Housewife	94	78.3
Professional (Teacher)	5	4.2
Business	1	0.8
Bank employee	1	0.8
Agriculture	19	15.9
Socioeconomic status (Modified B G Prasad classification)		
Class I	20	16.7
Class II	28	23.3
Class III	30	25
Class IV	28	23.3
Class V	14	11.7
DDS category		
High (≥6)	53	44.2
Medium(4-5)	56	46.6
Low (≤3)	11	9.2
Total	120	100

Globally low birth weight contributes to 40-60% of infant mortality.[1] Maternal nutrition is crucial in determining the birth weight of neonate.[2]

A Systematic Review on dietary diversity, nutrient intake and nutritional status of pregnant women, conducted by Ndung'u[10], observed that increased dietary diversity is thought to increase the probability of a healthier diet and positive anthropometric outcomes in Africa. Another study, documented that

Table 2: Association between the DDS and Anaemia

DDS group	hb classification		Total
	Moderate Anaemia (7.1-9.9 gm)	Normal (>10 gm)	
Low DDS	1	10	11
	9.1%	90.9%	100.0%
Moderate DDS	2	54	56
	3.6%	96.4%	100.0%
High DDS	5	48	53
	9.4%	90.6%	100.0%
Mean DDS ± SD	5.38±1.302	6.9±1.206	P=0.007

Table 3: Association between the DDS and Birth weight of the Baby

DDS group	Birthweight		Total
	Low birth-weight (<2.5 kg)	Normal birth-weight (>2.51 kg)	
Low DDS	5	6	11
	45.5%	54.5%	100.0%
Moderate DDS	33	23	56
	60.0%	40.0%	100.0%
High DDS	19	34	53
	35.8%	64.2%	100.0%
Total	57	63	120
	47.9%	52.1%	100.0%

Chi square=6.3, p=0.03 Significant

Table 4: Distribution of study subjects according to the nutritional status and weight gain

Pre-Pregnancy BMI	N (%)	Weight gain Mean ± SD	Recommended weight gain*	p value
Under weight (≤18.5)	18	5.25±0.8	12.5-18	0.001
Normal (18.5-22.99)	61	7.34±1.2	11.5-16	0.001
Over weight (23.0-27.48)	32	5.85±2.3	7-11.5	0.01
Obese (≥27.5)	9	7.78±1.3	5-9	0.01

*Institute of Medicine and National Research council Recommendations.

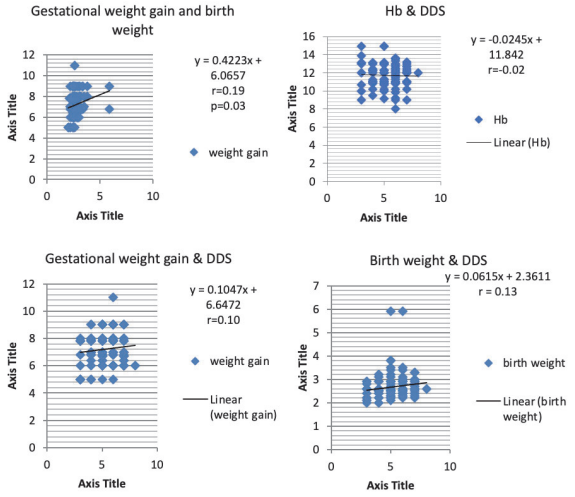

Figure 1: Correlation graphs between DDS, Gestational weight gain and the birth weight of the baby

there was an association between dietary diversity and respondent nutritional status. Pregnant women nutritional status had a positive linear relationship with intake of diversified diet. They also found that low maternal BMI were reported to be associated with low dietary diversity score, poor nutrient intake and significantly influenced the outcome of pregnancy, which is similar to our study.

Another Study conducted in Ethiopia, to study the predictors of undernutrition among pregnant women as household food insecurity and Low dietary diversity. This study concluded that low household food insecurity and low dietary diversity score were independently associated with undernutrition among pregnant women. Pregnant women who were from food insecure households were nearly two times more likely to be undernourished compared with pregnant women who were from food secured households. They found that the pregnant women who were married early, with food insecure households and who had low dietary diversity score were 2-4 times more likely to be malnourished, with AOR of 3.9, 2.3, 2.1 respectively.[11] Though we have not studied food security here, socio-economic status was used as a proxy-indicator for the same.

According to the study conducted by Komal M,[6] in the urban slums of Mumbai found a significant association between place of Antenatal visit, Dietary diversity score and birth weight of the baby ($p<0.05$). In this study the prevalence of Low birth weight was 15.7%, and women who delivered low birth weight babies were more involved in household domestic activities ($p<0.05$) compared to those who delivered normal weight babies. In this study, the women with high diet diversity scores were from better socio-economic background and vice versa with low dietary score, indicating household food insecurity among low socio-economic condition similar to our observation.

A retrospective cohort study was conducted in China, to evaluate the effects of maternal pre-pregnancy body mass index and gestational weight gain on neonatal birth weight. Compared with normal pre-BMI categories, underweight predicted an increased odds ratio of small-for-gestational age and decreased odds ratio for macrosomia and large for gestational age, and the results were opposite for over-weight.[12] In the present study, large for gestational age and macrosomia was not found, as we have excluded Gestational diabetes. Otherwise, it was found that there is a positive correlation between the gestational weight gain and birth weight of the baby.

A cohort study conducted in Indonesia, observed that pre-pregnancy body mass index, gestational weight gain is associated with the birth weight of the baby. Birth weight adjusted mean difference and the odds of macrosomia adjusted odds ratio are 205 and 13.45 respectively. Birth weight MD -139, significantly decreased in women with inadequate GWG compared to those with recommended GWG, while SGA OR 5.44 and for prematurity OR is 3.55.[13]

A study conducted in China, to study the effects of pre-pregnancy body mass index and gestational weight gain on neonatal birth weight found that neonatal birth weight is positively affected by both maternal pre-BMI and Gestational weight gain.[14] which is similar to the present study.

A cross sectional study conducted in Kenya , found that 19 % were under nourished and 16% were anaemic, and it was positively correlated with nutrient intake and nutritional status.[9]

A hospital based cohort study conducted in Madhya Pradesh, found that poor calorie and protein deficiency led to the low birth weight babies. Even anemic mothers had adverse effect on the birth weight of the baby.[15]

A prospective cohort study was done in Ghana , with the aim to explore the dietary diversity of pregnant women and its association with pregnancy outcomes among women in Northern Ghana. They observed that the pregnant women who consumed food with low diversity had increased chances of low birth weight babies compared to their counterpart.[16]

A prospective cohort study conducted in Norway, found that both increased or decreased weight gain compared to IOM recommendations were associated with adverse fetal and pregnancy outcomes. The Odds ratio of low birth weight increased when the gestational weight gain is less than the IOM recommendations and odds ratio of large for gestational age, gestational diabetes, preecclampsia increased as the gestational weight gain is more than the IOM recommendations.[17]

Conclusion and Recommendation

Hence there is clear evidence that Dietary diversity is associated with Gestational weight gain and in turn birth weight of the baby. Hence it is very important to provide nutrition education to pregnant women along with iron and folic acid supplementation. Nutrition education should include facts about the different food groups, its advantages will help to include diverse food items in their diet. The quality and quantity of nutrition are very important during pregnancy. Healthy diets include the most diverse foods, and balance in eating food provides maternal and fetal health and reduces the prevalence of Low birthweight.

Limitations of the study

This study was conducted in North Karnataka, which has its own dietary practices, with limited dietary diversity. Hence this type of study needs to be done at various population levels and different ethnicity, which may provide us with a fair idea about the impact of DD on Health as well as pregnancy.

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