



ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCES SCHOOL
OF MEDICINE DEPARTMENT OF MEDICAL PHYSIOLOGY

The Association Between Low Maternal Serum Magnesium And Calcium Levels
With Preterm Delivery And Low Birth Weight; In East Gojjam Zone, Amhara,
Ethiopia

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ABBREVIATION AND ACRONYM

ANC: Antenatal Care

BMI: Body Mass Index

Ca: Total Calcium

Ca²⁺: Ionized calcium

EDHS: Ethiopian Demographic and Health Survey

FIGO: International Federation of Gynecology and Obstetrics

LBW: Low Birth Weight

LMICs: Low and Middle Income Countries

Mg²⁺: Magnesium ion

MNCH: Maternal Newborn and Child Health

NBW: Normal birth weight

AOR: Adjusted odds ratio

PTD: Preterm Delivery

SGA: Small for Gestational Age

SPSS : Statistical Package for the Social Sciences

SSA: Sub-Saharan Africa

SST: Serum Separator Tube

TASH: Tikur Anbesa Specialized Hospital

WHO: World Health Organization

ABSTRACT

Background: Micronutrients like magnesium and calcium have a significant impact on the health of pregnant women and the growing fetus. They are involved in protein synthesis, bone matrix development, energy metabolism, regulation of muscle contraction, and hormone secretion. The deficiency of these micronutrients in pregnant women could be associated with adverse birth outcomes like low birth weight and preterm delivery. However, their association with preterm delivery and low birth weight in Ethiopia has not been determined.

Objective: To determine the association between maternal serum magnesium and total calcium levels with preterm delivery and low birth weight in East Gojjam Zone, Amhara, Ethiopia.

Methods: An institutional-based cohort study was conducted among 196 pregnant women with preterm births(24), term births(172), normal birth weight(168) and low birth weight(28) from June 2019 to December 2020. A questionnaire adopted from EDHS and FIGO was used to collect data on the socio-demographic characteristics, anthropometric and obstetric conditions of pregnant women. 5ml of blood was collected and the maternal serum Mg²⁺ and Ca levels were measured using a chemistry analyzer; UniCel Dxl. All statistical data were performed by using SPSS version 25.0. Independent t test was used to compare the means of magnesium and calcium levels between the groups. Binary logistic regression was used to predict the occurrence of preterm delivery and/or due to hypomagnesemia and/or hypocalcemia. A P-value < 0.05 was used to declare statistical significance.

Results: The mean \pm SD age of the study participants was 25.98 \pm 4.37 (range:16-38).The majority were married (99%), between 21-30 years (73.0%), house wives (41.3%), had completed senior secondary education (31.3%) and live in urban areas (85.8 %) with medium income (45.5%). Moreover, more than half of the participants (52.6%) were primigravida.The prevalence of PTD and LBW in the study population was 12.2 % and 14.28 % respectively. Maternal serum magnesium level was significantly associated with preterm delivery (t=-6.323, P<0.001)and LBW (t=-3.34, P=0.01). Maternal total calcium level was not significantly associated with preterm delivery (X²=1.768 P=0.184), t=-0.779, P=0.437)) but it was significantly associated with LBW(X²=6.075, P=0.014).

Conclusion: Maternal magnesium level was significantly associated with PTD and LBW while maternal serum total calcium was significantly associated with LBW. The results demonstrated how important maternal Mg²⁺ and total calcium levels are in influencing low birth weight and preterm delivery.

Keywords :Preterm delivery, Low birth weight, Magnesium, Calcium

1. Introduction

1.1. Background

Preterm delivery (PTD) is birth occurring after 22 weeks of gestational age and before 37 weeks whereas low birth weight (LBW) is birth weight <2500g from 37 weeks of completed gestation. Both PTD and LBW represent major public health problems worldwide due to their association with the catastrophic effects of morbidity and mortality (Taha *et al.*, 2020). LBW is associated with postnatal health problems on children and their offspring, creating a generational cycle of LBW (Shibata *et al.*, 2021). According to Potdar *et al.* (2014), it causes a rise in adult chronic disease among survivors, a decrease in childhood cognitive function, and an increase in perinatal death and stunting.

PTD continues to be the second most prevalent cause of death for children under five, after pneumonia, and the greatest direct cause of newborn death. Moreover, it has some long-term effects on preterm survivors throughout life, including visual and hearing impairment; neurodevelopmental effects like cognitive impairment, motor impairment and cerebral palsy, chronic lung disease of prematurity, chronic cardiovascular non-communicable diseases (WHO, 2012).

Inadequate micronutrient consumption or reserves during pregnancy can cause problems for the mother, including anemia, hypertension, labor issues, and even death. Additionally, the fetus is impacted, leading to improper organ development, low birth weight, congenital abnormalities, decreased immunocompetence, stillbirth, and preterm delivery (Moore, 2010). The health of mothers and children can be negatively impacted by deficiencies in iron, calcium (Ca^{+2}), magnesium (Mg^{2+}), zinc (Zn), copper (Cu), selenium (Se), vitamins A, B-complex, D, and E, which are common in LMICs (WHO, 2004; Gernand *et al.*, 2016). Low birth weight, premature delivery, and preeclampsia are among the pregnancy, delivery, and fetal development issues linked to magnesium (Mg^{2+}) and calcium (Ca^{+2}) shortages (Moore, 2010; Kumar and Singh, 2019).

Energy-demanding metabolic processes, nucleotide metabolism, protein synthesis, membrane integrity, nervous tissue conduction, neuromuscular excitability, muscle contraction, hormone secretion, and intermediate metabolism are just a few of the numerous biochemical and physiological processes in which Mg^{2+} is crucial (Fanni *et al.*, 2011; Parizadeh *et al.*, 2013; Konrad and Schlingmann, 2017).

Mg²⁺ acts as a physiological Ca²⁺ antagonist within cells, and as a result, the Mg²⁺/Ca²⁺ ratio is of major importance for the activity of Ca²⁺-ATPases and other Ca²⁺ transporting proteins. It is also involved in the active transport of K⁺ across cell membrane. Therefore, Mg²⁺ regulates muscle contraction, nerve impulse conduction, vascular tone and normal heart function (F de Baaij, J Hoenderop and M Bindels, 2015; Rigo *et al.*, 2017).

Mg²⁺ deficiency during pregnancy is associated with pre-eclampsia, pre-term delivery and low birth weight. It causes preterm delivery due to uterine hyperexcitability leading to initiation of uterine contractions and is intensified in situations of maternal stress. Moreover, hypomagnesemia may lead to neuromuscular excitability resulting in muscle cramps and uterine hyperactivity (Jenabi *et al.*, 2017). Mg²⁺ deficiency may reduce placental vascular flow. Therefore, it is possibly responsible for placental insufficiency, and so, it affects fetal development consequently, it might have a relationship with birth weight (Pourarian, Jahromi and Takmil, 2014).

Ca²⁺ plays an essential role in bone mineralization, nerve impulse transmission, muscular contraction, blood coagulation, hormone secretion, and signal transduction (Blaine, Chonchol and Levi, 2015; Buppasiri *et al.*, 2015; Mousa, Naqash and Lim, 2019). Ca²⁺ demand in a non-pregnant state is 600 mg/day which rises to 1,200 mg/day during pregnancy (Kant *et al.*, 2019). Pregnant women's demand for Ca²⁺ increases because it is actively transported across the placenta (Mousa, Naqash and Lim, 2019). This increased amount of Ca²⁺ is needed for the growth and development of the bones and teeth of the fetus. Due to hemodilution, serum Ca²⁺ level decreases during the second and third trimesters of pregnancy (Kant *et al.*, 2019).

Maternal Ca²⁺ deficiency has been associated with high blood pressure; pre-eclampsia, neonatal low bone mass, poor fetal growth, LBW, PTD, small for gestational age and neonatal death (Ajong *et al.*, 2019; Kant *et al.*, 2019; Mousa, Naqash and Lim, 2019; Ajong, Kenfack, Ali, Yakum, Ukaogo, *et al.*, 2022).

1.2. Statement of the problem

Around 15 million babies are born prematurely worldwide each year, and 965,000 of them pass away as neonates, according to the WHO and other research (Chawanpaiboon et al., 2019; Chasekwa et al., 2022). Preterm birth rates were highest in North America and Africa, where they were 10.6% and 11.9%, respectively (Gebreslasie, 2016). Additionally, according to WHO estimates, about 20 million low-birth-weight babies are born annually (WHO, 2015). According to Beck et al. (2010), 13% of all newborns born in Sub-Saharan Africa (SSA) are thought to be born LBW. Although they account for 20% of all births worldwide, preterm and term low birth weight (LBW) newborns account for 50% of the 2.6 million neonatal fatalities that occur each year (Chasekwa et al., 2022).

PTD and LBW are major problems in Ethiopia. 320,000 babies are born prematurely in Ethiopia each year, and 24,000 children under five pass away as a direct result of preterm problems, according to the country's profile of preterm and low birth weight prevention and care (Gebreslasie, 2016; Degno et al., 2021). An estimated 11–13% of all babies in Ethiopia are born with low birth weight, according to the Ethiopia's Demographic and Health Surveys (EDHS, 2011; EDHS, 2016).

1.3.Rationale and significance of the study

Despite tremendous improvements in maternal, neonatal, and child health (MNCH) over the past 20 years, adverse birth outcomes including PTD and LBW remain a serious worldwide health concern, particularly in low- and middle-income nations. Nutritional inadequacies are the primary cause of LBW and PTD, according to various research. Ethiopian pregnant women are more vulnerable to nutritional inadequacies because of physiological changes related to fetal growth and development, which raise the need for nutrients. Nonetheless, the majority of research conducted in Ethiopia has focused on identifying sociodemographic and economic variables that contribute to unfavorable birth outcomes. It is still unknown if micronutrients such as Mg^{+2} and Ca^{+2} are linked to poor birth outcomes. Thus, the purpose of this study was to determine the relationship. The finding of the study will provide basic information for program managers, policy makers and other stakeholders who are responsible for reducing maternal as well as fetal adverse birth outcomes.

2. Literature review

2.1 . Effect of maternal serum magnesium level on PTD and LBW

An observational research conducted in Pakistan revealed that pregnant women's Mg^{2+} levels were low. 150 primigravida pregnant women, 20-35 years of age, willing to participate in this study were recruited during the second and third trimesters of their pregnancies. Of the sixty people in the second trimester, 35 (58.3%) had low serum Mg^{2+} levels (below the 1.8 mEq/L standard range), and the other 25 (41.7%) had normal serum Mg^{2+} levels. Of the 90 individuals in the third trimester, 40 (44.4%) had low serum Mg^{2+} levels, while the remaining 40 had normal serum Mg^{2+} levels (1.8–2.9 mg/dl) with a non-significant P value (0.13) (Zulfiquar *et al.*, 2012).

A nested case-control study carried out in Iran revealed that there was a correlation between Mg^{2+} levels and premature birth. In this study, data were analyzed using the odds ratio (OR), t-test, and X². Stressful events linked to preterm birth ($P < 0.02$) and the frequency of prenatal visits ($P = 0.008$) were related to PTD. Preterm birth was linked to low serum Mg^{2+} levels OR=4.75, CI 95%. Moreover, the low serum Mg^{2+} level was associated with premature birth OR=4.75, CI 95% (Khani *et al.*, 2010). Another case-control study from India also indicated that preterm labor was associated with low maternal serum Mg^{2+} levels. According to this study, preterms had lower serum Mg^{2+} levels than term neonates ($p < 0.0001$). In addition, preterms were VLBW ($P = 0.004$) and LBW ($P = 0.0001$) than term neonates (Anand *et al.*, 2019).

In another study from Iran, the serum Mg^{2+} levels of women who experienced preterm labor were compared to those of women who were pregnant normally at the same age. In addition, the relationship between the serum Mg^{2+} levels and the women's response to magnesium sulfate ($MgSO_4$) was examined. The mean and standard deviation of serum Mg^{2+} level in the preterm labor (case group) was 1.80 ± 0.17 and in the term labor (control group) was 1.97 ± 0.19 , which demonstrated a significant difference ($P = 0.04$). This study suggests that a serum Mg^{2+} level may be a useful predictor of premature labor. Moreover, it can assist in the selection of people who benefit from $MgSO_4$ as a tocolytic drug in cases of preterm labor. To prevent preterm labor in patients with low serum Mg^{2+} levels, Mg^{2+} supplements may be beneficial (Lotfalizadeh *et al.*, 2018). Moreover, an observational study carried out in West Iran indicated that low maternal serum Mg^{2+} levels are associated with unfavorable pregnancy outcomes, such as preterm labor and low birth weight. According to the study's findings, the duration of gestational age and average birth weight was significantly lower in PTD compared to term labor ($P < 0.001$). The mean (SD) level of maternal serum Mg^{2+} was 2.12 (0.27) and 1.95 (0.16) in the term and preterm deliveries, respectively ($P = 0.004$). Maternal serum Mg^{2+} level was positively correlated with both gestational age ($r = 0.3305$) and neonatal weight ($r = 0.2975$), and negatively correlated with the length of the

first and second stages of labor ($r=-0.0184$ and $r=-0.0445$, respectively) (Jenabi *et al.*, 2017). However, another case-control study from Iran showed that the mean maternal Mg^{2+} level did not significantly correlate with either gestational age ($P=0.56$) or neonatal sex ($P=0.43$), nor showed any correlation with maternal parity ($P=0.52$), education ($P=0.75$), or occupation ($P=0.59$). Furthermore, no significant correlation ($P=0.79$) was found between the birth weight of each group and the mother's serum Mg^{2+} level (Pourarian, Jahromi and Takmil, 2014). Moreover, other case-control study conducted in Iran revealed that there was no discernible difference between the serum Mg^{2+} levels of mothers of low birth weight infants and mothers of normal weight infants. The two groups' maternal Mg^{2+} concentrations were 0.86 ± 0.11 m.mol/l and 0.94 m.mol/l respectively, without significant difference ($P=0.09$) (Parizadeh *et al.*, 2013).

A cross-sectional study conducted in Bangladesh indicated that there was a substantial correlation between low serum Mg^{2+} levels and preterm births ($P=0.01$). A student unpaired t-test was used to compare the mean blood Mg^{2+} value between the two groups, and a Pearson's correlation coefficient test was used to determine the relationship between the serum Mg^{2+} level and BMI, gestational age, and gravida in premature labor participants (Ferdous *et al.*, 2022). Another cross-sectional study conducted in Bangladesh showed that pregnant women with preterm labor had a considerably lower serum Mg^{2+} level than women with term labor (0.90 ± 0.37 versus 1.69 ± 0.33 ; $p<0.001$). The preterm labor group and term labor group did not differ in terms of mean age (25.43 ± 4.62 years against 24.40 ± 3.99 years; $p=0.332$) or gestational age (31.31 ± 1.78 weeks versus 31.34 ± 1.84 weeks; $p=0.974$) (Syed, Rahman and Islam, 2023). Moreover, another cross-sectional case-control study carried out in Nigeria also revealed that there was a link between low serum Mg^{2+} and premature labor. According to this study, low serum Mg^{2+} in varied degrees was present in 36% of the study participants. Preterm labor was 1.83 times more likely to occur in pregnant women with low serum Mg^{2+} levels (less than 1.6 mg/dL). A statistically significant difference ($p < 0.05$) was seen in the mean serum Mg^{2+} levels between the two groups (Okunade, Oluwole and Adegbesan-Omilabu, 2014).

In India, a prospective case-control study revealed that the mean serum Mg^{2+} in preterm labor participants was significantly lower (1.59 ± 0.83 mg/dl), while the mean serum Mg^{2+} in term labor participants was 2.55 ± 0.40 mg/dl. Socioeconomic status, gestational age, maternal age, and parity have no difference in serum Mg^{2+} levels between the preterm and term labor groups. According to the study's findings, preterm labor can be predicted using serum Mg^{2+} levels. This study suggested that premature labor can be prevented by taking a simple Mg^{2+} supplement, which

could offer a quick and low-cost way to lessen the issues associated with preterm labor (T. and Maddipati, 2020). Furthermore, an American-based prospective case-control study found that women in preterm labor had significantly lower serum Mg^{2+} levels than those who delivered their babies at term ($1.552 \pm .658$ mg/dl versus 1.81 ± 0.735 mg/d) (Mahmoud, Saleh and Khalaf, 2016).

A systematic review conducted in China also depicted that consuming enough Mg^{2+} during pregnancy could help reduce the incidence of preterm delivery. Results from eleven observational studies generally support the notion that serum Mg^{2+} levels and preterm birth rates are inversely correlated ($r = -0.68$; $P < 0.001$) (Zhang *et al.*, 2021).

2.2. Effect of maternal serum total calcium level on PTD and LBW

A cross-sectional study done in India indicated that the mean serum Ca level was significantly lower in mothers who gave birth to LBW ($P=0.03$). Neonatal mortality, preterm birth, and pre-eclampsia were not associated with serum Ca levels. The mean Ca intake from diet and Ca level in the serum were 796.4 ± 360.4 mg/day and 9.56 ± 0.94 mg/dl, respectively. Hypocalcaemia had a prevalence of 23.9% (95% CI: 20.8 – 27.2%). Dietary Ca consumption was not correlated with serum Ca level (p -value = 0.36). Mothers of LBW newborns had considerably lower mean blood Ca levels. Preterm birth, neonatal mortality, and pre-eclampsia were not linked to serum Ca levels (Kant *et al.*, 2019).

However, there was no statistically significant differences between women who received Ca^{2+} supplementation and those who did not in terms of reducing preterm births, according to a research review done in Thailand (risk ratio (RR) 0.86, 95% confidence interval (CI) 0.70 to 1.05; 13 studies, 16,139 women; random-effects model). In addition, the two therapy groups did not significantly differ in infant low birthweight (RR 0.93, 95% CI 0.81 to 1.07; six trials, 14,162 infants; random-effects model). Nonetheless, mothers in the Ca supplementation group gave birth to slightly higher birthweight infants than women in the control group (mean difference (56.40, 95% CI 13.55 to 99.25; 21 trials, 9202 women; random-effects model) (Buppasiri *et al.*, 2015). Another matched case-control study conducted in Nepal concluded that maternal and obstetric factors, including the ingestion of iron, folic acid, and Ca^{2+} , were determinants for the incidence of LBW. The results of a multivariable conditional logistic regression analysis showed that the main risk factors for low birth weight were iron and folic acid consumption for ≤ 60 days [AOR 5.47, 95% CI: 2.73-10.95), ($p < 0.001$)], iron and folic acid consumption for 61-120 days [AOR 3.04, 95% CI: 1.90-4.87), ($p < 0.001$)], and no Ca consumption [AOR 3.00, 95% CI: 1.78-5.04), ($p < 0.001$)] (Yadav *et al.*, 2020)

Another cross-sectional hospital-based study carried out in Cameroon revealed that fetal birth weight had no significant association with low serum Ca level ($P=0.468$) (Ajong *et al.*, 2019). Furthermore, another prospective cohort study conducted in Spain also showed that serum

Ca level had no correlation with PTD($P=0.355$) and LBW($P=0.758$) (María Pérez-Castillo *et al.*, 2020).

Another cross-sectional study conducted in Bangladesh showed that maternal total calcium level is significantly associated with neonatal birth weight in term deliveries where the birth weight of neonates reduces when maternal serum calcium level reduces($P<0.0001$) (Afroje et al ;2023).

A similar study, in Bangladesh, also showed that Ca status in cord blood might be associated with the birth length of newborns(birth weight, $P=0.022$; birth length, $P=0.001$). This study concluded that Ca levels during gestation may affect fetal growth (Doi et al;2011).

2.3. Conceptual framework

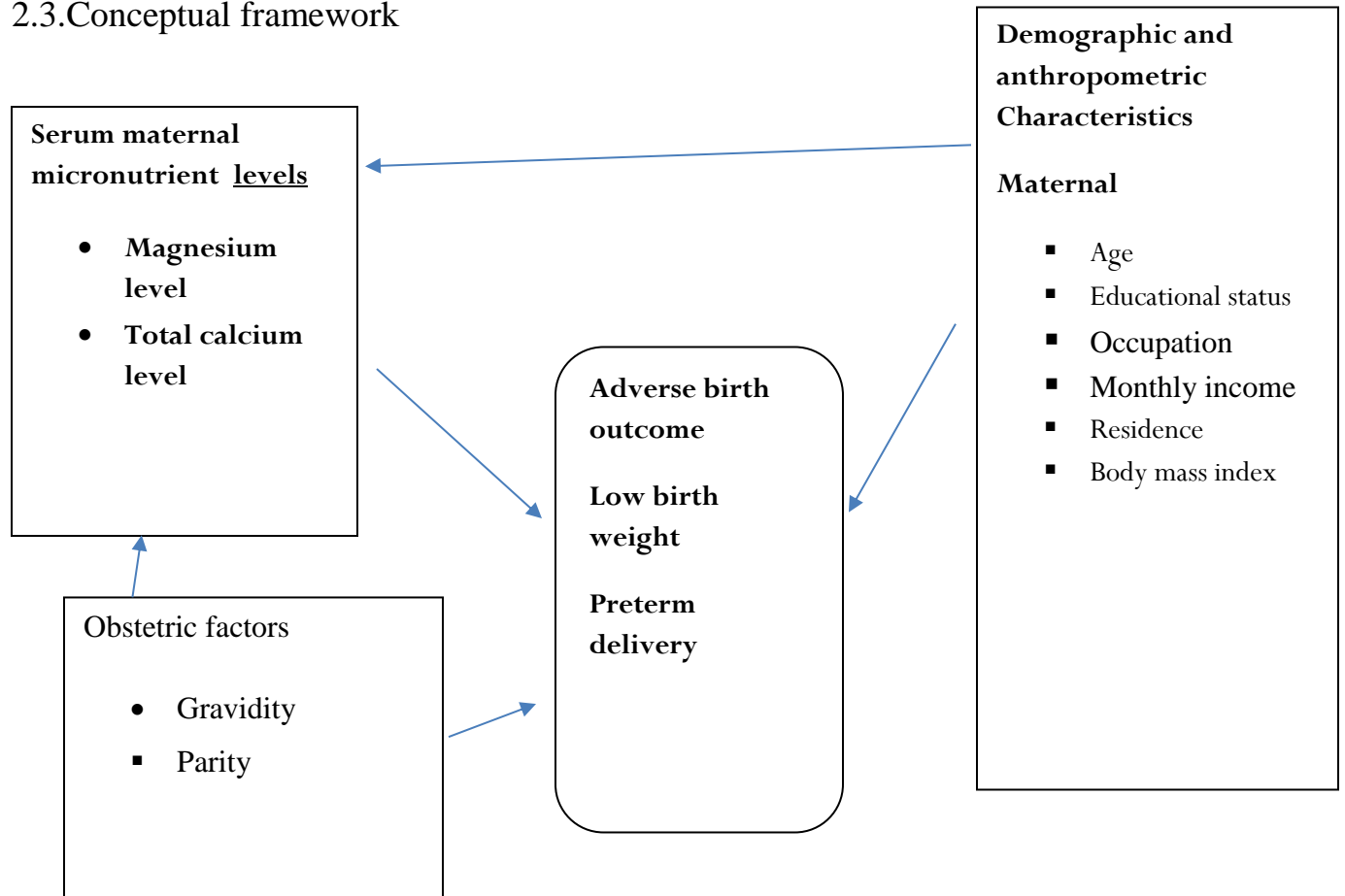


Figure 2 Conceptual framework to determine the association of maternal serum magnesium and total calcium levels with PTD and LBW

The incidence of LBW and PTD is affected by maternal sociodemographic , anthropometric , obstetric and micronutrient levels including magnesium and total calcium. The maternal serum magnesium and calcium levels can also be affected by sociodemographic and obstetric factors.

2.4. Research questions

2.4.1. Is maternal Ca level associated with the LBW and/ or PTD?

2.4.2. Is maternal Mg²⁺ level associated with LBW and/or PTD?

2.5. Research hypothesis

2.5.1. Null hypothesis: There is no association between maternal Ca and/or Mg²⁺ levels with LBW and/or PTD.

2.5.2. Alternative hypothesis

There is a significant association between maternal serum Ca and /or Mg²⁺ levels with LBW and /or PTD.

3. Objectives

3.1. General objectives

To determine the association between maternal serum magnesium and total calcium levels with preterm delivery and low birth weight.

3.2. Specific objectives

1. To determine the association between maternal serum magnesium level and preterm delivery.
2. To determine the association between maternal serum magnesium level and low birth weight.
3. To determine the association between maternal serum total calcium level and preterm delivery.
4. To determine the association between maternal serum total calcium level and low birth weight.
5. To determine the association between low birth weight and preterm delivery.

4. Methods and Materials

4.1. Study area and period

The investigation was carried out in four randomly chosen Woredas in the East Gojjam Zone. Four health centers (Dejen, Beteseb Memria, Hedase, and Debre Markos Health center) and two primary hospitals (Shegaw-Mota and Lumame Hospitals) were chosen in a methodical and random manner. The pregnant women who participated were then chosen at random from June 2019 to December 2020, taking into account the percentage of pregnant women in the chosen woredas and hospitals/health centers situated in the aforementioned Amhara Regional State zone. Debre Markos, the Zone's capital, is situated 300 kilometers from Addis Ababa in North West Ethiopia. With an estimated 3,800,000 people, East Gojjam Zone is the second-largest administrative entity in the Amhara Region based on demographic projections for 2013.

4.2. Study design

A facility-based cohort study was conducted in the two hospitals and four health centers in the East Gojjam zone.

4.3. Population

4.3.1. Target population

All pregnant women who live in East Gojjam.

4.3.2. Source population

All pregnant women who have regular follow up in selected hospitals and health centers found in East Gojjam.

4.3.3. Study population

The study population was pregnant women between 15-49 years of age who fulfilled the inclusion criteria and who came to selected health facilities for antenatal care before 17 weeks of pregnancy.

4.3.4. Study unit

A consenting pregnant woman who fulfills the inclusion criteria.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

All consenting pregnant women who were less than 17 weeks along in their pregnancy and who were scheduled to give birth at the designated medical facilities.

4.4.2. Exclusion criteria

HIV/AIDS, diabetes mellitus, severe anemia, chronic renal, liver, and gastrointestinal diseases that are likely to impact micronutrient status, multiple pregnancies, chronic hypertension, or a history of pre-eclampsia, as well as poor obstetric histories, are among the exclusion criteria.

4.5. Sample size determination and sampling technique

4.5.1. Sample size determination

The sample size was determined using a single population proportion taking 13.2 % (Kebede *et al.*, 2021), as the prevalence of LBW in the study area and with the assumptions of significance level ($\alpha=5\%$) and 95% confidence level.

$$n = \frac{(Z_{\alpha/2})^2 p (1 - p)}{d^2}$$

which gives a sample size of 180 by adding a 10% nonresponse rate the total sample size was 198. After excluding 2 participants due to insufficient serum sample, the final sample size was 196.

4.5.2. Sampling technique

A Systematic random sampling technique was used to select the health facilities and study participants.

4.6. Study variables

4.6.1. Dependent variables: Low birth weight and Preterm delivery

4.6.1. Independent variables

1. Maternal serum Mg^{+2} and total calcium
2. Sociodemographic and anthropometric characteristics

Mother's age, educational status, occupational status, residence, BMI

3. Obstetric factors: Parity, gravidity

4.7. Data collection process and instruments

4.7.1. Data collection instruments

A pre-tested structured interviewer-administered questionnaire was used to collect data on socio-demographic characteristics of pregnant women, their anthropometric and obstetric conditions that are adopted from questioners of EDHS and FIGO (FIGO, 2015)(EDHS, 2016). To ensure uniformity, the questionnaire was initially created in English, translated into Amharic, and then returned to English.

4.7.2. Data collection process

Trained midwives who provide antenatal care at the selected medical facilities collected the data. A few days before to the data collection period, the principal investigator trained data collectors on the contents of the data collection method. After that, questionnaires were given to every randomly chosen medical facility in the area. Pregnant women who visited the ANC unit before 17 weeks of pregnancy gave their assent and were chosen as participants based on the number of

pregnant women who visited the ANC unit overall. One pregnant woman was chosen from every two pregnant women that attended the ANC in each hospital until the necessary number of participants was reached.

The pregnant women's anthropometric measurements, such as their height and weight, were taken to the closest 0.1 kg and centimeter, respectively, when they were not wearing shoes. Their blood pressure was measured using sphygmomanometer and setehoscope when they are seated in a chair with their feet on the floor. By dividing their weight in kilograms by the square of their height in meters, their BMI was determined. Within an hour of the babies' delivery, their weights were recorded using a digital scale. Every anthropometric and blood pressure measurement was taken twice. Pregnancy outcomes, including as low birth weight and preterm delivery, were documented immedietly after delivery. Lastly, the cohort's pregnant women were divided into two groups according to their birth weight and gestational age at delivery: term births/preterm births and normal weight/low birth weight.

4.7.3. Sample collection and processing

A total of 5 ml venous blood was collected from mothers into a serum separator tube (SST) under a complete aseptic technique by phlebotomists. The collected blood was allowed to clot properly and was centrifuged in a centrifuge at 10,000 rpm for five minutes to get clear serum. The serum was collected into an Eppendorf tube and stored at -80 until it was analyzed for Mg^{2+} and Ca levels. The maternal serum mg^{2+} and Ca levels were measured using a chemistry analyzer; UniCel DxI , an access immune assay system. By running quality control material alongside the study participant's sample, the analyzer's performance was managed.

4.8. Operational definition and definition of terms

Hypocalcemia: total calcium level less than 8Mg/ml

Hypomagnesemia:magnesium level less than 1.6 Mg/ml

Parity: refers to the number of births after 20 weeks of gestation

Nulliparity : A woman who has never carried a pregnancy beyond 20 weeks of gestation.

Primiparity: A woman who has given birth once to a fetus or neonate that reached at least 20 weeks of gestation or weighed ≥ 500 grams, regardless of whether the child was born alive or stillborn.

Multiparity : A woman who has given birth two or more times to fetuses or neonates that reached at least 20 weeks of gestation or weighed ≥ 500 grams, regardless of whether the children were born alive or stillborn.

Gravidity: refers to the total number of pregnancies regardless of its outcome.

Primigravida: A woman who is pregnant for the first time.

Multigravida : A woman who has been pregnant two or more times, regardless of the outcome of the pregnancies.

4.9. Data quality assurance

According to the laboratory's routine, quality assurance checks were carried out every day in the laboratory. The collection and processing of the specimens were done in accordance with standard operating procedures. Every reagent used was made in accordance with the manufacturer's instructions and its expiration date was checked.

4.10. Data process and analysis

Data entry was done using Epi Data version 3.2, which was then exported to SPSS version 25.0. For numerical variables, descriptive statistics were displayed as means \pm SD and range. Frequencies and percentages were used to display categorical variables. To assess the relationship between categorical variables, chi-square was employed. The means of continuous variables were compared between the groups using the independent t test. To forecast the likelihood of a poor birth outcome brought on by hypomagnesemia and hypocalcemia, binary logistic regression was employed. Additionally, it was done to find the independent factors linked to PTD and LBW and to account for any relevant confounders. Confounding was examined, and if the regression coefficients' (β) percentage change is less than 20%, there isn't a confounder. Statistical significance was declared when the P-value was less than 0.05.

4.11. Ethical consideration

The Department of Medical Physiology, School of Medicine, College of Health Sciences, Addis Ababa University's research and ethical committee granted ethical approval. Before beginning data collecting, the Amhara Regional Health Bureau's formal consent was acquired and the Medical Director of each chosen healthcare facility was briefed on the study's purpose. If able to write, all participants were requested to offer written informed consent; if unable, they were asked to use an inked thumbprint on the consent form in front of an impartial witness. Every respondent received information on the study's purpose as well as assurances regarding the study's confidentiality, disadvantages and advantages.

5. Result

The study consisted of 196 pregnant women with ages ranging from 16 to 38 years (\bar{x} =25.98, SD=4.37). The majority of participants were married (99%), between 21-30 years (73.0%), house wives (40.8%), had completed senior secondary education (31.6%) and live in urban areas (86.7%) with medium income (45.5%). Moreover, more than half of the participants (52.6%) were primigravida, while the remaining 47.4% were multigravida. Most of the participants were nulliparous (57.7%). The prevalence of PTD and LBW in the study population was 12.2% and 14.3% respectively. Hypomagnesemia was prevalent in 27% participants while the prevalence of hypocalcemia was only 5.1% (Table 1)

Table 1: Distribution of study participants by their sociodemographic, anthropometric, obstetric, magnesium and calcium variables

Sociodemographic, anthropometric, obstetric variables, magnesium and calcium levels	N	%	Mean ± SD	Range
Age				
<21	25	12.8	25.98 ± 4.37	16-38
21-30	143	73		
31-40	28	14.2		
Education				
Cannot read and write	24	12.2	3814 ± 1845	1500-10000
Can read and write	7	3.6		
Primary education (1-5)	13	6.6		
Juniors secondary education (6-8)	20	10.2		
Senior secondary education (9-12)	61	31.6		
TVET	16	8.2		
Diploma or higher	54	27.6		
Monthly income (in birr)				
<2500	51	26	3814 ± 1845	1500-10000
2500-5000	89	45.5		
>5000	56	28.5		
Occupation				
Housewife	80	40.8	21.54 ± 2.97	15.17-30.30
Farmer	12	6.1		
Local alcohol seller	4	2.0		
Civil servant	45	23.0		
Job seeker	13	6.6		
Private/ merchant	40	20.4		
Student	2	1.0		
Residence				
Rural	26	13.3	21.54 ± 2.97	15.17-30.30
Urban	170	86.7		
Gravidity				
Primigravida	103	52.6	38.17±2.12	31-42
Multigravida	93	47.4		
Parity				
Nulliparous	113	57.7	2916.32±465.69	1600-4000
Primiparity	45	23		
Multiparity	38	19.3		
BMI			21.54 ± 2.97	15.17-30.30
Gestational age at birth				
<37 weeks	24	12.2	38.17±2.12	31-42
37-42 weeks	172	87.8		
Birth weight				
<2500 gram	28	14.28	2916.32±465.69	1600-4000
2500-400 gram	168	85.72		
Magnesium level				
<1.7 Mg/dl	53	27.0	1.74±0.17	0.90-2.60
1.7-2.8 Mg/dl	143	73.0		
Calcium level				
<8.0M g/dl	10	5.1	8.61±0.71	5.0-10.0
8-10 Mg/dl	186	94.9		

Table 2: Association of sociodemographic variables to PTD and LBW

Sociodemographic variables	PTD	Term	Statistical interpretation	LBW	NBW	Statistical interpretation
	N (%)	N (%)		N (%)	N(%)	
Age						
<21 years	4 (16.7)	21 (12.2)	X ² =0.406,p=0.816	3 (10.7%)	22(13.1)	X ² =1.651 p=0.438
21-30	17 (70.8)	126 (73.3)		23 (82.1)	120(71.4)	
31-40	3 (12.5)	25 (14.5)		2 (7.1)	26(15.5)	
Mean ±SD	24.54±4.13	26.19 ±4.38	t=1.735,P=0.084	26.14±4.47	25.97 ±4.33	t*=0.194,p=0.847
Range (min-max)	20-35	16-38		19-38	16-38	
Education						
Can not read and write	5 (20.8)	19 (11.1)	X ² =8.234 p=0.221	7 (25.0)	17 (10)	X ² =20.892 p=0.002
Can read and write	1 (4.2)	6 (3.5)		4 (14.3)	3 (1.8)	
Primary education(1-5)	2 (8.3)	11 (6.4)		1 (3.6)	12 (7.2)	
Juniors econdary education(6-8)	2 (8.3)	18 (10.5)		5 (17.9)	15 (9.0)	
Senior secondary education(9-12)	6 (25.0)	56 (32.3)		5 (17.9)	56 (33.5)	
TVET	3 (12.5)	13 (7.6)		1 (3.6)	15 (9.0)	
Diploma or higher	5 (20.8)	49 (28.6)		5 (17.9)	49 (29.3)	
Income (birr/month)						
<2500	18 (31.6)	27 (22.1)	X ² =5.186 p=0.269	7 (28)	38 (24.7)	X ² =0.158 p=0.997
2500-5000	27 (47.4)	55 (45.1)		11 (44)	72 (46.7)	
>5000	12 (21.1)	40 (32.8)		7 (28)	44 (28.6)	
Occupation						
Housewife	7 (29.1)	74 (43.0)	X ² =3.929 P=0.686	9 (32.1)	71 (42.6)	X ² =6.096 P=0.412
Farmer	2 (8.3)	10 (5.8)		3 (10.7)	9 (5.3)	
Local alcohol seller	1 (4.3)	3 (1.7)		2 (7.1)	2 (1.1)	
Civil servant	5 (20.8)	40 (23.3)		6 (21.4)	39 (23.3)	
Job seeker	2 (8.3)	11 (6.4)		2 (7.1)	11 (6.4)	
Private/ merchant	7 (29.1)	33 (19.2)		6 (21.4)	34 (20.2)	
Student	0 (0)	1 (0.9)		0 (0)	2 (1.1)	
Residence						
Rural	4 (16.7)	22 (12.8)	X ² =0.275 P=0.600	3(10.7)	23 (13.7)	X ² =0.185 P=0.667
Urban	20 (83.3)	150 (87.2)		25(89.3)	145 (86.3)	

*=unpaired t test to compare the maternal mean ages between the groups

Table 2 shows the relation of socio-demographic variables with PTD and LBW. In PTD, 70.8% of participants were between the ages of 21 and 30; in term delivery 73.3% of participants were within the same age range. The mean age was 26.19 ± 4.38 in term and 24.54 ± 4.13 in PTD. This demonstrated that the two groups' mean ages ($t=-1.735, P=0.084$) and age groups ($X^2=0.406, P=0.816$) did not differ significantly. The age range of 21–30 years old accounted for 82.1% of LBW, while the same age group accounted for 71.4% of NBW. LBW and NBW had mean ages of 26.14 ± 4.47 and 25.97 ± 4.33 , respectively. Age groups ($X^2=5.714, P=0.222$) and mean ages ($t=0.194, P=0.847$) did not differ significantly between the two.

A quarter (25.0%) of women in PTD and 32.3% of women who gave birth at term were completed senior secondary education (9-12). One-fourth of participants in LBW can not read and write but most of the women who gave birth to NBW neonates belonged to the senior secondary education (33.5%). A significant correlation was found between education level and LBW ($X^2=20.892, P=0.002$), but not between education level and PTD ($X^2=8.234, P=0.221$). NBW neonates were more likely to be born to women with more educational attainment.

In addition, type of occupation was not significantly associated with PTD ($X^2=3.929, P=0.686$) and LBW ($X^2=6.096, P=0.412$). Most of the participants in PTD (29.1%), term (43.0%), LBW (32.1%) and normal birth weights (42.6%) were housewives.

Regarding income per month, it was not significantly associated with either PTD ($X^2=5.186, p=0.269$) or LBW ($X^2=0.158, p=0.997$). Most of the participants belonged to the middle income (2500-5000 birr) in PTD (47.4%), the term (45.1%), LBW (44%) and NBW (46.7%). Furthermore, the majority of women in PTD (83.3%), terms (87.2%), LBW (89.3%), and NBW (86.3%) were from urban regions. This showed that residency had no significant relationship with either LBW ($X^2=0.185, P=0.667$) or PTD ($X^2=0.275, P=0.600$).

Table 3: Association of BMI to PTD and LBW

	Preterm	Term	Statistical test	Low birth weight	Normal birth weight	Statistical test
	Mean ± SD	Mean±SD	Unpaired t test	Mean±SD	Mean±SD	Unpaired t test
BMI(Kg /m ²)	22.44±2.92	21.67± 2.67	t=1.490 P=0.138	21.66± 2.66	21.51 ±3.03	t=0.218 p=0.818
Range	17.01-28.03	15.17-30.30		15.55-27.18	15.17-30.30	

The mean BMI was found 22.44±2.92 Kg/m² in PTD, 21.67± 2.67 Kg/m² in term, 21.66± 2.66 Kg/m² in LBW and 21.52 ±3.03 Kg/m² in NBW. There was no significant association of BMI with PTD (t=1.490,P=0.138) and LBW (t=0.218,P=0.818).

Table 4: Relationship of obstetrical variables to PTD and LBW

	Preterm	Term	Statistical analysis	LBW	NBW	Statistical analysis
	N(%)	N(%)	X ²	N(%)	N(%)	X ²
Parity						
0	17(70.8)	96(55.8)	X ² =1.965 p=0.374	13(46.4)	100(59.5)	X ² =1.693 p=0.429
1	4(16.7)	41(23.8)		8(28.6)	37(22.0)	
≥ 2	3(12.5)	35(20.4)		7(25.0)	31(18.1)	
Gravida						
1	17(70.8)	86(50)	X ² =3.67 p=0.056	12(42.9)	91(54.2)	X ² =1.231 p=0.267
≥2	7(29.2)	86(50)		16(57.1)	77(45.8)	

Table 4: Showed the association of obstetrical variables to PTD and LBW. We observed that about 70.8% women who delivered preterm and 50% women who delivered at term were primigravida. Most of the study participants in PTD(70.8%) and term(55.8%) were nulliparous. This suggested that PTD was not significantly associated to parity(P=0.374) and gravidity(P=0.056). Furthermore, 46.4% women delivered to LBW neonates and 59.5% of women delivered to NBW neonates were nulliparous. Regarding gravidity, 54.2% of women who gave birth to NBW were primigravida while the remainin 45.8% were multigravida. In women who gave birth to LBW neonates, the majority were multigravida(≥2)(57.1%). However, LBW was not significantly associated to parity(P=0.429) and gravidity(P=0.267) .

Table 5: Association of serum magnesium (Mg²⁺) to PTD and LBW

Serum magnesium level(Mg/dl)	PTD	Term	Statistical analysis	LBW	NBW	Statistical analysis
	N (%)	N (%)		N (%)	N (%)	
<1.7	17(70.8)	36(20.9)	X ² =26.58, P<0.001 OR = 9.8, 95% CI [3.54, 23.81]	14(50)	39(23.2)	X ² =15.78,P<0.001 OR = 3.31, 95% CI [1.43, 7.53]
1.7-2.8	7(29.2)	136(79.1)		14(50)	129(76.8)	
Total	24(12.2)	172(87.8)		28(14.28)	168(85.8)	
Mean ±SD	1.56±0.20	1.77±0.15	t=-6.13	1.66±0.16	1.76±0.17	t=-3.05
Range (min-max)	0.90-1.90	1.49-2.60	P<0.001	1.10-1.90	0.90-2.60	p=0.003

Table 5 showed that the relationship of maternal Mg²⁺ with PTD and LBW. The prevalence of hypomagnesemia in this study was 27.0%. It was observed that 70.8% of women who delivered preterm and 20.9% women who delivered at term had <1.7 mg/dl of serum Mg²⁺ level. The mean serum Mg²⁺ was found 1.56±0.20 and 1.77±0.15 in women with PTD and term delivery respectively. This showed that there was a significant association of maternal serum Mg²⁺ level with PTD (P<0.001). Women with hypomagnesemia (<1.7 mg/dL) were around 9.8 times more likely to experience preterm delivery compared to those with normal Mg²⁺ levels (1.7–2.8 mg/dL) (OR = 9.8, 95% CI [3.54, 23.81]). In addition, 50% of women who delivered to LBW and 23.2% of women who delivered to NBW had <1.7 mg/dl of serum Mg²⁺ level. The mean serum Mg²⁺ was found 1.66±0.16 and 1.76±0.17 in women delivered to LBW and NBW respectively. This showed that there was a significant association of maternal serum Mg²⁺ level with LBW (P<0.001). Moreover, women with low Mg²⁺ levels (<1.7 mg/dL) were nearly three times more likely to have LBW infants compared to those with normal Mg²⁺ levels (1.7–2.8 mg/dL) (OR = 3.31, 95% CI [1.43, 7.53]).

Table 6: Association of total calcium(Ca) to PTD and LBW

Serum total calcium(mg/dl)	PTD	Term	Statistical analysis	LBW	NBW	Statistical analysis
	N (%)	N (%)	$X^2=3.1$ P=0.079	N (%)	N (%)	$X^2=5.69$
<8.0	3(12.5)	7(4.1)	OR=3.34, CI[0.80, 14.02], p = 0.095.	4(14.3)	6(3.6)	P=0.017 OR =4.5, 95% CI[0.58, 0.845], p = .027.
8-10	21(87.5)	165(95.9)		24(85.7)	162(96.4)	
Mean \pm SD	8.42 \pm 0.93	8.64 \pm 0.67	t=-0.763 p=0.079	8.5 \pm 1.0	8.64 \pm 0.65	t=-0.924 p=0.356
Range (min-max)	6.0-9.0	5.0-10.0		5.0-9.0	5.0-10.0	

Table 6 shows the association of serum maternal calcium with preterm delivery and low birth weight. The prevalence of hypocalcemia in this study was 5.1 %. It was noticed that 12.5% of women who delivered preterm and 4.1% of women who delivered at term had <8 mg/dl of serum Ca level. The mean serum Ca level was found 8.42 \pm 0.93 and 8.64 \pm 0.67 in women with PTD and women delivered at term respectively. There was no significant association of maternal calcium level with PTD (P=0.079). Women with total low calcium levels (<8 mg/dL) were not significantly more likely to experience PTD compared to those with normal calcium levels (8–10 mg/dL), OR = 3.34, 95% CI [0.80, 14.02].

However, 14.3 % of women who delivered to LBW neonates and 3.6 % of women who delivered to NBW neonates had <8 mg/dl of serum Ca level .The mean serum Ca level was found 8.5 \pm 1.0 and 8.64 \pm 0.65 in women delivered to LBW neonates and NBW neonates respectively. This reflected that chi square analysis showed hypocalcemia was associated with a high risk of LBW ($X^2=5.69$, P=0.017). While the independent t-test did not show a significant mean difference in calcium levels between LBW and NBW groups (t=-0.924, P=0.356). Binary logistic regression analysis confirmed that hypocalcemia remained a significant predictor of LBW after adjusting for confounders including maternal age, BMI, monthly income, educational status , place of residence and magnesium levels (OR =4.5, 95% CI[0.58, 0.845], p = .027).

Table 7:Relationship of PTD with LBW

Gestational age at birth	LBW	NBW	Mean \pm SD	Statistical association
	N(%)	N (%)		X ² , unpaired t-test
PTD(<37 weeks)	8(28.6)	16(9.5)	2658.3 \pm 464.3	X ² =8.103,P=0.004 t*=-2.954,P=0.004
Term (37-42 weeks)	20(71.4)	152(90.5)	2952.3 \pm 455.7	

*-Unpaired student test to compare the mean birth weight in preterm and term neonates

Table 8 shows the relationship between PTD and LBW. We observed that 28.6% of LBW neonates were preterm whereas only 9.5 % NBW neonates were preterm. The mean neonatal birth weight was 2658.3 \pm 464.3 and 2952.3 \pm 455.7 in PTD and term respectively. These results showed that PTD was significantly associated with LBW (P=0.004).

Table 8: Association of socioeconomic and obstetrical variables to Mg²⁺ and Ca

Socioeconomic, obstetrical variables	Magnesium level(mg/dl)		Statistical analysis	Calcium level(mg/dl)		Statistical analysis
	<1.7 mg/dl	1.7-2.8mg/dl		<8.0	8-10	
Age	N (%)	N (%)		N(%)	N (%)	
<21	10(18.9)	15(10.5)	X ² =4.94 P=0.085	0(0)	25(13.0)	X ² =3.909 P=0.142
21-30	38(71.7)	105(73.4)		10(100)	133(71.5)	
31-40	5 (9.4)	23(16.1)		0(0)	28(15.5)	
BMI						
<18.5	6(12.2)	21(17.1)	X ² =4.89 P=0.087	0(0)	27(16.7)	X ² =2.052 P=0.358
18.5-24.9	37(75.6)	86(71.9)		7(70)	116(71.6)	
>24.9	6(12.2)	16(13.0)		3(30)	19(11.7)	
Monthly income						
<2500	13(26.5)	32(25.2)	X ² =0.68 P=0.711	1(10)	44(26.5)	X ² =0.705 P=0.703
2500-5000	24(49.0)	56(44.1)		4(40)	76(45.8)	
>5000	12(24.5)	39(30.7)		5(50)	46(27.7)	
Residence						
Rural	6(11.3)	20(14.0)	X ² =0.24 P=0.625	2(20)	24(12.9)	X ² =0.409 P=0.522
Urban	43(88.7)	123(86.0)		8(80)	162(87.1)	
Parity						
0(Nulliparity)	33(62.3)	79(54.9)	X ² =0.71 P=0.700	5(50)	108(56.5)	X ² =0.337 P=0.845
1(Primiparity)	11(20.8)	36(25)		3(30)	42(23.3)	
≥2(Multiparity)	19(17.0)	29(20.1)		2(20)	36(20.2)	
Gravidity						
Primigravida	30(56.6)	72(50)	χ ² =0.59p =0.44	5(50)	98(52.7)	X ² =0.28 P=0.868
Multigravida	23(43.4)	72(50)		5(50)	98(47.3)	

Table 8 shows the association of sociodemographic and obstetrical variables to maternal serum Mg²⁺ and calcium levels. 71.7% of women with hypomagnesemia and 73.4% of women with normal Mg²⁺ levels were in the age group of 21-30 years old. This indicated that there was no statistically significant association of age group with Mg²⁺ level (P= 0.085). All of hypocalcemic women and 71.5% of women with normal Ca were in the age group of 21-30 years old. However, the difference is not significant(P=0.142).

Among participants with hypomagnesemia 75.6 % had normal BMI and 71.9 % of participants with normal Mg²⁺ are in the normal range of BMI. This suggested that BMI has no association

with Mg^{2+} level ($P= 0.087$).Furthermore, 70% of women with hypocalcemia and 71.6 % of women with normal Ca were belonged to the normal range of BMI.This also suggested that BMI has no association with total calcium level ($P= 0.358$).

About 49.0 % of women with hypomagnesemia and 44.1 % of women with normal Mg^{2+} levels had medium income level(2500-4999 birr/month). This suggested that monthly income is not associated to Mg^{2+} level($P= 0.615$).About half of women with hypocalcemia(50%) were belonged to high income(>5000 birr/month) while most women with normal Ca (45.8%) had medium income.However, the difference is not statistically significant($P = 0.703$).

Around 88.7% of women with hypomagnesemia and 86.0% of women with normal Mg^{2+} levels were lived in urban areas. Likewise, most women with hypocalcemia (80%) and most women with normal Ca (87.1%) live in urban areas. These results showed that residence had no association with Mg^{2+} level($P = 0.700$) nor with calcium level($P=0.522$).

62.3% of women with hypomagnesemia and 54.9% of women with normal Mg^{2+} levels were nulliparous. Likewise, half of women with low calcium levels (50%) and most women with normal calcium levels (56.5%) were also nulliparous. These results indicated that parity had no association with magnesium level($P=0.700$) nor with calcium level($P =0.704$).

Regarding gravidity, 56.6% of women with hypomagnesemia and 50 % of women with normal Mg^{2+} levels were primigravida. Likewise, 50% of women with hypocalcemia and 52.7% of women with normal normal Ca levels were primigravida. These results indicated that gravida had no association with Mg^{2+} level($P =0.442$) nor with calcium level($P =0.591$).

6. Discussion

In this study, LBW and PTD prevalences are 14.28 % and 12.2 % respectively. These results are similar to previous reports that indicated a higher proportion of PTD and LBW rates in LMICs (WHO, 2012; Imdad and Bhutta, 2013; UNICEF & WHO, 2019; Madzorera et al., 2020). PTD prevalence ranges from 4.4% to 18 %, according to studies conducted in Ethiopia and other sub-Saharan African countries, underscoring the persistent public health concern (Abadiga et al., 2021; Sendeku et al., 2021; Fetene et al., 2022; Ayele and Moyehodie, 2023). The prevalence of PTD in Ethiopia ranges from 13.2 %-16.9% (Abadiga *et al.*, 2021) . Similarly, the LBW prevalence of 14.28 % is consistent with the WHO's estimation for LMICs, where LBW rates are close to 15% (UNICEF & WHO, 2019) and with Ethiopian studies that reported LBW prevalence ranging from 9.6% to 28.3% across different regions of the country (Habtegiorgis *et al.*, 2023; Endalamaw *et al.*, 2018; Birhanie *et al.*, 2020; Katiso *et al.*, 2020; Kassaw *et al.*, 2021; Kebede *et al.*, 2021, EDHS 2011, EDHS, 2016).

While hypocalcemia was less prevalent (only 5.1%), hypomagnesemia was observed in 27 % of pregnant women. The hypocalcemia in this study was smaller than studies in Nigeria (Bako et al ; 2019) and Cameroon(Ajong, Kenfack, Ali, Yakum, Onyidinma, *et al.*, 2019) which reported prevalences of total hypocalcemia among pregnant women 29.0 % and 56.7% respectively. The possible reasons for these differences could include the small sample size and higher number of urban participants than the aforementioned studies. Moreover, these studies had larger sample sizes, included participants from diverse socioeconomic backgrounds, and focused on women in the later stages of pregnancy, resulting in low serum micronutrient levels due to hemodilution. The study's hypomagnesemia prevalence of 27.0 % is in line with data from comparable populations in LMICs, where a lack of dietary diversification frequently results in inadequate dietary magnesium intake (Fanni *et al.*, 2011; Okunade, Oluwole and Adegbesan-Omilabu, 2014; Morton, 2018)). These findings highlight the significance of assessing pregnant women for Mg²⁺ shortage and giving supplements to women at-risk.

Hypomagnesemia was found to be strongly associated with both PTD and LBW in this study. PTD was 9.8 times as common in women with low magnesium levels. This result is consistent with researches showing that magnesium deficiency raises the risk preterm delivery and fetal growth restriction (Okunade, Oluwole, and Adegbesan-Omilabu, 2014; Mahmoud, Saleh, and Khalaf, 2016; Anand et al., 2019; T. and Maddipati, 2020; Ferdous et al., 2022; Syed, Rahman and Islam, 2023). Additionally, the likelihood of delivering LBW babies was almost four times higher for women with low magnesium levels. This result is comparable to other study findings (Takaya and Kaneko, 2011; Salehiniya, Mohamadkhani-Shahri and Sabet-Birjandi, 2014; Santoso et al., 2023). These correlations may be explained by the fact that Mg²⁺ lowers uterine contractility and preserves placental vascular function, contributing to fetal development and uterine relaxation. The study's strong correlation between hypomagnesemia and LBW, however, differs from research done in Iran that found no statistically significant link between the two conditions (Pourarian, Jahromi and Takmil, 2010; Parizadeh et al., 2013). The possible difference may be on

the study design and time of blood collection. These are case-control studies that collected blood in the first 24 hours after delivery.

Maternal Ca levels did not substantially correlate with PTD in contrast to magnesium. This is inconsistent with a study undertaken in China that concluded maternal serum calcium could be a predictor for PTD (Zhang *et al.*, 2024). The possible reason for this difference could be its enrolment at 32 weeks of gestation (late pregnancy) while this study enrolled women at early pregnancy.

A significant correlation was seen between hypocalcemia and LBW in the present study. This is similar to previous studies (Doi *et al.* 2011; Afroje *et al.* 2023) that found a statistically significant correlation between serum total calcium level and neonatal birth weight. Some other studies however did not find a significant association between maternal total calcium level and LBW (Ajong *et al.*; 2019). The probable justification for this difference could be they enrolled women at 37 weeks of gestation and above and had a significantly larger sample size when compared to the present study.

The study found no significant correlations between PTD and sociodemographic factors (age, income, level of education, occupation, residence) or obstetric history (gravidity, parity). LBW has also not been associated with age, income, occupation, residence, gravidity and parity. However, it is significantly associated with the level of education. The majority of participants were in the 21–30 age range, nulliparous, had completed senior secondary education, were housewives, and lived in an urban area. These characteristics could reflect nutritional deficiencies rather than sociodemographic factors as the primary causes of PTD and LBW. These results are in agreement with studies by (Okunade, Oluwole and Adegbesan-Omilabu, 2014; Mahmoud, Saleh and Khalaf, 2016; T. and Maddipati, 2020; Syed, Rahman and Islam, 2023), which found sociodemographic factors had less of an impact on the incidence of PTD or LBW. Nevertheless, these findings contradict research by Ayele and Moyehodie (2023) that found maternal age, educational attainment, family income, and multigravida to be significantly associated with PTD, as well as research by Ejeta Chibsa *et al.* (2024) and Demelash *et al.* (2015) that found LBW to be significantly associated with place of residence, maternal age, monthly income, and education. This discrepancy might be due to the small sample size, lack of variability in sociodemographic factors and study design of this study. The other studies were case-control studies with more participants from rural areas than the participants of the present study. Moreover, maternal dietary and biochemical parameters may be more important in influencing pregnancy outcomes in this study than sociodemographic factors, which could account for the difference.

There was a significant correlation between birth weight and gestational age; 28.6 % of LBW babies were born prematurely. This outcome is consistent with research that indicated a strong correlation between LBW and premature delivery (Khani et al., 2009; Jenabi et al., 2015; Doi et al., 2011). This finding aligns with existing pieces of evidence showing preterm delivery as a major determinant of LBW. This reflects that interventions aimed at prolonging gestation may significantly reduce the burden of LBW in similar populations.

7. Limitations and strengths of the study

7.1. Limitations of the study

The Mg^{2+} and total calcium have been analyzed after long storage at $-80^{\circ}C$ which may have an effect on the serum level of these minerals. Moreover, most of the residents are from urban areas indicating less representation of the rural pregnant women which could even increase the prevalence of PTD and LBW than what is identified in this study.

7.2. Strengths of the study

This study has identified the association between serum Mg^{2+} and total calcium with PTD and low birth weight in east Gojjam Zone, Ethiopia for the first time. It provides a comprehensive understanding of the factors influencing PTD and LBW, including sociodemographic and biochemical aspects. Its emphasis on maternal nutrition made it possible to gain particular insights into how nutritional deficits affect the course of pregnancy .

8. Conclusion

This study found a strong correlation between maternal Mg^{2+} and low birth weight (LBW) and preterm delivery (PTD). The likelihood of experiencing PTD and LBW was considerably higher in women with low magnesium levels (<1.7 mg/dL). Maternal serum calcium levels, on the other hand, indicated a significant association with LBW. The results demonstrate how important maternal Mg^{2+} and total calcium levels are in influencing low birth weight.

9. Recommendations

The findings emphasize the need for routine screening and management of magnesium and calcium levels during antenatal care. Therefore, Policy makers should support routine screening of serum magnesium and calcium levels during antenatal care as a predictive marker for PTD and LBW. Future research should conduct longitudinal and multicenter studies to validate findings and explore the role of calcium and other micronutrients in adverse birth outcomes. Magnesium and calcium supplementation could be a cost-effective intervention to reduce the risks of PTD and LBW. Pregnant women should be advised to include magnesium and calcium rich diets in their daily meal.

10. References

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ANNEXES

Annexe I: consent form(Amharic version)

የምርምርውን ገለጻ አንብቤያለሁ እና ወደ የተረዳሁት ቋንቋ ተተርጉሞኛል.
በተጨማሪም ከተመራማሪው ጋር በመስራቴ ደስተኛ ነኝ። የእኔ ተሳትፎ
በፈቃደኝነት ላይ የተመሰረተ መሆኑን ተረድቻለሁ። በምርምር ጥናቱ ውስጥ ስለ
ተካፈሉበት ዓላማ፣ ስልቶች፣ ስጋቶችና ጥቅሞች በቂ ግንዛቤ አለኝ።በፈቃዴም
5ሚ.ሊ ደም ለምርምሩ ለመስጠጥ በፈቃደኝነት ተስማምቻለሁ። በማንኛውም
ጊዜ የዚህ ጥናት አካል መሆኑን ላቆም እንደምችል ተረድቻለሁ። ራሴን ለመጠበቅ
የዚህን የፈቃድ ቅጽ ወረቀት ተቀብያለሁ።

ቀን.....
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.....

(ከ18 አመት በታችለሆኑ እናቶች ብቻ) የምስክሩ ደብዳቤ (አማርኛ ትርጉም) (Assent form,
Amharic Version) የምርምርውን ገለጻ በቤተሰብ እገዛ እና በተመራማሪው አማካይነት
አንብቤያለሁ እንዲሁም ወደ የተረዳሁት ቋንቋ ተተርጉሞኛል. በተጨማሪም
ከተመራማሪው ጋር በመስራቴ ደስተኛ ነኝ። የእኔ ተሳትፎ በፈቃደኝነት ላይ የተመሰረተ
መሆኑን ተረድቻለሁ። በምርምር ጥናቱ ውስጥ ስለ ተካፈሉበት ዓላማ፣ ስልቶች፣ ስጋቶችና

ጥቅሞች በቂ ግንዛቤ አላች። በፈቃዴም 5ሚ.ሊ ደም ለምርምሩ ለመስጠጥ በፈቃደኝነት ተስማምቻለሁ። በማንኛውም ጊዜ የዚህ ጥናት አካል መሆኑን ለቆም እንደምችል ተረድቻለሁ። ራሴን ለመጠበቅ የዚህን የፈቃድ ቅጽ ወረቀት ተቀብያለሁ።

ቀን.....

.....

ስም.....

.....

አሻራ.....

..... የምስክር

ቃል

እኔ..... በተመራማሪው የተነገረውን

ገለጻ በደንብ ተረድቻለሁ ይህንንም በፊርማዬ አረጋግጣለሁ።

የምስክር

ፊርማ.....

.....

ስም.....

.....

Annexe II:consent form (English version)

I have read the research description and it has been translated into a language I understand. I am also happy to work with the researcher. I understand that my participation is voluntary. I have sufficient understanding of the purpose, strategies, risks and benefits of participating in the research study. I voluntarily agree to drink 5 ml of blood for the research. I understand that I can stop being a part of this study at any time. I have received a copy of this consent form for my own protection.

Date.....

Name.....

Signature

Witness name, signature.....

(Only for mothers under 18 years old) The letter of the witness (English version) (Assent form, English version) I read the description of the research with the help of the family and the researcher and it was translated into the language I understood. I am also happy to work with the researcher. I understand that my participation is voluntary. I have sufficient understanding of the purpose, strategies, risks and benefits of participating in the research study. I voluntarily agree to drink 5 ml of blood for the research. I understand that I can stop being a part of this study at any time. I have received a copy of this consent form for my own protection.

Name....., Signature

Date.....

Witness's word

I.....I understand the explanation given by the researcher well and I confirm this with my signature.

Name

Signature, Date.....

Annex III: Questionnaire (Amharic version)

የመጠይቁ መግቢያ: የተከበሩ የዚህ ቃለ መጠይቅ ፈቃደኛ ተሳታፊ የሽይታሚንና የሚኒራሎች በርግዝና ጊዜ ህጻኑ ከሚከሰቱ ጉዳዮች ላይ ያለው ተዛምዶ ላይ ጥናት እያደረግን ሲሆን በመጠይቁ ላይ ፈቃደኛ ሆነው ታማኝና ውድ ይሆኑ ሃሳብዎን እንዲገልጹልን እንጠይቅዎታለን ለዚህም እናመሰግንዎታለን። በዚህ መጠይቅ ላይ በሚነግሩን ነገር ላይ ሚስጥራዊነቱን እንጠብቃለን። በድጋሚ ፈቃደኛ ስለሆኑ እናመሰግናለን።

የጠያቂው ባለሙያ ስም _____ ሙያ _____

የማግኘትም እና ካልሾም በርግዝና ወቅት ከመውለጃ ጊዜ እና ከህፃኑ ክብደት ጋር ያለው ተዛምዶ : በምስራቅ ጎጃም ዞን ወረዳ

I. የተጠያቂዎ እናት ማህበራዊ ምጣኔ ሀብታዊና ስነ ህዝባዊ መረጃዎችን በተመለከተ።

ተ.ቁ	ጥያቄ	
1.	ወረዳ	1. ስም _____ 2. መለያ ኮድ
2.	ሆስፒታል	1. ስም _____ 2. መለያ ኮድ
3.	የተጠያቂዎ እናት	1. ስም _____ 2. መለያ ኮድ
4.	የተጠያቂዎ እናት መለያ ኮድ (ቁጥር)	
5.	ስልክ ቁጥር ተጨማሪ ስልክ ቁጥር	1 _____ 2 _____
6.	የካርድ ቁጥር(MRN)	_____
7.	የመኖሪያ ቦታ	1. ገጠር 2. ከተማ

8.	የተጠያቂዎ እናት ቋሚ የመኖሪያ ቀበሌ	_____
9.	የተጠያቂዎ እናት እድሜ የተወለዱበት ወርና አመት (as at last birthday-in yrs)	ወር አመት
10.	የተጠያቂዎ እናት እድሜ (በአመት)	
11.	የጋብቻ ሁኔታ	1. ያገቡ 2. ያላገቡ 3. የተፋቱ 4. ባል የሞተባት
12.	መደበኛ ትምህርት ተምረዋል?	1. አወ 2. አይደለም

13.	የትምህርት ደረጃ (ያጠናቀቁት)	1. ማንበብ እና መጻፍ አልችልም 2. ማንበብ እና መጻፍ እችላለሁ 3. የመጀመሪያ ደረጃ(1-6) 4. መካከለኛ ደረጃ(6-8) 5. ከፍተኛ ደረጃ(9-12) 6. ቴክኒክ እና ሙያ(12(ከደረጃ 1-3 7. ዲፕሎማ/ዲግሪ/ከዚያ በላይ
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14.	አጠቃላይ የቤተሰብዎ የወር ገቢ በብር ስንት ነው?	
16.	ሐይማኖት	1. ኦርቶዶክስ 2. ሙስሊም 3. ፕሮቴስታንት ሌላ ካለ ይጠቀስ _____
17.	ብሄር	1. አማራ 2. ኦሮሞ 3. ትግሬ ሌላ ካለ ይጠቀስ _____

18.	ሥንት ልጆች አለዎት እስካሁን?	
19.	ስንተኛ እርግዝናዎ ነው?	

20.	ስንት ጊዜ ወለዱ?	□□□
21.	የስንት ሳምንት እርግዝና ነዉ አሁን ያለዎት?	□□□

22.	<p>በቅድመ ወሊድ ክትትል ጊዜ ከሚከተሉት ምርመራዎች ቢያንስ አንዱን አርገዋል?</p> <p>A. ደም ግፊት መለካት</p> <p>B. የሽንት ምርመራ</p> <p>C. የደም ምርመራ</p> <p>D. የምክር አገልግሎት ስለአመጋገብ ስርአት</p>	<p>A. 1=አወ; 2=አይደለም</p> <p>B. 1=አወ; 2=አይደለም</p> <p>C. 1=አወ; 2=አይደለም</p> <p>D. 1=አወ; 2=አይደለም</p>
23.	የስራ ሁኔታ	<p>1. የቤት እመቤት</p> <p>2. ገበሬ</p> <p>3. አልኮል ሻጭ(አረቄ፤ ጠላ</p> <p>4. የመንግስት ሰራተኛ</p> <p>5. ስራ ፈላጊ ሌላ ካለ ይጠቀስ_____</p>

II. Section B- የእናት በማህጸን ያለዉ ወይንም አዲስ የተወለደዉ ልጅ ልኬቶችን በተመለከተ.

24.	የርግዝና ጊዜ (ስንት ሳምንት)	□□□
25.	ክብደት በኪ.ግ □□□ ቁመት በ ሴንቲ ሜትር □□□□	
26.	የደም ግፊት ሲስቶል □□□□ mmHg ዲያስቶል □□□□ mmHg.	
27.	የተጠያቂዋ እናት የጨመረችዉ ክብደት ከርግዝናዉ ዘመን ጋር ሲነጻጸር (Weight of the mother for gestational age)	<p>1. ዝቅተኛ</p> <p>2. ጤናማ</p> <p>3. ከፍተኛ</p>
28.	የናት የእጅ ጡንቻ ስፋት በሴንቲ ሜትር(ሚክ) Women mid upper arm circumference	□□□
29.	በርግዝና መካከል ያለ የጊዜ ልዩነት Inter pregnancy intervals (months).	□□□
30.	የልጁ እድሜ በመጨረሻዉ የወር አበባ ልኬት ወይንም በአልትራ ሳውንድ Ultrasound scan result (last menstrual cycle) fetal age last menstrual cycle (wks).	□□□
31.	የልጁ እድገት ከርግዝና ሳምንቱ/ እድሜዉ ጋር ሲነጻጸር (Fundal height for gestational age)	1. ዝቅተኛ

		2. ጤናማ 3. ከፍተኛ
32.	የእናት የሆድ መጠን (Abdominal Circumference) (1=normal or better; 0= below normal)	1. ዝቅተኛ 2. ጤናማ 3. ከፍተኛ
33.	የልጁ የጭንቅላት ስፋት በሴንቲ ሜትር ለእድሜው (1=ተመጣጣኝ ወይም ከፍ ያለ ነው፤ 0= ዝቅተኛ ነው) (Ultrasound scan result fetal head circumference cm) for gestational age (1=normal or better; 0= below normal)	_ _
34.	የልጁ ቁመት በሴንቲ ሜትር ለእድሜው (1=ተመጣጣኝ ወይም ከፍ ያለ ነው፤ 0= ዝቅተኛ ነው) (Ultrasound scan result fetal length (in cm) for gestational age)	_ _

ስለጥናቱ መጠይቁን ከሞሉት እናት የሚሰጥ አጠቃላይ አስተያየት ካለ

_____ እናመሰግናለን።

Annexe IV:Questionarie(English Version)

I. Socio-Economic and Demographic Information of the Respondent's Mother.

SN	Question	
1	Woreda	3. Name _____ 4. Code
2	Hospital	2. Name _____ 3. Code
3	Women's code	1. Name _____ 2. Code
4	ID no. of women	
5	Telephone No. Alternative telephone	_____ _____
6	Place of residence	1.Rural 2.Urban
7	Pregnant women's age
8	Marital status	1.Single 2.Married 3.Widowed 4.Divorced
9	Have you had regular education?	1.Yes 2.No
10	Educational level	1.can not read and write 2.can read and write 3.Elementary(1-6) 4.Medium(7-8) 5.High school(9-12) 6.TVET 7.Diploma and above
11	Monthly family income(in birr)	
12	Religion	1.Orthodox 2.Muslim 3.Protestant

		4.Other
13	Ethnicity	1.Amhara 2.Oromo 3.Tigray 4.Other
14	How many children do you have so far?	
15	Gravidity	
16	Parity	
17	How many weeks pregnant are you now?	
18	Did you get at least one of the following tests during prenatal care? A. Blood pressure measurement B. Urinalysis C. Blood test D. Diet counseling	A.1:Yes 2:No B.1:Yes 2:No C.1:Yes 2:No D.1:Yes 2.No
19	Occupation	1.Housewife 2.Farmer 3.Local alcohol seller 4.Civil servant 5.Job seeker 6.Private /merchant 7.Student

SECTION B:MATERNAL ,FETAL AND NEONATAL MEASUREMENTS

		Amount
21.	Gestational age	
22.	Mother's weight (Kg).....Height(cm).....	
23.	Blood pressure,systole.....,diastole	
24.	Mothers weight for gestational age	1. Low 2. Normal 3. High
25.	Women mid upper arm circumference(MUAC)	
26.	Inter pregnancy intervals (months).	
27.	Ultrasound scan result (last menstrual cycle) fetal age last menstrual cycle (wks)	
28	Fundal height for gestational age	A.Low B.Normal C.High
29	Abdominal Circumference	1.Normal or better 2.below normal
30	Ultrasound scan result fetal head circumference cm) for gestational age	1.Normal or better 2.Below normal
31	Ultrasound scan result fetal length (in cm) for gestational age	

II. Section H. Questions used to assess pregnancy outcomes

	Pregnancy out come	Duration/ amount/ length
128.	Date of delivery	Day Month Year
129.	Gestational age at birth(weeks)	
130.	Birth Weight(BW in gm)	
131.	Head circumference in cm	
132.	Baby length in cm	
133.	APGAR score	

134.	Duration of pregnancy	<u>weeks</u>
135.	Duration of labor	<u>mins</u>
136.	PTD(Preterm Delivery	1=Yes 2= NO
137.	Still birth	1=Yes 2= NO
138.	First week neonatal mortality	1=Yes 2= NO
139.	Maternal mortality	1=Yes 2= NO
140.	Baby sex	1=M 2= F
141.	Mode of delivery	
142.	Need for augmentation of delivery	
143.	Post partum hemorrhage	
144.	Other adverse outcomes if any	
145.	Total number of ANC follow ups you did?	
146.	Which dangerous signs of newborn health were observed?	<ol style="list-style-type: none"> 1. Feeding less 2. Too hot or too cold 3. Too sleepy 4. Convulsion 5. Fast breathing 6. Umblical red/PU 7. Pus in eye