Association between maternal thyroid function during early pregnancy with pregnancy outcomes: Preeclampsia, preterm birth, and low birth weight among pregnant women aged between 20-40 years old in Bhaktapur district in the Kathmandu valley in Nepal.

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ABSTRACT

Background: Thyroid dysfunction affects up to 5–7% of all pregnancies. Many studies from Western, high-income countries have reported that hypothyroidism and hyperthyroidism, both overt and subclinical types, have been associated with the adverse pregnancy outcomes of preeclampsia, preterm birth, and low birth weight (LBW). The burden of these outcomes globally remains high, especially in low- and middle-income countries such as Nepal. In Nepal, a lower-middle-income country in South Asia, hospital-based studies have shown a high prevalence of thyroid dysfunction among pregnant women in different regions. Data from developing countries, including Nepal, are limited, so further research is needed to investigate the association between thyroid dysfunction and adverse maternal and fetal outcomes in Nepal.

Aim of the study: The aim of this study was to determine the prevalence of thyroid dysfunction and the outcomes of preeclampsia, preterm birth, and LBW. Furthermore, we aimed to investigate the association between maternal levels of the thyroid hormones thyroid-stimulating hormone (TSH) and free thyroxine (FT4) in early pregnancy and the pregnancy outcomes: preeclampsia, gestational age at delivery, preterm birth, birth weight, and LBW.

Material and methods: This is a secondary analysis using a retrospective cohort design with data from a randomized control trial conducted among pregnant women aged between 20 and 40, from early pregnancy not later than 15 weeks pregnant, residing in Bhaktapur district, the Kathmandu Valley in Nepal. We performed descriptive statistics to describe the population and applied multivariate logistic and linear regression analyses to investigate the possible associations between the markers of thyroid function and the adverse pregnancy outcomes.

Results: In total, 767 mother-infant pairs were included in the analyses. We found that 7.6% of the participants were thyroid peroxidase antibody (TPOAb) positive, 0.4% had overt hypothyroidism, 2.5% had subclinical hypothyroidism, 2% had subclinical hyperthyroidism, and 0.3% had overt hyperthyroidism. Furthermore, the prevalence of preeclampsia, preterm birth, and LBW was found to be 0.39%, 9.1%, and 9.7%, respectively. No significant association was found between maternal levels of TSH or FT4 in early pregnancy and the pregnancy outcomes gestational age at delivery, preterm birth, birth weight, and LBW.

Conclusion: This study included a healthy cohort and had very few cases of exposure and outcomes. Further research is needed to determine the prevalence of thyroid dysfunction in early pregnancy and the adverse outcomes of preeclampsia, preterm birth, and LBW and to assess their association in larger population-based cohort studies in Nepal.

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List of Abbreviations

HIC	High income country
ATA	American Thyroid Association
ACOG	American College of Obstetricians and Gynecologists
WHO	World Health Organization
UNICEF	United Nations International Children's Emergency Fund
UN	United Nations
hCG	Human chorionic gonadotropin
TH	Thyroid Hormones
TSH	Thyroid-stimulating hormone
T3	Triiodothyronine
T4	Thyroxine
FT4	Free Thyroxine
FT3	Free Tetraiodothyronine
TBG	Thyroid binding globulin
TPOAb	Thyroid peroxidase antibody
Under-5	Children under 5 years of age
LBW	Low birth weight
SDG	Sustainable development goals
USG	Ultrasonography
LT4	Levothyroxine
THHG	Transient Hyperthyroidism of Hyperemesis Gravidarum
VCAM-1	Vascular cell-adhesion molecule 1
TRAB	Thyrotropin receptor antibody
SSA	sub-Saharan Africa

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1. INTRODUCTION

1.1. Background

1.1.1. Thyroid function during pregnancy

The thyroid gland undergoes physiological changes during pregnancy, which include enlargement of the gland and increased vascularization.[1] These changes are normal and reversible but profoundly impact the thyroid gland and its function.[2] For pregnant women with sufficient iodine status, an approximate 10% enlargement is seen, and an even more significant increase—between 20% and 40%—is seen in iodine-deficient women.[2] Changes are also seen in the two pregnancy-related hormones, human chorionic gonadotropin (hCG) and estrogen.[3] During early pregnancy, the placenta produces hCG, which can bind to thyroid-stimulating hormone (TSH) receptors in the thyroid tissue and stimulate the thyroid gland to release increased amounts of thyroid hormones: thyroxine (T4) and Triiodothyronine (T3).[4] The elevated levels of hCG during the first trimester, therefore, lead to a transient increase in free thyroxine (FT4) levels and decreased TSH levels.[4] The hCG concentration peaks around the 10th and 11th weeks of gestation and starts to decline thereafter to a more stable level from the 12th week onward until the end of pregnancy.[5] Furthermore, during pregnancy, elevated estrogen levels and, thus, estrogen stimulation lead to an increase in thyroid-binding globulin (TBG) levels, a protein that binds to and transports the thyroid hormones T3 and T4 in the blood.[3, 6] The circulating TBG levels rise a few weeks after conception, around 7 weeks of gestation, and reach their peak by around 16 weeks of gestation, thus reaching a more stable level mid-pregnancy.[3, 6] The increase in TBG results in decreased FT4 levels, stimulating the pituitary gland's secretion of TSH and the production of thyroid hormones.[4]

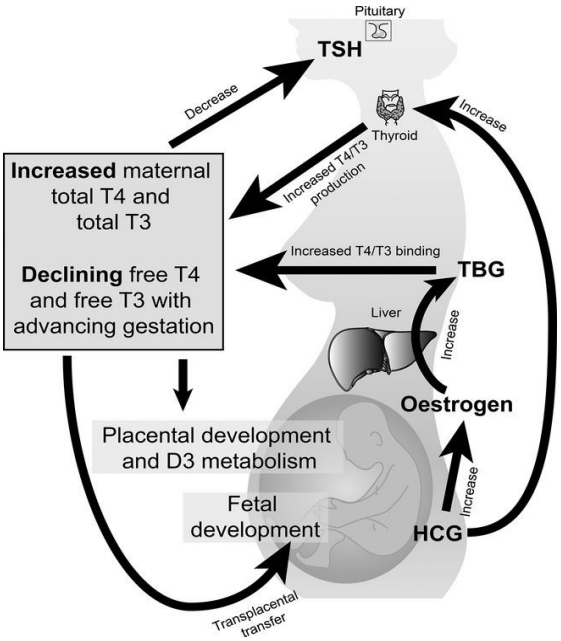

Figure 1: The normal physiological changes of the maternal thyroid function. [7]

Abbreviations: T4, thyroxine; T3, Triiodothyronine; free T4, free thyroxine; free T3, free triiodothyronine; D3, deiodinase type 3 enzymes; TSH, thyroid stimulating hormone, thyroid-binding globulin (TBG); human chorionic gonadotropin (hCG)

The optimal functioning of the thyroid gland during pregnancy ensures a sufficient supply of thyroid hormones to both mother and fetus, playing a significant role in maternal and fetal health.[8] Thyroid hormones are crucial for placental development, blood pressure regulation, and endothelial function.[9] In addition, during early gestation, the fetus solely relies on the thyroid hormones supplied by the mother.[4] Thyroid hormones are developmental factors that play a vital role in the fetal developmental process, neurodevelopment, and brain maturation by directly impacting fetal metabolism.[10, 11] They are crucial for regulating numerous processes, including the metabolism, growth, and development in most tissues, the different processes of placental development and function, the growth of the fetus, and the expression of neuropeptides involved in the onset of labor.[11]

1.1.2. Thyroid dysfunction during pregnancy

Thyroid dysfunction is a condition in which the adequate functioning of the thyroid gland is affected.[6] It is prevalent among women of reproductive age and, at times, newly diagnosed during pregnancy.[12-14] The two main diagnoses are hypo and hyperthyroidism[6], which are defined and classified in Table 1 below.

Table 1: Definitions and classification of maternal thyroid function status.

Euthyroidism	"A condition where the thyroid gland functions normally and produces adequate amounts of thyroid hormones."[6]
Hypothyroidism	"A condition characterized by an underactive thyroid gland that leads to insufficient production of thyroid hormones."[6, 13]
• Subclinical Hypothyroidism	Mild hypothyroidism: Increased TSH and normal FT4 concentrations.[6]
• Overt Hypothyroidism	Clear hypothyroidism: Elevated TSH and decreased FT4 concentrations.[6]
Hyperthyroidism	"A condition characterized by an overactive thyroid gland that leads to overproduction of thyroid hormones."[6]
• Subclinical Hyperthyroidism	Mild Hyperthyroidism: Decreased TSH and normal FT4 concentrations.[6]
• Overt Hyperthyroidism	Clear Hyperthyroidism: Decreased TSH and increased FT4 levels.[6]

Abbreviations: TSH, thyroid stimulating hormone; FT4, free thyroxine.

1.1.3. Thyroid dysfunction diagnosis during pregnancy

It may be difficult to clinically diagnose thyroid dysfunction during pregnancy, as pregnancy can cause overlapping symptoms such as fatigue, anxiety, constipation, muscle cramps, weight gain, increased heart rate, palpitations, heat intolerance, hand tremors, and systolic murmur.[8] These common symptoms are observed in both hypo and hyperthyroidism.[8]

As for biochemical detection, the TSH and hormone levels in pregnant women may vary based on ethnicity, race, and the iodine status of the tested population.[6, 15] Therefore, based on the American Thyroid Association (ATA) and the American College of Obstetricians and Gynecologists (ACOG) recommendation, it is recommended to use population- and trimester-specific ranges to assess thyroid hormones and function in pregnant women.[6, 15] However, in the absence of trimester-specific TSH values for the specific population, these guidelines recommend using an upper TSH reference limit of 4.0 mU/L.[6] Population-specific reference ranges were calculated for Nepalese pregnant women in early pregnancy and used in this study to assess thyroid function.[16] They are presented in Table 2 below.

Table 2: Population-specific thyroid function reference ranges.

Thyroid Hormones	Normal reference range for the population
TSH (μIU/ml)	0.05 – 3.69
FT4 (pg/ml)	8.98 – 15.28
TPOAb positivity (IU/ml)	≥30

Abbreviations: TSH, thyroid stimulating hormone; FT4, free thyroxine; TPOAb, thyroperoxidase antibodies.

1.1.4. Thyroid dysfunction and adverse pregnancy outcomes

Thyroid dysfunction during pregnancy can significantly affect maternal and fetal outcomes. [3, 8, 12, 17-20] Recent research has shown that both hypo- and hyperthyroidism—including milder forms—during pregnancy are associated with an increased risk of maternal and fetal complications, including preeclampsia, preterm birth, and low birth weight (LBW).[3, 8, 12, 17-20] The definitions and the characteristics of the adverse pregnancy outcomes of preeclampsia, preterm birth, and LBW are presented in Table 3 below.

Table 3: Definitions and characteristics of pregnancy outcomes.

Pregnancy outcome	Definition and characteristics
Preeclampsia	A pregnancy-related hypertensive disorder. The ACOG defines preeclampsia as "a blood pressure of more than 140/90 mmHg at intervals of four hours after the 20th week of pregnancy, as well as proteinuria greater than or equal to 300 mg in 24 hours of urine collection."[21]
Preterm Birth	As defined by the WHO: "babies born before 37 weeks of pregnancy are completed."[22]
Low birth weight	As defined by the WHO: "weight at birth less than 2500 g (5.5lb)."[22]

Abbreviations: ACOG, American College of Obstetricians and Gynecologists; WHO, World Health Organization.

1.1.4.1. Hypothyroidism and adverse pregnancy outcomes

Hypothyroidism during pregnancy is more common than hyperthyroidism.[8] An inadequate supply from the mother can lead to irreversible adverse fetal effects, including growth retardation, cognitive-, motor-, speech-, and hearing defects.[23] The negative impact of overt hypothyroidism on pregnancy outcomes has been well documented, as most existing studies have shown that overt hypothyroidism has been associated with harmful impacts on fetal neurocognitive development and adverse pregnancy outcomes of preeclampsia, preterm birth, and LBW.[6, 8, 15]

Subclinical hypothyroidism is considered to be the most common type of thyroid dysfunction during pregnancy.[8] Its prevalence varies from one setting to another depending on ethnicity, other demographics, and iodine intake.[24] The adverse effects of subclinical hypothyroidism on maternal and fetal pregnancy outcomes remain unclear, with studies reporting both adverse effects and no effects.[6-9, 11, 18] A systematic review and individual-participant data meta-analysis conducted by Toloza et al. reported an association between maternal subclinical hypothyroidism and a higher risk of preeclampsia[9] explaining that the low availability of thyroid hormones may lead to an insufficient anti-inflammatory environment in the developing placenta. This leads to placental vascularity disturbances, which might have an impact on the development of pregnancy-induced hypertension, including preeclampsia.[9] This alone increases the risk of preterm birth.[25] According to Parizad Nasirkandy et al., the inflammatory process caused by hypothyroidism, in association with an altered cytokine regulation network in the uterus and the absence of the pair-control inflammatory processes, can increase the risk of preterm birth and, therefore, potentially LBW.[26]

1.1.4.2. Hyperthyroidism and adverse pregnancy outcomes

Hyperthyroidism is less common during pregnancy.[8, 27] However, excessive maternal supply of thyroid hormones can also lead to adverse fetal outcomes such as fetal and neonatal hyperthyroidism, rapid heart rate leading to heart failure, enlarged thyroid, and poor weight gain.[3]

Hyperemesis gravidarum—severe nausea and vomiting during pregnancy—may cause transient hyperthyroidism, which has been found to be more prevalent in Asian populations than in European populations.[8] Furthermore, hyperthyroidism is also prevalent in women with Grave's disease.[8] Grave's disease is an autoimmune condition requiring medical management

in which the excess stimulation caused by the TSH receptor antibodies (TRAbs) leads to a pathologic increase in FT4 levels through binding to the thyroid receptors and, thus, causing their activation.[27]

While results are unclear on the associations between milder forms of hyperthyroidism and adverse pregnancy outcomes, studies have shown that overt hyperthyroidism can lead to endothelial cell dysfunction by disrupting the protective mechanisms against endothelial damage.[6, 9, 27-29] The metabolic changes in the mother caused by hyperthyroidism can, in turn, negatively impact the growth of the placenta and the fetus.[27] Elevated FT4 concentrations in overt hypothyroidism during early pregnancy have been associated with increased vascular resistance in both the maternal and fetal placental compartments, potentially contributing to the development of preeclampsia and also leading to preterm birth and LBW.[9]

1.1.5. Global health relevance

Thyroid dysfunction affects all populations globally and can potentially lead to severe health implications if left untreated.[30] Based on the global estimates for thyroid dysfunction, in 2020, the age-standardized incidence rates for the world population were 10.1 per 100,000 women and 3.1 per 100,000 men, indicating a higher prevalence among women than men.[31]

Among women of reproductive age, both thyroid dysfunction and thyroid autoimmunity affect up to 5–7% of all pregnancies.[18, 32] As previously described, thyroid dysfunction during pregnancy is associated with a higher risk for negative pregnancy outcomes, including preeclampsia, preterm birth, and LBW.[3, 8, 12, 17-20] These outcomes contribute to maternal, neonatal, and children under 5 years of age (under-5) morbidity and mortality, which are especially high in low- and middle-income countries.[33-36]

Preeclampsia affects up to 2–8% of pregnancies worldwide.[37] It is responsible for over 50,000 maternal deaths and 500,000 fetal deaths worldwide every year.[37] In developing countries, the incidence is seven times higher than in developed countries[38] ; it is responsible for 9–26% of maternal deaths in low-income countries (LIC) and 16% of maternal deaths in high-income countries (HIC).[37] It is a significant public health problem due to its frequency of occurrence and its association with maternal and perinatal morbidity and mortality.[25, 39]

Preterm birth increases the risk of neonatal and under-5 morbidity and mortality[35, 40, 41] as preterm birth infants are more susceptible to developing respiratory distress, apnea, temperature instability, seizures, hypoglycemia, impaired neuro-development, and feeding

difficulties.[42] Despite continuous efforts, preterm birth remains a global challenge.[40] It is estimated that 12–15 million newborns worldwide are born preterm each year, of which one million die due to complications.[35, 43] In 2020, around 13.4 million newborns were born preterm worldwide, with southern Asia and sub-Saharan Africa (SSA) having the highest estimated burden, accounting for 65%.[40] Preterm birth is one of the primary causes of LBW.[22, 40]

Birth weight is an essential indicator of fetal growth and development and one of the critical predictors of prenatal morbidity and mortality.[22] LBW is, together with prematurity, the leading cause of child morbidity and mortality in low- and middle-income countries.[22, 41, 44] Globally, it is estimated that 15–20% of newborns are born having LBW.[22] Over 20 million newborns are born with LBW yearly, and global estimates show that around three-quarters of these births occur in South Asia and SSA, with South Asia having the highest burden.[22, 44] LBW is associated with short- and long-term health consequences contributing to neonatal and under-5 morbidity and mortality.[22]

1.1.6. Burden in Nepal

Nepal, a developing, lower-middle-income country and a previously iodine-deficient area, has achieved optimal iodine intake for its population through a successful universal salt iodization program.[45] The country has seen significant improvements in key population and health indicators over the past decades, including a decline in child mortality and malnutrition rates and an increase in antenatal and delivery care services and uptake.[46] However, the burden of thyroid dysfunction among pregnant women and adverse pregnancy outcomes continue to be high.

There is a lack of prevalence studies of thyroid dysfunction in the general pregnant population in Nepal, but hospital-based studies are available in some regions. These studies describe a high prevalence varying between 17–34% across the different regions.[47-50] Furthermore, the burden of the adverse pregnancy outcomes has also been shown to be high in Nepal. Preeclampsia has been found to affect 2.6% of pregnancies[51], and according to the 2024 Nepalese Annual Health Report, hypertensive disorders of pregnancy were the leading cause of maternal deaths, with a national prevalence of 26%.[52] Furthermore, preterm birth and LBW are the leading causes of neonatal mortality in Nepal.[52] The prevalence of preterm birth within the general population has not been determined; however, hospital-based studies estimated a

range between 13 to 16%.[53-55] Moreover, based on a USAID report in 2015, it was estimated that around 81,000 preterm births occur every year in Nepal.[56] In 2020, the prevalence of LBW was 20% in Nepal.[57]

1.2. Problem statement

According to estimated trajectories for achieving the UN's sustainable development goals (SDG) by 2030, Nepal's maternal mortality ratio, neonatal, and under-5 mortality rates are higher than set targets.[58] The SDG target for the maternal mortality ratio is less than 70 per 100,000 live births; however, in Nepal, it is 174 per 100,000 live births.[58, 59] The target for neonatal mortality is less than 12 per 1,000 live births, whereas the rate is 21 per 1,000 live births in Nepal.[46, 58]. As for the under-5 mortality rate, the SDG target is less than 25 per 1,000 live births, while the rate is 33 per 1,000 live births in Nepal.[46, 58]

Thyroid dysfunction may develop during pregnancy in pregnant women with no prior history of thyroid disease.[3] If left undiagnosed or untreated, it can lead to significant maternal and fetal adverse effects, including preeclampsia, preterm birth, and LBW.[3, 8, 12, 17-20] These outcomes have a high contribution to maternal, neonatal, and under-5 mortality, especially in low- and middle-income countries.[33, 34]

Most research on this topic has been conducted in high-income countries. Data available from developing countries is limited; however, in India, a neighboring South Asian country, studies found that thyroid dysfunction during pregnancy was not only more prevalent among Indian women than in Western countries but also that the pregnancy outcomes were more adverse.[60, 61] Further, the prevalence of thyroid dysfunction has been shown to be high among pregnant women in various regions of Nepal. There is a lack of research addressing the association between thyroid function and pregnancy outcomes among this population. This omits a significant portion of pregnant women in Nepal who might be at greater risk of the adverse outcomes.

1.3. Rationale

Thyroid dysfunction during pregnancy is a health problem that affects both the mother and the fetus. It can lead to adverse pregnancy outcomes that contribute to an increase in maternal, neonatal, and under-5 morbidity and mortality. Previous hospital-based studies in Nepal have indicated a high prevalence of maternal thyroid dysfunction, and the burden of adverse

pregnancy outcomes is reported to be high. Furthermore, the maternal mortality ratio and neonatal and under-5 mortality rates in Nepal are higher than the SDG goals. Existing studies exploring the associations between maternal thyroid function and the adverse pregnancy outcomes preeclampsia, preterm birth, and LBW are mainly from high-income countries, and it is important to assess whether the high prevalence of thyroid dysfunction in pregnancy may contribute to the high burden of adverse pregnancy outcomes in Nepal. The results of this study may add to the existing knowledge gap and inform healthcare providers, which could contribute to reducing the burden of adverse maternal and fetal outcomes. This rationale has led to the following hypotheses:

- Approximately 10% of the study participants have thyroid dysfunction during pregnancy, and the adverse pregnancy outcomes preeclampsia, preterm birth, or LBW affected approximately 5–20% of births.
- Maternal thyroid function during early pregnancy is associated with the risk of preeclampsia, having a preterm birth, and giving birth to an infant with LBW.

1.4. Research question

The following research questions were formulated based on our background knowledge as well as our review of existing literature:

1) What is the prevalence of thyroid dysfunction and the adverse pregnancy outcomes preeclampsia, preterm birth, and LBW among pregnant women aged between 20 and 40 in Bhaktapur district, the Kathmandu valley in Nepal?
2) Are there any associations between markers of maternal thyroid function in early pregnancy and the adverse outcomes of preeclampsia, preterm birth, and LBW among pregnant women aged between 20 and 40 in Bhaktapur district, the Kathmandu valley in Nepal?

2. STUDY AIM

This retrospective cohort study aimed to determine the prevalence of thyroid dysfunction and the adverse pregnancy outcomes of preeclampsia, preterm birth, and LBW. Furthermore, we aimed to investigate the associations between markers of maternal thyroid function in early pregnancy and the adverse outcomes among pregnant women aged between 20 and 40 in Bhaktapur district, the Kathmandu Valley in Nepal, from March 2017 to October 2020.

2.1. Specific objectives

1. To determine the prevalence of thyroid dysfunction in pregnant women.
2. To determine the prevalence of pregnancy outcomes: preeclampsia, preterm birth, and LBW.
3. To investigate the association between markers of maternal thyroid function measured in early pregnancy and the risk of preeclampsia.
4. To investigate the association between markers of maternal thyroid function measured in early pregnancy and the risk of preterm birth.
5. To investigate the association between markers of maternal thyroid function measured in early pregnancy and the risk of giving birth to an infant with LBW.

3. METHODOLOGY

3.1. Study design

This is an observational study using data from a randomized controlled trial (RCT) as a retrospective cohort study. The RCT included 800 mother-infant pairs and aimed to investigate the effect of maternal vitamin B12 supplementation during pregnancy and through 6 months postpartum on early child development and growth.

3.2. Study setting

The study site was Bhaktapur, located 1400m above sea level and 15km east of Kathmandu, Nepal's capital city. Bhaktapur is an ancient city listed as a UNESCO World Heritage Site. It is a peri-urban, agriculturally based city mostly populated by the Newar ethnic group. It is characterized by a primarily homogeneous community of mixed Hindus and Buddhists but also by migrant workers from diverse ethnic groups.

The local climate is hot and humid from May to August while dry and cool from October to March. The food grown in the community is the main food source for the residents of Bhaktapur. Their eating patterns vary based on the season, field workload, and food availability: rice is the staple food, and leafy green vegetables are usually consumed in the winter and spring.

3.3. Study population

Our study population was comprised of healthy, 20- to 40-year-old pregnant women and their infants. The women were recruited during early pregnancy, no later than 15 weeks. They resided in Bhaktapur municipality as well as the surrounding areas in the Bhaktapur district in Nepal. The recruitment period was between March 2017 and October 2020.

3.4. Sample size calculation

This study was a secondary analysis from an RCT, and the initial sample size was 800 pregnant women. The statistical power of the outcomes included in this thesis was further calculated using results from an Indian study where the differences in proportions of preeclampsia, preterm birth, and LBW between euthyroid women and those with thyroid dysfunction were assessed.[62] The highest statistical power was expected to be achieved with the outcomes of preeclampsia and LBW. The sample size was expected to give approximately 99% power to detect a difference of 13% and 18%, respectively (Figures 2 and 4). A two-sample proportion formula was used for the 'power' function in the statistical analysis software, STATA.[63]

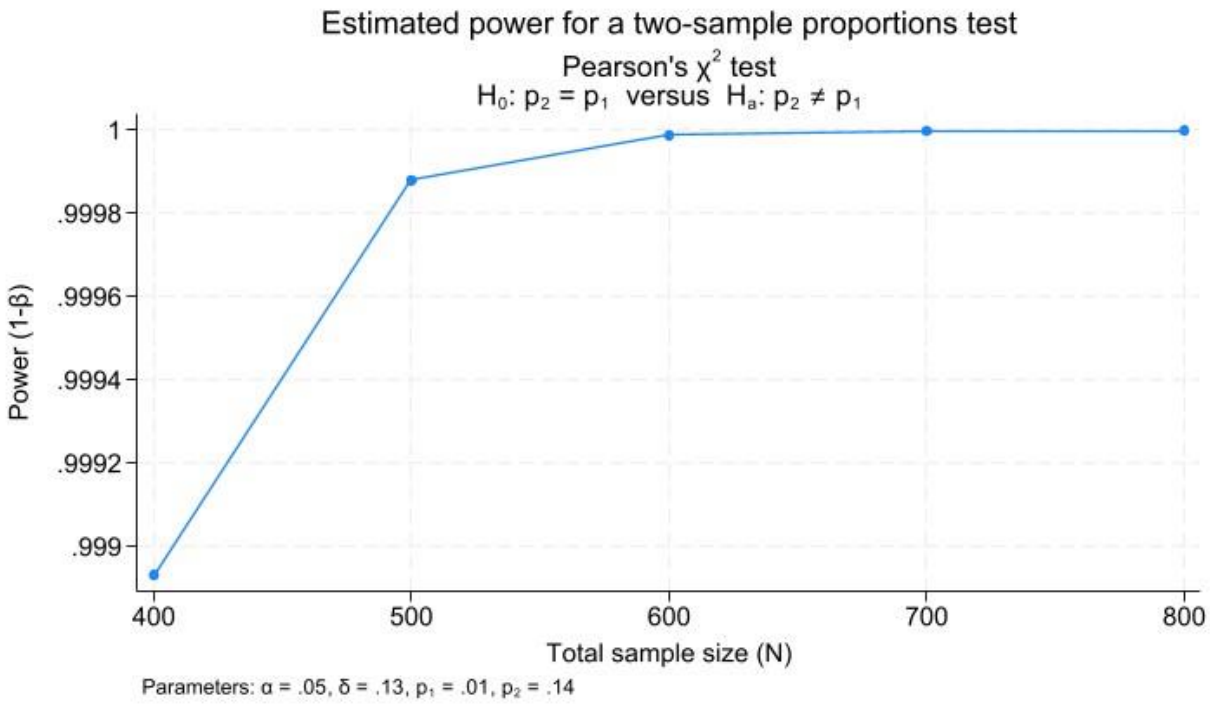

Figure (2): Estimated power for a two-sample proportion test for preeclampsia.

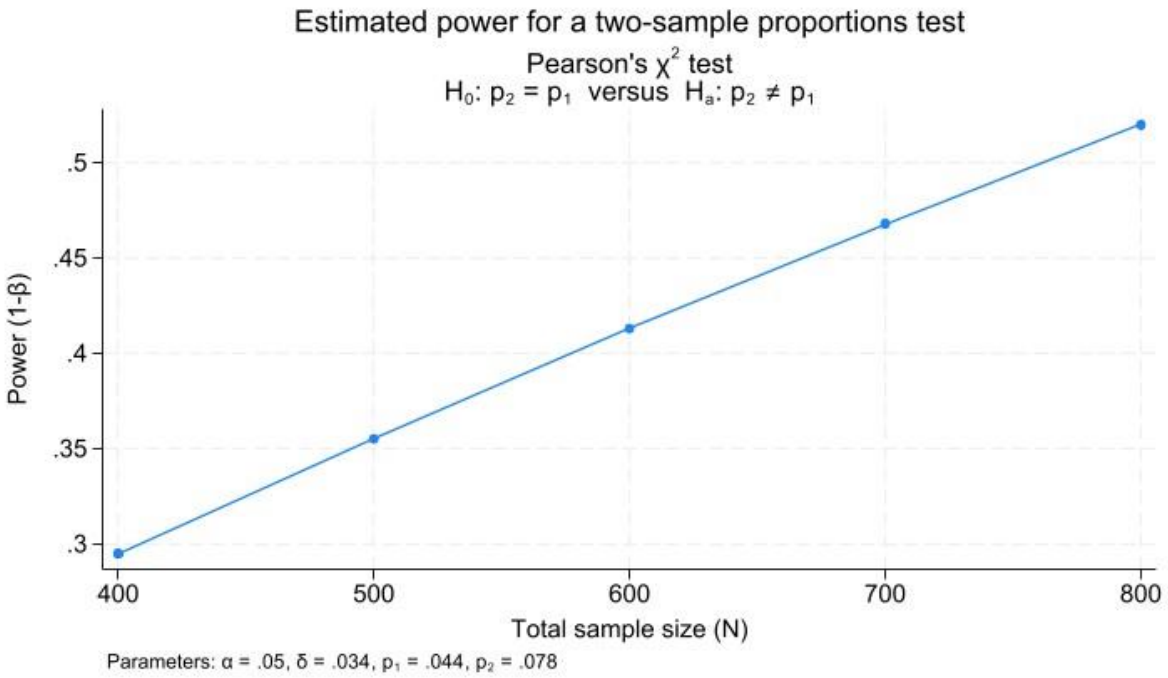

Figure (3): Estimated power for a two-sample proportion test for preterm birth.

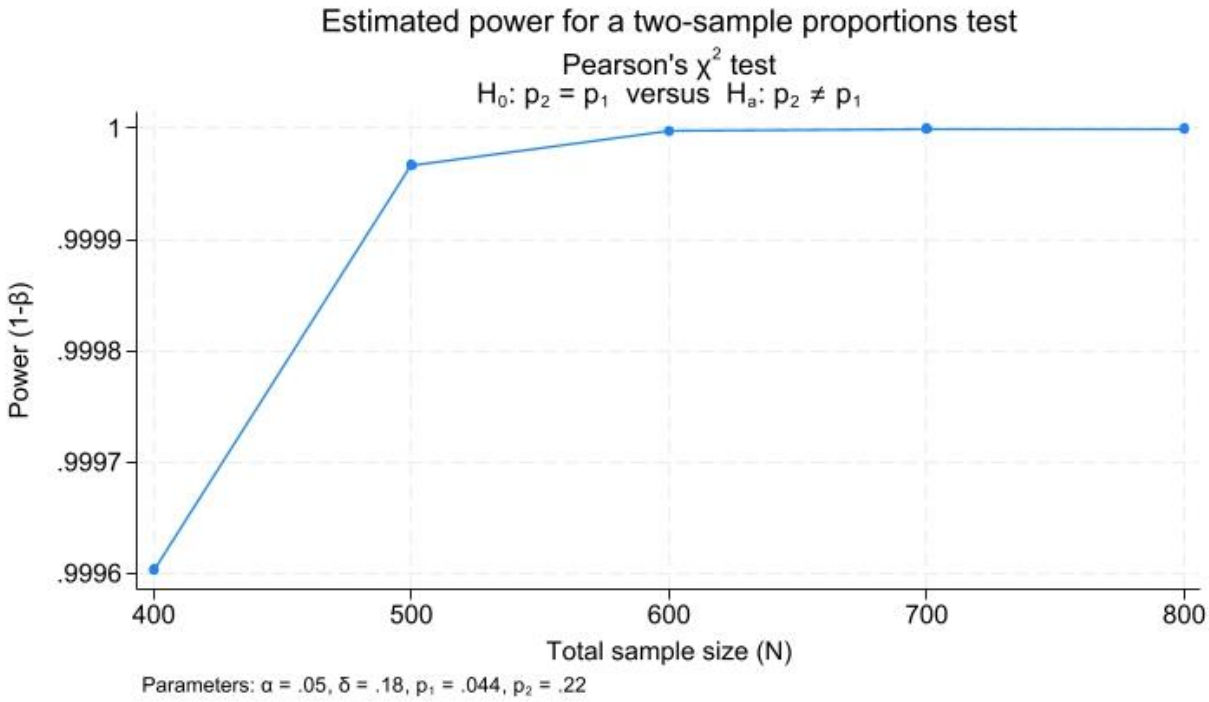

Figure (4): Estimated power for a two-sample proportion test for low birth weight.

3.5. Sampling and recruitment process

The sampling method used was purposive sampling. In the main RCT, the pregnant mothers were identified using a hospital-and community-based surveillance system. Newly married couples' records were available in the study area and updated quarterly. The identified women were screened for eligibility criteria and provided with detailed information about the study, including its duration, the collection of biological samples, and the follow-up plan.

The inclusion and exclusion criteria for the RCT.

Inclusion criteria:

1. 20 to 40-year-old pregnant women from early pregnancy, not later than 15 weeks pregnant.
2. Residing in Bhaktapur municipality and surrounding areas in the Bhaktapur district.
3. The availability of informed consent.

Exclusion criteria:

1. Taking dietary or multivitamin supplements having vitamin B12
2. Cases of chronic disease under treatment such as diabetes, hypertension, Crohn's disease, tuberculosis, diabetes, hypothyroidism or hyperthyroidism, pernicious anemia, tuberculosis, and current use of anticonvulsant drugs.
3. Women with multiple pregnancies.
4. Cases for whom the pregnancy outcomes were unknown, such as those who did not deliver at the specified hospital.
5. Cases of severe anemia (hemoglobin concentration <7 g/dL). The concentration of hemoglobin level in the blood was analyzed at enrolment using HemoCue.
6. Suffering from any condition that requires treatment with vitamin B12, such as pernicious anemia and strict vegans.

This study included 767 pregnant women out of the 800 enrolled at baseline. Thirty-three participants were excluded due to missing information on the outcomes of gestational age at delivery and birth weight resulting from loss to follow-up due to illness, miscarriage, death, and other non-specified reasons. (Figure 5)

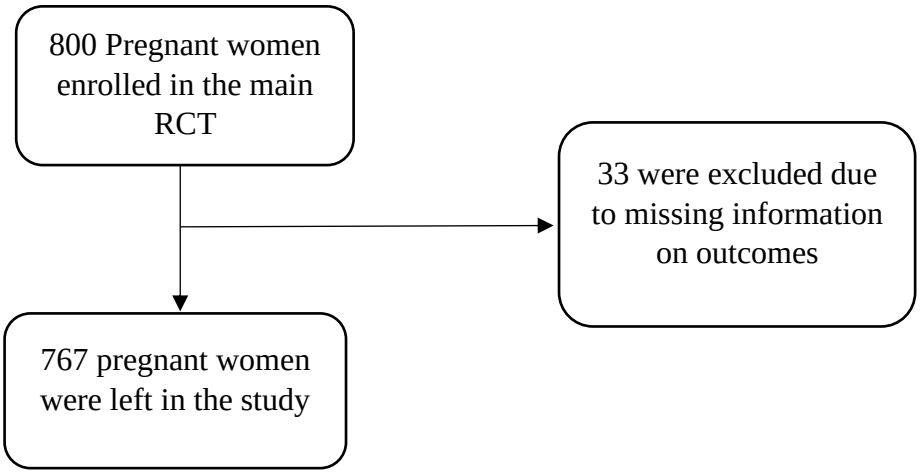

Figure (5): Flow chart of study participants.

3.6. The inclusion and exclusion criteria for this master thesis project

Inclusion criteria:

- The pregnant women recruited in the main RCT.

Exclusion Criteria:

- Participants with missing data on outcomes.

3.7. Data collection methods and tools

The data was collected during the RCT at several time points. Maternal background information and blood samples were collected at enrollment. Interviews were conducted to gather maternal background information, and field workers filled out a structured demographic and health form.

Blood samples of 3 milliliters were obtained from the pregnant women following the aseptic measures and standard protocol in an Ethylenediaminetetraacetic acid (EDTA) vial. First centrifuged at around 700 *g* at room temperature for a duration of 10 minutes, the plasma was then separated and transferred into storage vials and stored at −70°C prior to analysis.

During the first home visit, as soon as possible after birth, the field workers interviewed the mothers again, collecting information about the baby's delivery and any labor/delivery complications, including the outcome variables of interest, using the structured delivery report form.

3.7.1. Biochemical analyses of thyroid function markers

The biochemical analyses of the blood samples were performed at the Institute of Medicine at Tribhuvan University in Kathmandu, Nepal. Using a Chemiluminescence auto analyzer, specifically the Snibe Maglumi 800 model, researchers in the biochemical laboratory analyzed the thyroid function markers: TSH, FT4 and TPOAb. For TSH and TPOAb, a Sandwich immunoluminometric assay design was utilized, whereas for FT4, a competitive immunoluminometric assay design was utilized.

Different quantification limits were used for TSH, FT4, and TPOAb, which are presented in Table 4 below.

Table 4: Quantification limits for TSH, FT4, and TPOAb

Thyroid function biomarker	Quantification Limits
TSH (μIU/mL)	0.001–100
FT4 (pg/mL)	1.0–120
TPOAb (IU/mL)	0.38–1,000

Abbreviations: TSH, thyroid stimulating hormone; FT4, free thyroxine; TPOAb, thyroperoxidase antibodies.

The coefficients of variation, for both inter- and intra-assays, of the aforementioned thyroid function biomarkers ranged from 2.98 to 6.01%, as presented in Table 5. Furthermore, the tests were conducted after ensuring that the quality control values were acceptable (within 1 standard deviation, SD). A maximum of 40 blood samples at a time were thawed, handled, and analyzed. Prior to running each sample, both internal and external controls were included and processed.

Table 5: The inter- and intra-assay coefficients of variation of the thyroid function biomarkers

Thyroid Function Biomarker	Coefficients of variation (%)
TSH	2.98
FT4	6.01
TPOAb 45.17 IU/mL 110.66 IU/mL	 4.79 3.69

Abbreviations: TSH, thyroid stimulating hormone; FT4, free thyroxine; TPOAb, thyroperoxidase antibodies

3.8. Data management

The data was collected on paper in structured forms designed for the RCT by the fieldworkers. The forms were checked manually by the study supervisors before computer entry. The data was entered twice by two different operators within a period of one week of data collection, either in a local database or in a cloud-based data management system (iForm). The collected data was stored in Kathmandu's data management center (DMC). Access to the data was granted only to the supervisors of the study and the computer data entry staff. The data were processed several times to identify inconsistencies between the variables and the forms. The system also detected entries that were out of range and notified them of missing data.

For the purposes of this study, I was granted access to a de-identified file containing participant data in the University of Bergen's (UiB) Secure Access to Research Data and E-infrastructure (SAFE) server. SAFE is a system developed by UiB's IT department to securely handle sensitive personal data in research. It guarantees the protection of sensitive personal data, ensuring that confidentiality, integrity, and availability are upheld during research.

3.9. Study variables

Our secondary analysis used the analyzed blood sample tests of TSH, FT4, and TPOAb and registered preeclampsia diagnosis, gestational age at birth, birth weight, infant sex, parity maternal age, and socio-economic status (WAMI index, described in section 3.9.3 of covariates). The study variables were divided into exposure variables, outcome variables, and covariates.

3.9.1. Exposure variables:

Markers of thyroid function

Maternal TSH and FT4 concentrations were analyzed to evaluate maternal thyroid function. According to the population-specific reference ranges for thyroid function in early pregnancy presented in Table 2 [(16)], the thyroid hormones TSH and FT4 were categorized into low, normal, and high levels.

Furthermore, the pregnant women were grouped based on their thyroid function status—euthyroid, hypothyroid (subclinical and overt), or hyperthyroid (subclinical and overt), as presented in Table 1—determined by their TSH and FT4 concentrations in Table 2.

3.9.2. Outcome variables:

The outcome variables preeclampsia (categorized as yes or no), gestational age at delivery (in weeks), and birth weight (in grams) were collected when the mothers gave birth.

The field workers gathered the mothers' preeclampsia diagnoses through the delivery report form (DRF) that they received when they were discharged from the hospital. They conducted interviews with the mothers after delivery, at the first home visit, and as early as possible after birth. The gestational age at delivery was calculated using an ultrasound due date and the baby's birth date. The hospital where the participating mother gave birth registered the birth weight immediately after birth. Different types of scales/weights were used to obtain the birth weight.

Preeclampsia was assessed as a dichotomous variable (yes or no), whereas gestational age and birth weight were assessed as continuous variables and further categorized. Gestational age was dichotomized into preterm birth (yes or no), which was defined as having birth in less than 37 weeks of gestation. Birth weight was dichotomized into LBW (yes or no), which was defined as having a birth weight of less than 2500 grams.

3.9.3. Covariates:

Based on the existing literature, the potential covariates were predefined as infant sex, parity, maternal age, WAMI index, and TPOAb.[36, 64-74] The covariates of infant sex and parity were assessed as dichotomous. Infant sex was categorized into male and female (1 = male, 2 = female), whereas parity was dichotomized as primiparous (0) and multiparous (1 or more).

Maternal age (in years), WAMI index (range between 0 and 1), and concentration of TPOAb (IU/ml) were assessed as continuous variables in our linear and logistic regression models. The WAMI index indicates the participants' socioeconomic status, which includes four parts: improved water and sanitation access, assets ownership, maternal education, and income.[75] In our study, we calculated the WAMI index in STATA using the data gathered on access to safe water and sanitation, assets ownership, maternal education, and household income at the baseline interview.

For the first part of the WAMI index, water and sanitation, access to safe water was categorized as yes/no and considered safe if the source was from bottled water, tap water, or tanker supply. As for sanitation, also categorized as yes/no, it was considered safe when participants had access to toilet facilities with proper drainage into a septic tank. For the second part of the

WAMI index, ownership of assets was categorized as yes/no. It was assessed based on having a separate kitchen and bedroom, ownership of land, and other assets such as a motorbike, TV, freezer, PC, microwave, and car. As for the third part of the WAMI index, maternal education was categorized into eight groups ranging from 0 to a maximum of 18 years of schooling. For the final part of the WAMI index, monthly average income earned by the entire household was also categorized into eight groups, ranging from 2000–1000000 Nepalese Rupees (NPR). The four scores of the different WAMI parts were obtained, each carrying an equal weight when constructing the WAMI index. These scores were summed up and then divided by 32 to obtain the WAMI index range from 0 to 1, where a higher index indicates a higher socioeconomic status.

These covariates could potentially be associated with both maternal thyroid function and the study outcomes. In Figure 6 below, the relationship is presented in the directed acyclical graph (DAG) system, a software used for drawing and identifying potential sources of bias that might alter the study's findings, helping to understand the relationship between exposure, outcomes, and covariates. The DAG system shows how maternal thyroid function is linked with green arrows to the adverse outcomes of preeclampsia, preterm birth, and LBW, indicating a possible direct relationship. The covariates of infant sex, parity, maternal age, WAMI index, and TPOAb are linked with pink arrows to both the exposure and the outcomes, as they could be potentially associated with both.

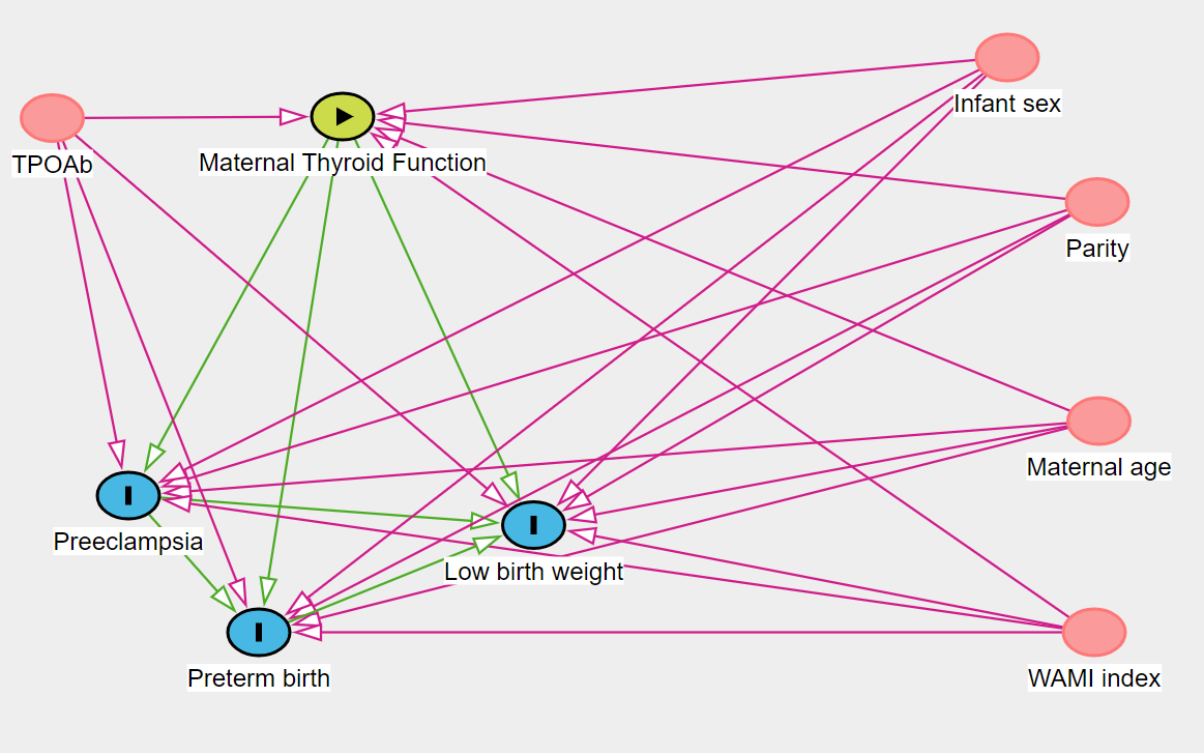

Figure (6): Directed acyclical graph (DAG) of covariates.

3.10. Statistical analysis

The data analysis was performed using the software STATA version 18.0/SE in the SAFE server. The statistical analysis was divided into descriptive analysis and analysis of association, including linear and logistic regression.

3.10.1. Descriptive statistics:

Frequencies and percentages were used to summarize the categorical variables and to describe the population and the outcomes of preeclampsia, preterm birth, and LBW. The maternal age and TPOAb were also categorized for this purpose. Maternal age was categorized into four age groups: 20–24, 25–29, 30–34, and 35–40 years old, whereas TPOAb was classified as either positive (≥30 IU/mL) or negative(<30(IU/mL).

Mean and standard deviation (SD) were used to summarize the normally distributed continuous variables, while median and interquartile range (IQR) were used to summarize TSH and TPOAb, as they were non-normally distributed. One outlier was identified in the maternal TSH hormone data. The participant belonged to the subclinical hypothyroid group and gave birth to a LBW baby. The outlier was dropped, the analysis was run again to re-assess, and only minor

changes were detected. Therefore, it was included in the analysis. In addition, one of the participants did not have a WAMI index due to missing data; for this participant, their WAMI index was imputed using the mean (0.7).

3.10.2. Association between maternal markers of thyroid function in early pregnancy and outcomes of pregnancy

1. Linear regression analyses

Linear regression analyses were used to investigate the association between maternal TSH and FT4 concentrations and the continuous outcomes of gestational age at delivery in weeks and birth weight in grams. The association between maternal concentrations of TSH (μIU/ml) and FT4 (pg/ml) and the continuous outcomes of gestational age at delivery and birth weight was non-linear. Therefore, the non-linear associations were assessed using both cubic and linear splines of TSH and FT4 and with both specified and unspecified knots. However, splines did not display a better fit for the regression models (birth weight and gestational age), so a linear regression model was used for the analysis. Separate multivariate models were used to assess the association between maternal TSH levels and the outcomes, as well as FT4 levels and the outcomes. These models were run separately because there is an association between TSH and FT4, where TSH is the main stimulus for thyroid hormone production, including T4, by the thyroid gland.[76]

2. Logistic regression analyses

Logistic regression analyses were used to investigate the association between maternal concentrations of TSH (μIU/ml) and FT4 (pg/ml) and the categorical outcomes of preterm birth (0=no, 1=yes) and LBW (0=no, 1=yes). Furthermore, as described above, separate multivariate models were run to assess the association between maternal TSH levels and the outcomes and FT4 levels and the outcomes.

3.11. Potential risks and benefits

One potential risk of the main study was collecting blood samples from the participants during the RCT project. The participants were at risk of slight pain or discomfort while blood was drawn. However, blood samples from pregnant women were obtained following the aseptic measures and standard protocol. The study staff had received training before the initiation of the main project. Furthermore, the fieldwork was monitored by skilled supervisors, medical doctors, and gynecologists.

Regarding the benefits of the study, participants were not compensated financially, nor did they receive any direct benefits for their contributions to the main RCT study. However, the study participants may have benefitted from receiving regular blood tests checking their hemoglobin levels, blood group, venereal disease research laboratory test (VDRL), hepatitis B, and human immunodeficiency virus (HIV) status, as per the protocol of the government of Nepal. Furthermore, the enrolled pregnant mothers benefitted from receiving supplements such as iron, folic acid, and calcium, as well as deworming medicine. Iron supplementation was also given to infants if their hemoglobin values were low. In addition, treatment for illnesses like diarrhea, fever, acute respiratory infections, and otitis media was provided when required. Those who needed additional assessment and care were directed to tertiary hospitals like Tribhuvan University Teaching Hospital. Following Nepali government guidelines, study staff members also offered education on exclusive breastfeeding and complementary feeding. Regular height and weight measurements of participating infants were taken, identifying those at risk of malnutrition.

A benefit of our current study is that we used secondary data analysis, so no additional data or blood samples were needed from the participants.

3.12. Ethical considerations

Ethical approval from the National Health and Research Council, Nepal (NHRC 253/2016) and the Regional Committee for Medical and Health Research Ethics of Western Norway (2016/1620/REK vest) has been obtained. Furthermore, the RCT is registered in clinical trials.gov, which serves as a database for clinical studies that are both publicly and privately funded and are conducted globally. The consent forms for the RCT were filled out by gynecologists included in the study or supervisors in the husband's presence. The participants were provided with a copy of the consent form and the information sheets. A separate provision for consent was included in the consent form for the biological samples. In the case of illiterate participants, thumbprints were obtained after getting a signature from an impartial witness who was not part of the research team.

In addition, the current study was reported and registered in Risk and Risk Experience in Research Projects (RETTE), as per UiB protocols. RETTE is UiB’s risk and compliance system for managing and overseeing the handling of personal data in research and student projects conducted at the university.

4. RESULTS

4.1. Socio-demographic characteristics

Out of the 800 pregnant women enrolled at baseline, 767 participants are included in the current study, having a follow-up rate of 95%. The 33 participants excluded had missing information on the included outcomes (Figure 5).

Table 6 shows the socio-demographic characteristics of the 767 mother-infant participants. More than three-fourths (n=599, 78.1%) of the participants stated that they belonged to the Newar ethnic group. The mean age of the pregnant women was 27.6 years. Approximately half of the infants were male (n=397, 51.8%), and the mean WAMI index was 0.7.

Table 6: Demographic characteristics of the pregnant women residing in the Bhaktapur municipality and the surrounding areas between March 2017 and October 2020. (N=767)

Socio-Demographic Characteristics	n (%)
Ethnicity	
Brahmin	24 (3.1)
Chhetri	51 (6.7)
Newar	599 (78.1)
Gurung/Rai/Magar	8 (1)
Tamang/lama	64 (8.3)
Chudahari/Madhesi/Muslim	7 (0.9)
Dalit (Backward caste)	12 (1.6)
Others	2 (0.3)
Maternal Education (Years)	
No Education (0-2)	15 (2)
Pre-primary (3-4)	17 (2.2)
Primary (5-9)	136 (17.7)
Lower Secondary (10-12)	422 (55)
Secondary (13-14)	6 (0.8)
Higher Secondary (15-16)	126 (16.4)
Higher Education (>17)	45 (5.9)
Maternal age (Mean, SD)	27.6 (4)
Maternal age (years)	
20-24	186 (24.3)
25-29	355 (46.3)
30-34	184 (24)
35-40	42 (5.4)
WAMI Index (Mean, SD)	0.7 (0.1)
Infant sex	
Male	397 (51.8)
Female	370 (48.2)

Data are presented as frequency (%) or mean ± SD.

Abbreviations: SD, standard deviation; IQR, interquartile range; WAMI index (range 0-1), "a measure of the household socioeconomic status based on access to improved Water/sanitation, Assets, Maternal education, and Income."[75]

4.2. Medical history and clinical information characteristics

The medical history and clinical information characteristics of the enrolled pregnant women (n=767) are presented in Table 7. The participants' mean BMI was 23.7 kg/m^2. Approximately half (n=373, 48.6%) were primiparas, and 394 (51.4%) were multiparas. The mean gestational age at enrolment was 11.1 weeks.

Of the total included women, 18 (2.4%) had low TSH levels (<0.05 μIU/ml), and 24 (3.1%) had high TSH levels (>3.69 μIU/ml) according to the population-specific reference ranges (Table 2).[16] The median concentration of TSH was 1.2 μIU/ml. Further, 20 (2.6%) of the women had low FT4 levels (<8.89 pg/ml), and 20 (2.6%) had high FT4 levels (>15.28 pg/ml). The mean concentration of FT4 was 11.8 pg/ml. We found that 58 (7.6%) of the women were TPOAb positive (≥30 IU/mL), only three women (0.4%) had overt hypothyroidism, 19 (2.5%) had subclinical hypothyroidism, two (0.3%) had overt hyperthyroidism and 15 (2%) had subclinical hyperthyroidism according to their thyroid function values measured at baseline.

As for medical history, 129 (16.8%) of the women had a history of having one or more abortions, and four women (0.5%) had a history of having one or more stillbirths.

Table 7: The participants' medical history and clinical information characteristics (N=767).

Clinical Information & Medical History	n (%)
Clinical Information	
BMI (kg/m^2) (Mean, SD)	23.7 (3)
Gestational age at blood sampling (Weeks) (Mean, SD)	11.1 (2.8)
Thyroid Hormones	
TSH (µIU/ml) (Median, IQR)	1.23 (0.81/1.91)
TSH (µIU/ml)	
Low (<0.05 µIU/ml)	18 (2.4)
Normal (0.05-3.69 µIU/ml)	725 (94.5)
High (>3.69 µIU/ml)	24 (3.1)
FT4 (pg/ml) (Mean, SD)	11.84 (2.1)
FT4	
Low (<8.89 pg/ml)	20 (2.6)
Normal (8.89-15.28 pg/ml)	727 (94.8)
High (>15.28 pg/ml)	20 (2.6)
Maternal Thyroid Function Status	
Euthyroid	689 (89.8)
Hypothyroid Subclinical[a]	19 (2.5)
Hypothyroid Overt[b]	3 (0.4)
Hyperthyroid Subclinical[c]	15 (1.9)
Hyperthyroid Overt[d]	2 (0.3)
TPOAb (IU/ml) (Median, IQR)	3.2 (1.66/5.62)
TPOAb (IU/ml)[e]	
Positive	58 (7.6)
Negative	709 (92.4)
Parity	
0	373 (48.6)
≥1	394 (51.4)
Medical History	
Number of Abortions (≥1)	129 (16.8)
Number of Stillbirths (≥1)	4 (0.5)
Using medication at inclusion	
Yes	158 (20.6)

Data are presented as frequency (%), mean ± SD, or Median and IQR.
Abbreviations: SD, standard deviation; IQR, interquartile range; BMI, body mass index; TSH, thyroid stimulating hormone; FT4, free thyroxine; TPOAb, thyroperoxidase antibodies. [a]Characterized as elevated TSH and normal FT4.[b]Characterized as elevated TSH and declined FT4. [c]Characterized as decreased TSH and normal FT4. [d]Characterized as decreased TSH and elevated FT4. [e]TPOAb positivity was classified as ≥30 IU/mL.

4.3. Outcome-related characteristics and prevalence of the pregnancy outcomes: preeclampsia, preterm birth, and LBW

The mean birth weight of the infants was 3015 grams, and the mean gestational age at delivery was 38.5 weeks (Table 8). A total of 74 (9.7%) of the women gave birth to LBW babies, and 70 (9.1%) had a preterm birth. There were three cases of preeclampsia (0.4%).

Table 8: **Outcome-related characteristics and prevalence of the pregnancy outcomes: preeclampsia, LBW, and preterm birth of the participants (N=767).**

Outcomes	n (%)
Preeclampsia/Eclampsia	3 (0.4)
Gestational age at delivery (Weeks) (Mean, SD)	38.5 (1.8)
Preterm Birth (<37 weeks of gestation)	70 (9.1)
Birth weight (grams) (Mean, SD)	3015.1 (457)
LBW (<2500g)	74 (9.7)

Data are presented as frequency (%) or mean ± SD.
Abbreviations: SD, standard deviation; LBW, low birth weight.

4.4. Association between maternal levels of TSH and FT4 in early pregnancy and preeclampsia.

Preeclampsia was reported in only three of the pregnant women included in our study. Therefore, the number of preeclampsia cases was insufficient to investigate its potential association with maternal levels of TSH and FT4 in early pregnancy.

4.5. Association between maternal levels of TSH and FT4 in early pregnancy and gestational age at delivery.

We did not find a significant association between the maternal TSH or FT4 hormone levels in early pregnancy and gestational age at delivery (Coeff TSH: 0.003, 95% CI: -0.05, 0.06, Coeff FT4: - 0.02, 95% CI; -0.08, 0.04, Table 9). The 95% confidence interval (CI) indicates that if we had performed the study 100 times, in 95 of them, the values would lie in these intervals, which could indicate both positive and negative associations.

In the bivariate model, the covariate maternal age was the only one significantly associated with gestational age at delivery. However, in the multivariate models, the 95% CI was -0.08,

0.00, indicating that a one-year increase in maternal age may cause a slight or no reduction in gestational age at delivery. (Table 9)

Table 9: Bivariate and multivariate linear regression model of the association between maternal levels of TSH and FT4 in early pregnancy and gestational age at delivery (N=767).

	Bivariate			**Multivariate (TSH & Covariates)**			**Multivariate (FT4 & Covariates)**		
Gestational age at delivery (weeks)	**Crude Estimates**			**Adjusted Estimates**			**Adjusted Estimates**		
	Coeff.	**P-value**	**95% CI**	**Coeff.**	**P-value**	**95% CI**	**Coeff.**	**P-value**	**95% CI**
TSH	0.002	0.95	-0.05, 0.06	0.003	0.90	-0.05, 0.06	-	-	-
FT4	-0.01	0.72	-0.07, 0.05	-	-	-	-0.02	0.50	-0.08, 0.04
Infant sex									
Male (n=397)	**Ref**			**Ref**			**Ref**		
Female(n=370)	0.25	0.06	-0.01, 0.50	0.24	0.07	-0.02, 0.50	0.25	0.06	-0.01, 0.51
Parity									
Primipara(n=373)	**Ref**			**Ref**			**Ref**		
Multipara(n=394)	-0.22	0.10	-0.48, 0.04	-0.08	0.61	-0.39, 0.23	-0.08	0.63	-0.38, 0.23
Maternal age (years)	**-0.04**	**0.01**	**-0.08, -0.01**	**-0.04**	**0.05**	**-0.08, 0.00**	**-0.04**	**0.04**	**-0.08, 0.00**
WAMI Index	-0.41	0.40	-1.37, 0.55	-0.44	0.38	-1.43, 0.55	-0.41	0.42	-1.40, 0.58
TPOAb	0.00	0.80	0.00, 0.00	0.00	0.85	0.00, 0.00	0.00	0.83	0.00, 0.00

The multivariate model was adjusted for infant sex, parity, maternal age, WAMI index, and TPOAb.
Abbreviations: CI, confidence interval; TSH, thyroid stimulating hormone; FT4, free thyroxine; WAMI index (range 0-1), "a measure of the household socioeconomic status based on access to improved Water/sanitation, Assets, Maternal education, and Income"[75]; TPOAb, thyroid peroxidase antibodies.

4.6. Association between maternal levels of TSH and FT4 in early pregnancy and preterm birth.

We did not find any significant association between maternal TSH hormone levels and the risk of having preterm birth (adjusted odds ratio [OR] of 1.02, 95% CI; 0.94 –1.12, p=0.60, Table 10). Further, we found no significant association between maternal FT4 hormone levels and the risk of preterm birth (adjusted OR of 0.99, 95% CI; 0.87–1.12, p=0.86).

In the multivariate regression models, we found that maternal age was associated with the risk of having a preterm birth, with an adjusted OR of 1.08 (95% CI; 1.00–1.16, p=0.03). This indicates that with a year of maternal age increase, the odds of having a preterm birth increase by 8 percentage points.

We did not find an association between the covariates- infant sex, parity, WAMI index, and TPO antibodies (TPOAb) and the risk of preterm birth (Table 10).

Table 10: Bivariate and multivariate logistic regression model of the association between maternal levels of TSH and FT4 in early pregnancy and preterm birth. (N=767)

	Bivariate			**Multivariate (TSH & Covariates)**			**Multivariate (FT4 & Covariates)**		
Preterm Birth (N=70)	**Crude Estimates**			**Adjusted Estimates**			**Adjusted Estimates**		
	OR	**P-value**	**95% CI**	**OR**	**P-value**	**95% CI**	**OR**	**P-value**	**95% CI**
TSH	1.01	0.78	0.93 – 1.10	1.02	0.60	0.94 –1.12	-	-	-
FT4	0.98	0.74	0.86 – 1.11	-	-	-	0.99	0.86	0.87 – 1.12
Infant sex									
Male (n=397)	**Ref**			**Ref**			**Ref**		
Female (n=370)	0.65	0.09	0.39 – 1.07	0.63	0.08	0.38 – 1.05	0.63	0.08	0.38 – 1.05
Parity									
Primipara(n=373)	**Ref**			**Ref**			**Ref**		
Multipara(n=394)	0.83	0.46	0.51 – 1.36	0.62	0.12	0.34 – 1.14	0.63	0.13	0.35 – 1.15
Maternal age (years)	1.05	0.11	0.99 – 1.12	1.08	0.03	1.01 – 1.16	1.08	0.03	1.01 – 1.16
WAMI Index	5.95	0.06	0.92 – 38.61	3.98	0.16	0.59 – 26.96	4.05	0.15	0.60 – 27.50
TPOAb	1.00	0.92	1.00 – 1.00	1.00	0.84	1.00 – 1.00	1.00	0.87	1.00 –1.00

The multivariate model was adjusted for infant sex, parity, maternal age, WAMI index, and TPOAb.
Abbreviations: CI, confidence interval; OR, odds ratio; TSH, thyroid stimulating hormone; FT4, free thyroxine; WAMI index (range 0-1), "a measure of the household socioeconomic status based on access to improved Water/sanitation, Assets, Maternal education, and Income"[75]; TPOAb, thyroid peroxidase antibodies.

4.7. Association between maternal levels of TSH and FT4 in early pregnancy and birth weight.

We did not find a significant association between maternal TSH hormone levels and infant birth weight (Coeff: -12.92, 95% CI; -26.50, 0.67, p=0.06, Table 11). Similarly, we did not find a significant association between maternal FT4 levels and infant birth weight (Coeff: -10.80, 95% CI; -26.15, 4.55, p=0.17).

In the multivariate regression models, the covariate infant sex was the only one significantly associated with infant birth weight, with a bivariate estimate of 129.8 and a 95% confidence interval of -194, -66, indicating that females had an average reduction of 130 grams compared to males (Table 11).

Table 11: Bivariate and multivariate linear regression model of the association between maternal concentration of thyroid hormones TSH and FT4 measured in early pregnancy and birth weight (N=764).

	Bivariate			**Multivariate (TSH & Covariates)**			**Multivariate (FT4 & Covariates)**		
Birth Weight	**Crude Estimates**			**Adjusted Estimates**			**Adjusted Estimates**		
	Coeff.	**P-value**	**95% CI**	**Coeff.**	**P-value**	**95% CI**	**Coeff.**	**P-value**	**95% CI**
TSH	-12.36	0.07	-25.92, 1.20	-12.92	0.06	-26.50, 0.67	-	-	-
FT4	-12.31	0.12	-27.60, 2.99	-	-	-	-10.80	0.17	-26.15, 4.55
Infant sex									
Males (n=397)	**Ref**			**Ref**			**Ref**		
Females(n=370)	**-129.84**	**0.00**	**-194.16, -65.51**	**-128.18**	**0.00**	**-192.57, -63.80**	**-125**	**0.00**	**-189.64, -60.34**
Parity									
Primipara(n=373)	**Ref**			**Ref**			**Ref**		
Multipara(n=394)	9.70	0.77	-55.27, 74.67	14.26	0.72	-63.44, 91.95	10.16	0.80	-67.38, 87.71
Maternal age (years)	2.67	0.52	-5.44, 10.78	1.60	0.74	-7.85, 11.04	1.36	0.78	-8.14, 10.85
WAMI Index	222.65	0.07	-18.89, 464.19	220.50	0.08	-27.57, 468.57	231.56	0.07	-17.66, 480.71
TPOAb	0.05	0.75	-0.24, 0.34	0.07	0.60	-0.21, 0.36	0.03	0.82	-0.25, 0.32

The multivariate model adjusted for infant sex, parity, maternal age, WAMI index and TPOAb.
Abbreviations: CI, confidence interval; OR, odds ratio; TSH, thyroid stimulating hormone; FT4, free thyroxine; WAMI index (range 0-1), "a measure of the household socioeconomic status based on access to improved Water/sanitation, Assets, Maternal education, and Income"[75]; TPOAb, thyroid peroxidase antibodies.

4.8. Association between maternal levels of TSH and FT4 in early pregnancy and LBW.

We did not find a significant association between maternal TSH hormone levels and the risk of having a LBW baby (adjusted OR of 1.09, 95% CI of 0.97–1.21, p=0.14, Table 12). Further, we did not find a significant association between maternal FT4 hormone levels and the risk of LBW (adjusted OR of 1.04, 95% CI of 0.94–1.15, p=0.41).

In the multivariate models, the covariates of infant sex, parity, maternal age, WAMI index, and TPO antibodies (TPOAb) were also not associated with the risk of having a LBW baby (Table 12).

Table 12: Bivariate and multivariate logistic regression model of the association between maternal concentration of thyroid hormones TSH, FT4 in early pregnancy and risk of LBW (N=764).

	Bivariate			Multivariate (TSH & Covariates)			Multivariate (FT4 & Covariates)		
LBW (N=74)	**Crude Estimates**			**Adjusted Estimates**			**Adjusted Estimates**		
	OR	**P-value**	**95% CI**	**OR**	**P-value**	**95% CI**	**OR**	**P-value**	**95% CI**
TSH	1.08	0.15	0.97 – 1.21	1.09	0.14	0.97 – 1.21	-	-	-
FT4	1.04	0.45	0.94 – 1.15	-	-	-	1.04	0.41	0.94 – 1.15
Infant sex									
Male(n=397)	**Ref**			**Ref**			**Ref**		
Female(n=370)	1.29	0.29	0.80 – 2.09	1.27	0.34	0.78 – 2.06	1.26	0.34	0.78 – 2.05
Parity									
Primipara(n=373)	**Ref**			**Ref**			**Ref**		
Multipara(n=394)	1.00	1.00	0.62 – 1.61	0.81	0.48	0.45 – 1.45	0.86	0.61	0.48 – 1.53
Maternal age (years)	1.03	0.36	0.97 – 1.09	1.04	0.23	0.97 – 1.12	1.04	0.28	0.97 – 1.12
WAMI Index	1.15	0.88	0.19 – 6.84	0.96	0.96	0.15 – 6.16	0.95	0.96	0.15 – 6.11
TPOAb	1.00	0.69	1.00 – 1.00	1	0.88	1.00 – 1.00	1	0.70	1.00 – 1.00

The multivariate model adjusted for infant sex, parity, maternal age, WAMI index and TPOAb.
Abbreviations: LBW; low birth weight; CI, confidence interval; OR, odds ratio; TSH, thyroid stimulating hormone; FT4, free thyroxine; WAMI index (range 0-1), "measure of the household socioeconomic status based on access to improved Water/sanitation, Assets, Maternal education, and Income"[75]; TPOAb, thyroid peroxidase antibodies.

5. DISCUSSION:

Studies on maternal thyroid dysfunction during pregnancy in Nepal are limited. To our knowledge, this is one of few studies investigating the associations between markers of thyroid function in early pregnancy and pregnancy outcomes in the country.

The present study determined the prevalence of thyroid dysfunction in early pregnancy and the pregnancy outcomes of preeclampsia, preterm birth, and LBW among healthy women in Bhaktapur district, Kathmandu Valley, Nepal. We found that 7.6% of the included women were TPOAb positive, 0.4% had overt hypothyroidism, 2.5% had subclinical hypothyroidism, 0.3% had overt hyperthyroidism, and 2% had subclinical hyperthyroidism. The prevalence of preeclampsia, preterm birth, and LBW was found to be 0.4%, 9.1%, and 9.7%, respectively. Furthermore, we assessed the association between maternal TSH and FT4 hormone levels in early pregnancy and pregnancy outcomes: gestational age at delivery, preterm birth, birth weight, and LBW. However, no significant associations were found. This section will cover an

interpretation of our findings as well as a discussion of the strengths and limitations of our study.

5.1. Prevalence of maternal thyroid dysfunction and pregnancy outcomes

5.1.1. Maternal thyroid function status

The current study revealed that, even without pre-existing thyroid conditions, cases of overt- and subclinical hypo- and hyperthyroidism were found among the enrolled pregnant women—consistent with existing literature that suggests thyroid dysfunction could develop for the first time during pregnancy.[12] The prevalence of hypothyroidism was low when compared to previous hospital-based studies in Nepal, where the prevalence ranges were between 19.5–31% for subclinical hypothyroidism and 1.1–13% for overt hypothyroidism.[47-49] This could be attributed to the healthy cohort of mothers in the current study. Another explanation could be the variations in the socio-demographic characteristics of the enrolled participants, such as ethnicity, educational level, and socioeconomic status, from one study setting to another, which could impact thyroid function.[15, 30] A study conducted by Pandeya et al. revealed that thyroid dysfunction was more prevalent in certain ethnicities in Nepal.[77] Their study findings indicated that the Chhetri and Brahmin ethnic groups exhibited a higher prevalence of thyroid dysfunction compared to other ethnicities[77], and these ethnicities were a minority in our study. The study explains that genetic components, in addition to intergenerational social and cultural factors linked to ethnicity, might influence thyroid hormone levels. Thus, certain ethnicities may be at a higher risk of developing thyroid dysfunction.[77] Furthermore, varying trimester-specific cut-off values for both TSH and FT4 used in different studies could explain the variation in the prevalence across the different regions.[6]

In addition, the prevalence of hyperthyroidism, both subclinical and overt, in the present study is comparable to findings from hospital-based studies conducted by the Kathmandu Medical College and Teaching Hospital (KMCTH) and Nepal Medical College and Teaching Hospital (NMCTH), which are located in Nepal's central region, Kathmandu.[48, 50] The prevalence in their studies varied between 1.8–2.6% for subclinical hyperthyroidism and 0.6–0.7% for overt hyperthyroidism.[48, 50] Hyperthyroidism is a less common condition during pregnancy than hypothyroidism, which could explain their low prevalence as well.[8, 27] However, both studies found high thyroid dysfunction prevalence, where most of the participants with thyroid

dysfunction had subclinical hypothyroidism, which is considered to be a more common type of thyroid dysfunction during pregnancy.[8]

5.1.2. Preeclampsia

The prevalence of preeclampsia/eclampsia in the current study was 0.39%, which is lower than the 2.6% reported in a recent meta-analysis of prevalence studies in Nepal.[51] The low prevalence in our study made it difficult to investigate whether there was an association between maternal thyroid dysfunction during pregnancy and the risk of developing preeclampsia, as though previous studies have found an association.[8, 9, 19] This was probably also due to the healthy cohort in our study, where women at increased risk of preeclampsia, including multiple pregnancies, were excluded from taking part in the RCT. Multiple pregnancies have been associated with a higher risk of pregnancy complications, including an increased risk of developing pregnancy-induced hypertension and preeclampsia[78], and women with multi-fetal pregnancies have a three to fourfold increased risk of developing preeclampsia than singleton pregnancies.[78, 79] Excluding women with multiple pregnancies likely contributed to our low prevalence findings.

Furthermore, the proportion of pregnant women attending antenatal care (ANC) visits as per the national protocol is inconsistent across the different provinces in Nepal.[52] With some having a lower uptake, these inconsistencies could lead to an increased risk of developing preeclampsia during pregnancy, explaining the variation in prevalence between different regions.[52]

5.1.3. Preterm birth

The prevalence of preterm birth was found to be 9.13% in the current study, which is lower than the 13 to 16% prevalence reported in hospital-based studies in Nepal.[53-55] Globally, the majority of preterm births occur in South Asian and Sub-Saharan countries, where most belong to lower-middle-income countries.[40, 80] In India, a bordering country of Nepal, a systematic review of the prevalence of preterm births and associated factors reported an overall high prevalence of above 15%,[81] which is also higher than the prevalence reported in the current study. Furthermore, in 2020, Bangladesh and Pakistan, also South Asian countries, had the highest reported global preterm birth rates of 16.1% and 14.4%, respectively.[40] The prevalence reported in the current study is lower than in other regions in Nepal as well as in other lower-middle-income countries in South Asia, such as India, Bangladesh, and Pakistan.

Similar to our findings on the prevalence of preeclampsia, this is likely due to the inclusion of healthy pregnant women in this study. Our healthy cohort had restrictive maternal age and exclusion of multiple pregnancies and women with chronic diseases, which are known factors associated with increased risk of preterm birth[37, 66, 67, 79, 80, 83, 84], which may explain the lower prevalence in the current study.

5.1.4. Low birth weight

The prevalence of LBW was 9.65% in the current study, which is again lower than the findings reported from hospital-based studies conducted in different regions of Nepal, where the prevalence varied between 15.3% and 23.6%.[82-84] The prevalence was also lower than the 20% UNICEF-WHO estimates of LBW in Nepal in 2020.[57] Across different regions of the world, the prevalence of LBW varies, with Southern Asia having the highest number of LBW births globally.[44] A systematic review and meta-analysis of LBW studies in India reported a pooled prevalence of 31%.[85] Further, a similar analysis in Bangladesh reported a pooled prevalence of 29%.[86] The much lower prevalence in the current study could again be explained by the restrictive maternal age and exclusion of multiple pregnancies and women with chronic diseases, which are also known risk factors for LBW.[36, 65, 66, 78, 87, 88] In addition, preterm birth is one of the primary causes of LBW,[22, 40] and its prevalence was also found to be low in this study.

5.2. Association between maternal levels of TSH and FT4 in early pregnancy and outcomes: Gestational age at delivery, preterm birth, birth weight, and LBW.

In the present study, during their early pregnancy, 2.4% and 2.6% of participants had low maternal TSH and FT4 levels, respectively, while 3.1% and 2.6% had high maternal TSH and FT4 levels, respectively. Therefore, the maternal concentrations of TSH and FT4 were analyzed as continuous due to the low number of participants in the categorized groups with low and high levels of TSH and FT4 compared to the reference group (normal level).

We did not find a significant association when we explored the association between maternal TSH and FT4 levels in early pregnancy and the outcomes of gestational age at delivery and preterm birth. A recent, large-scale meta-analysis of individual participant data (47,045 pregnant women)[11] reported significant associations between mild thyroid dysfunctions and the risk of having a preterm birth. Further, they reported a significant association between maternal levels of TSH and increased risk of having a preterm birth, where a 1-SD increase in maternal TSH levels increased the risk of having a preterm birth by 4%.[11] This study also

found a significant association between maternal FT4 level and the risk of having a very preterm birth (<32 weeks of gestation), where a 1-SD increase in FT4 level was associated with a lower risk of having a very preterm birth by 12%.[11]

We also did not find a significant association between maternal TSH and FT4 levels in early pregnancy and infant birth weight or risk of LBW. This is contradictory to findings in a well-performed systematic review and individual-participant data analysis study of 48,145 mother-infant pairs, which reported a significant inverse association between maternal TSH and FT4 levels and birth weight.[89] An increase of 1-SD in maternal TSH level was associated with a lower birth weight of around 6 grams, and each 1-SD increase in maternal FT4 levels was associated with a lower birth weight of around 21 grams.[89]

Our study had very few cases with thyroid function test abnormalities and few cases of the outcomes of preterm birth and LBW. As such, our healthy cohort, with very few cases of exposure and outcome, may explain the variation in the results between our study and the existing large meta-analysis studies. Moreover, most of the pregnant women in our study belonged to the euthyroid group. The very few cases of subclinical and overt hypo- and hyperthyroidism, therefore, limited our ability to explore the association between subclinical and overt hypo- and hyperthyroidism and the outcomes of preterm birth and LBW.

5.3. Methodological considerations

5.3.1. Strengths of the study

This retrospective cohort study utilized data from a well-designed RCT, which allowed us to have good-quality data. The data collection was standardized, and a double entry was made to eliminate errors with data entry. This adds to the internal validity of the study. The data was collected during early pregnancy, a critical stage for the development of the fetus, and the assessment of thyroid function. Ultrasound was used to estimate the gestational age of the pregnant women accurately and to avoid systematic error, thus ensuring greater reliability and internal validity for the study. In addition, to further add to the internal validity of this study, we used population-specific cut-offs for the maternal concentrations of TSH and FT4 to assess the thyroid function in early pregnancy, which is the preferred method according to the 2017 ATA guidelines for the "Diagnosis and Management of Thyroid Disease During Pregnancy and Postpartum".[6] The changes in the thyroid hormone levels during pregnancy differ greatly between different racial and ethnic groups, so the reference ranges for the TSH and FT4 may vary significantly in different populations. Population-based specific reference ranges should,

therefore, be defined through an assessment of the local population.[6] Furthermore, controlling for the covariates, which are extraneous factors that could influence the study's outcomes, assured internal validity.

5.3.2. Limitations of the study

There are also several limitations to our study. First and foremost, the birth weight registered at the hospital was measured using different scales, which could lead to information bias affecting the study's internal validity. Ideally, a standard scale, preferably an electronic scale that measures to the nearest 0.01 kg, should be used to prevent the risk of over- or underestimating birth weight.

In addition, our findings cannot be generalized to the general population of pregnant women in their early pregnancy in Nepal because the main RCT project used a non-probability sampling method. As our study population was not representative, and our study findings cannot be generalized to the general population of pregnant women in their early pregnancy in Nepal, this affects our study's external validity. Further, due to strict exclusion criteria, as mentioned earlier, the present study only included a healthy cohort of pregnant women. This led to few cases of thyroid dysfunction and a small number of outcomes that were too small to properly assess the association, affecting the precision of the estimates. Future studies should recruit a larger sample size that is representative of the population, including pregnant women with clinical complications that could lead to the adverse outcomes, to have more balanced groups (exposed vs. non-exposed and outcomes vs. no outcomes). This could help produce more precise estimates and generalizable results.

6. CONCLUSION AND RECOMMENDATIONS

In conclusion, the prevalence of maternal thyroid dysfunction and the outcomes of preeclampsia, preterm birth, and LBW were found to be low in the current study when compared to other existing studies in Nepal. The low number of cases of exposure and outcomes could be attributed to the healthy cohort of mothers, which is not representative of the general population of pregnant women in their early pregnancy in Nepal. Furthermore, although the current study did not find a significant association between maternal TSH and FT4 concentrations in early pregnancy and the outcomes of gestational age at delivery, preterm birth, birth weight, and LBW, our findings cannot be generalized to the general population of pregnant Nepalese women in their early pregnancy, as areas of uncertainty remain. The

association between maternal thyroid function and pregnancy outcomes is complex; many factors influence thyroid function and adverse pregnancy outcomes, and the results from other existing studies are heterogeneous.

Despite the limitations, this research can be seen as a first step towards investigating maternal thyroid function in early pregnancy and its association with adverse outcomes that, to our knowledge, have not been directly investigated in Nepal. Further research is needed to determine the prevalence of thyroid dysfunction and the adverse outcomes of preeclampsia, preterm birth, and LBW and assess the association in larger population-based cohort studies in Nepal.

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