MATERNAL THYROID HORMONAL STATUS IN PREELAMPSIA AND ECLAMPSIA AT KENYATTA NATIONAL HOSPITAL- A CASE CONTROL STUDY.

A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine in Human Pathology, University of Nairobi.

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DEDICATION

This work is dedicated to the whole humanity and in particular the women, who sometimes with insurmountable difficulties, carry their pregnancies to term with resilience and a lot of love for the unborn. These women truly understand the meaning of Mother Nature.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following:

My supervisors Prof Kigondu, Dr Amayo and Dr Qureshi for their unrelenting patience and guidance throughout the study period.

Nurses and other staffs in the antenatal clinic and obstetric wards of KNH.

Staff at the Immunology laboratory of the University of Nairobi especially Mr. Ernest Lutomia.

All my colleagues, most especially Dr Okinyi and Dr Asaava for their support and assistance throughout the study period.

My friend Okello Pamba for the editing and rearrangement that went into this book.

My wife Mghoi and daughter Becca for giving me the reason to give my best in all I do.

LIST OF ABBREVIATIONS

ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
BP	Blood Pressure
CHF	Congestive Heart Failure
CI	Confidence Interval
ELISA	Enzyme Linked Immunosorbent Assay
FT3	Free Triiodothyronine
FT4	Free Thyroxine
hCG	Human Chorionic Gonadotropin
HDL	High Density Lipoprotein
HELLP	Hemolysis Elevated Liver Enzymes Low Platelet
I-	Iodine
IQ	Intelligence Quotient
IQC	Internal Quality Control
KNH	Kenyatta National Hospital
LBW	Low Birth Weight
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoproteins.
NACB	National Academy of Clinical Biochemists
NHBPEP	National High Blood Pressure Education Program.
OH	Overt Hypothyroidism
OR	Odds Ratio
PMH	Pumwani Maternity Hospital

RR	Relative Risk
SCH	Subclinical Hypothyroidism
SD	Standard Deviation
SGA	Small-for- Gestational Age
T3/TT3	Total Triiodothyronine
T4/TT4	Total Thyroxine
TBG	Thyroid Binding Globulin
TH	Thyroid Hormones
TPO-Ab	Thyroid Peroxidase Antibodies
TSH	Thyroid Stimulating Hormone (Thyrotropin)

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ABSTRACT

Background

Preeclampsia and eclampsia are important causes of maternal and perinatal morbidity and mortality in developing countries. Although pregnancy is usually associated with mild hyperthyroxinemia, preeclamptic women are reported to have a high incidence of hypothyroidism that might correlate with the severity of preeclampsia. The mechanism of hypothyroidism in preeclamptic women has not been identified but the changes in thyroid function during pregnancy are accounted for by high circulating estrogens (leading to increased thyroid binding globulin). There are controversies about the mechanism and clinical significance of thyroid hormones in preeclampsia, which are attributed to decreased plasma protein concentrations and high levels of endothelin, a potent vasoconstrictor produced by vascular endothelium after vascular injury. The rationale for this study was to describe the correlation, if any, between thyroid hormone and preeclampsia among Kenyan women.

Aim: The study evaluated thyroid hormonal status in patients with preeclampsia and eclampsia; and matched normotensive non-proteinuric pregnant mothers at Kenyatta National Hospital.

Methods: This was a matched case control study conducted at KNH between February and April 2010, both months inclusive. Seventy (70) subjects, comprising of thirty-five (35) preeclamptics and eclamptics as subjects and a similar number of age- and- gestation matched healthy normotensive pregnant mothers as controls were studied. Demographic and medical data from the study subjects were obtained. Blood was drawn for estimation of serum thyroid stimulating hormone (TSH), serum free thyroxine (FT4) and serum free tri-iodothyronine (FT3) using Enzyme Immunoassay

Statistical analysis: The demographic data and hormone levels were analyzed using students t- test and chi-square test. To examine the association between TSH and preeclampsia odds ratio along with 95% confidence interval was estimated for TSH levels.

Results: Mean TSH was significantly higher in preeclamptic group when compared to controls ($p=0.047$). Mean value of serum FT4 was significantly higher in controls compared to subjects ($p=0.045$). However mean values of serum FT3 were similar between the two groups ($p=0.618$). All the values were within the manufacturers reference ranges except in three preeclamptic women who had levels of TSH > 5mIU/ml which is the upper limit of the normal. However, this was not statistically significant ($p=0.05726$).

Conclusions: In this study modest alterations were observed in maternal thyroid functions (namely serum TSH and FT4) in preeclampsia and eclampsia. The clinical significance and explanation of these findings requires further investigation.

1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Hypertensive disorders complicating pregnancy are common and constitute one of the deadly triads along with hemorrhage and infection that contribute significantly to maternal and perinatal morbidity and mortality. According to National Center for Health Statistics 2001, gestational hypertension was identified in 150,000 women in United States of America, or 3.7% of pregnancies (1)

Mati found that hypertension complicates pregnancies in 1.5-9% of Kenyan women in his series (2). Wanjohi found 58 eclamptic patients over a period of two years at Kenyatta National Hospital (KNH) (3), while Bansal who studied patients at the Pumwani Maternity Hospital (PMH) found prevalence rate of 3.7%. Most recently in 2002, Wangwe found prevalence at PMH of 2.9 % (4, 5). Kibaru, found a prevalence of hypertension in pregnancy of 5.4% at KNH (6).

Unlike in the developing countries, maternal deaths have become rare in developed world. However maternal morbidity remains greatest with preeclampsia and continues to be one of the leading causes of admission of pregnant woman into intensive care in the developed world (1). Makokha found preeclampsia / eclampsia to contribute to 3.1% of maternal mortality at the KNH, whereas Lawson in Nigeria found it to account for 10% of the maternal mortality (7, 8). More recently Obore found it to account for 9.4 % of deaths at KNH (9). The commonest causes of death are cerebral vascular complications, aspiration pneumonia, hypoxic encephalopathy, thrombo-embolism, hepatic rupture, renal failure and anesthetic accidents (1, 8).

How pregnancy initiates or aggravates hypertension remains unsolved despite decades of intensive research. Indeed, hypertensive disorders remain among the most significant and intriguing unsolved problems in obstetrics. (1).

Over the past twenty years there has been a major expansion of our knowledge regarding thyroid disorders associated with pregnancy. These advances relate to the optimal management of pregnant women who are on L- thyroxine therapy, the impact of iodine deficiency on the mother and developing fetus, the adverse effects of maternal hypothyroidism on mental development of their offspring and thyroid dysfunction associated with postpartum thyroiditis. Simultaneously, a doubling of miscarriage rate has been reported in the studies of anti-thyroperoxidase antibody-positive euthyroid women and increase in preterm deliveries has been found in women with subclinical hypothyroidism and / or thyroid autoimmunity. Pregnancy may affect the course of thyroid disorders

and conversely thyroid disorders may affect the course of pregnancy. Moreover, thyroid disorders (and their management) may affect both the pregnant woman and developing fetus (10).

There is still a raging debate regarding the thyroid functional changes in pre-eclampsia. Alterations in thyroid gland function have been correlated with the severity of preeclampsia by some and totally rejected by others. Kumar et al showed that odds ratio corresponding to TSH levels >5 mIU/L in the preeclampsia group compared to the normotensive controls was 4.85 and concluded that TSH is a strong associating factor for preeclampsia(11).However, a report from Jordan in 2003 found no significant differences in the levels of serum FT4, FT3 and TSH in preeclamptic patients and various healthy controls in different gestational age subgroups(12).Other reports found a significant correlation between thyroid hormones, especially elevated serum TSH ,and preeclampsia(13,14,15).The most consistent finding from different studies is the link between biochemical hypothyroidism and preeclampsia.

A large Colorado study showed a continuous graded increase in serum cholesterol over a range of serum TSH values from <0.3 to >60 mIU/L (16). It has been noted that mild thyroid failure can significantly increase systemic vascular resistance and impair cardiac systolic and diastolic function (17). Subclinical hypothyroidism, or mild thyroid failure, was shown to be an independent risk factor for both myocardial infarction and radiologically-visible aortic atherosclerosis in a Dutch study (18). The finding of impaired flow-mediated, endothelium-dependent vasodilatation even in subjects with borderline hypothyroidism or high-normal serum TSH values (19) is of potential importance; with the postulation that serum TSH elevation may directly cause endothelial dysfunction which is a proposed core pathogenetic mechanism in preeclampsia and eclampsia. Other evidence suggests that high levels of exposure to anti-angiogenic factors as in pre-eclampsia may be associated with increased risk for reduced thyroid function during and after pregnancy (20, 21, 22). The meta-analysis of the relevant studies and their references are shown in subsequent sections.

Literature Review

1.2.1 : Hypertensive Disorders in Pregnancy

A. Terminology, Classification and Diagnosis

The term gestational hypertension is used now to describe any form of new-onset pregnancy related hypertension. It was adopted by the working group of National High Blood Pressure Educational Program (NHBPEP) 2000, which proposed a classification system based on clinical simplicity to guide management (1).

The classification of hypertensive disorders complicating pregnancy by the working group of the NHBPEP is outlined below:

i. Gestational Hypertension (formerly pregnancy induced hypertension that include transient hypertension).

ii. Preeclampsia

iii. Eclampsia

iv. Preeclampsia superimposed on chronic hypertension

v. Chronic hypertension.

The diagnosis of Hypertensive Disorders Complicating Pregnancy as per NHBPEP (1) includes:

Gestational Hypertension.

Blood pressure (BP) ≥ 140/90mmHg for the first time during pregnancy

No proteinuria

BP that returns to normal before 12 weeks post-partum

May have other signs and symptoms of preeclampsia, for example, epigastric discomfort or thrombocytopenia.

Preeclampsia

The *minimum Criteria*

BP≥ 140/90mmHg after 20 weeks gestation

Proteinuria ≥ 300mg/24 hours (or ≥ 1 + dipstick)

Increased certainty of preeclampsia

BP≥ 160/110mmHg

Proteinuria 2.0g/24 hours (or ≥ 2+ dipstick).

Serum creatinine > 102.0µmol/L unless known to be previously elevated (reference range < 98µmol/L).

Platelets < 100,000 /per ml (reference range 150,000-450,000/ml).

Microangiopathic hemolysis demonstrated by increased Lactate dehydrogenase (reference range 150-250 IU/L).

Elevated Alanine transaminase & or Aspartate transaminase ≥ 70IU/L (reference range 5-40IU/L).

Persistent headache or other cerebral or visual disturbances.

Eclampsia

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

Superimposed preeclampsia (on chronic hypertension).

New onset proteinuria ≥ 300mg/24hours in hypertensive woman before 20 weeks gestation or a sudden increase in proteinuria or blood pressure or platelets count < 100,000/per ml in women with hypertension and proteinuria before 20 weeks gestation

Chronic hypertension

BP ≥ 140/90mmHg present before pregnancy or diagnosed before 20 weeks gestation, not attributable to gestational trophoblastic disease or hypertension first diagnosed after 20weeks gestation and persistent after 12 weeks postpartum.

This scheme and criteria differ importantly from older diagnostic schemes (23). Key features of preeclampsia category include: (i) Elimination of a change in BP as a diagnostic criterion (the group recommends using familiar cut-off of 140/90mmHg, instead; (ii) Elimination of edema as criterion, because this finding is so common in healthy pregnant women; and (iii) Absolute requirement for proteinuria (more than 300mg [0.3g/day]) for the diagnosis (24).For this study, the NHBEP scheme is used.

The severity of preeclampsia is assessed by frequency and intensity of abnormalities listed in Table 1.

Table 1: Indications of Severity of Hypertensive disorders during pregnancy (1)

Abnormality	Mild	Severe
Diastolic BP	<100 mmHg	≥ 110mmHg
Proteinuria	Trace to 1+	Persistent 2+ or more
Headache	Absent	Present
Visual disturbances	Absent	Present
Upper abdominal pain	Absent	Present
Oliguria	Absent	Present
Convulsion (Eclampsia)	Absent	Present
Serum Creatinine	Normal	Elevated(>102µmol/L)
Thrombocytopenia	Absent	Present
Liver enzyme elevation	Minimal	Marked(≥ x2 ULN)*
Fetal growth retardation	Absent	Obvious
Pulmonary edema	Absent	Present

*Upper limit of the normal ranges

Blood Pressure per se is not always a dependable indicator of severity. An adolescent patient may have convulsions with BP of 140/90mmHg and a proteinuria of 3+ whereas other patient may not convulse with much higher BP (1).

The syndrome of hemolysis elevated liver enzymes and low platelet count (HELLP) characterized by microangiopathic hemolytic anemia, liver dysfunction and thrombocytopenia occurs in severe eclampsia. Sibai and Decker diagnosed HELLP syndrome by the presence of the following laboratory findings and is a poor prognostic sign (25, 26).

a) Hemolysis defined by an abnormal peripheral blood smear, which includes burr cells and /or schistocytes with raised bilirubin levels ≥ 20.4µmol/L(reference range ≤ 18µmol/L) and /or LDH > 600IU/L.

b) Elevated liver enzymes, AST ≥70 IU/L

c) Platelet count < 100,000/ml.

Ngayu at KNH found HELLP syndrome to occur in 2.8% of patients with hypertensive disorders in pregnancy (27). Serum LDH and platelet count are the two most important clinical tools for disease assessment. LDH reflects both the extent of hemolysis and hepatic dysfunction. Materno-fetal complications cause 7-70% perinatal mortality rate and 1-24% maternal mortality rate (28).

B: ***Pathophysiological and pathogenetic mechanisms involved in Preeclampsia (Current Concept)***

A 2 stage model of preeclampsia has been proposed as useful concept to address its pathophysiology. In stage 1 of preeclampsia, reduced placental perfusion, is considered the "root cause". This then translates, in some but not all women into stage 2: the multisystemic syndrome of preeclampsia. Although much still remains unknown in both of these areas, the major questions raised are: (1) why does reduced placental perfusion result in preeclampsia in some women and (2) what parameters link stages 1and 2? (29)

I. Stage 1 of Preeclampsia: Reduced Placental perfusion

Clinical Evidence

More than 60 years ago, Ernest Page formalized the concept that placental perfusion was reduced in preeclampsia (30). Preeclampsia is more common in conditions with very large placentas, including multiple gestations and molar pregnancies. The concept advanced was that the excess of placental tissue could not be perfused adequately. Further support was provided by the increased frequency of preeclampsia in women with pre-existing medical conditions that were characterized by microvascular diseases including diabetes, hypertension and collagen vascular diseases. Doppler velocimetry of uterine vessels has demonstrated increased resistance in the vessels that supply the intervillous spaces of preeclamptic women (in early gestation) with predilection to develop preeclampsia (29).

In normal pregnancy the maternal uterine arteries that supply the intervillous space undergo striking modifications including a fourfold increment of vascular luminar diameter, loss of smooth muscle in the vessel wall and inner elastic lamina; with reduced responses to vasoactive stimuli. These changes result in a flaccid tube which provides a low resistance circuit to the intervillous space. The vascular remodeling is either not present or if it does occur is limited to superficial portions of the vessel located in the decidua in preeclampsia (31).

It appears that the placental site vascular remodeling is largely a result of trophoblastic invasion, in particular, endovascular invasion. Cellular control of cytotrophoblast invasion depends on interactions between decidua and fetal trophoblast. Local oxygen and immune- mediated interactions are primary determinants of the process; and their common mechanism may be through apoptosis. (32)

II. Stage 2 of preeclampsia: Maternal Syndrome- More than a Pregnancy induced hypertension.

Pathological and pathophysiological changes

Pathological changes in women with eclampsia present a common theme: they are all consistent with profoundly reduced perfusion. In the liver and adrenal glands, reduced perfusion is indicated by infarction, necrosis and intraparenchymal hemorrhage. Endocardial necrosis is present in the heart, similar to that present in hypovolemic shock, the prototypic-reduced perfusion disorder. The pathological changes in the kidney are termed glomerular endotheliosis and consist largely of marked swelling of glomerular endothelial cells sufficient to occlude the capillary lumen (33).

This is accompanied by minimal changes in renal podocytes. This change is important because it is present in no other form of hypertension, indicating preeclampsia is not merely unmasking of pregnancy hypertension. (29)

Perfusion is reduced in virtually any organ examined, including the uterus (34). Reduced uterine blood flow further reduces placental perfusion, resulting in a feed-forward loop consistent with the clinical course of preeclampsia. This disease never improves, only deteriorates and when it begins to worsen, it does so rapidly (29). Perfusion decreases secondary to vasospasm, activation of the coagulation cascade with formation of occlusive microthrombi and loss of fluid from vascular space (29). Quite importantly the increased pressor sensitivity, activation of coagulation cascade and loss of vascular integrity are evident in groups of women before clinical manifestation of the disorder. This has led to the concept that endothelial dysfunction, which could explain all of the changes described, is a central pathophysiological features on this disorder (34). Furthermore myriads of markers of endothelial injury or dysfunction (both biochemical and immunological) are present in women with preeclampsia and in many cases precede clinically evident disease supporting a causal role. (35)

Striking metabolic changes also characterize preeclampsia. These include a dyslipidemia characterized by the elevation of triglycerides, free fatty acids, LDL cholesterol and reduced HDL cholesterol (36). Insulin resistance, uric acid and other components of the metabolic syndrome are also increased in preeclampsia. (34)

Maternal- Fetal -Placental Interactions in Preeclampsia

Not all women with reduced placental perfusion develop preeclampsia. This has led to the concept that reduced placental perfusion must interact with maternal factors to result in clinical preeclampsia. These factors are proposed to be genetic, behavioral and environmental. They are modified by the normal physiological changes of pregnancy, of which increased inflammatory responses may be particularly relevant. Conditions recognized to increase the risk of preeclampsia include obesity, hypertension, diabetes, hyperhomocysteinemia, increased androgens and black race. (37)

Preeclampsia is clearly inherited. The frequency of preeclampsia in mothers, daughters, sisters and granddaughters is 2-5 times higher than in mother-in-law, daughters-in-law or control population. In summary of inheritance studies, Arngrimmson concluded that with the limitations, data were consistent with a major dominant gene with variable penetrance or multifactorial inheritance. (38)

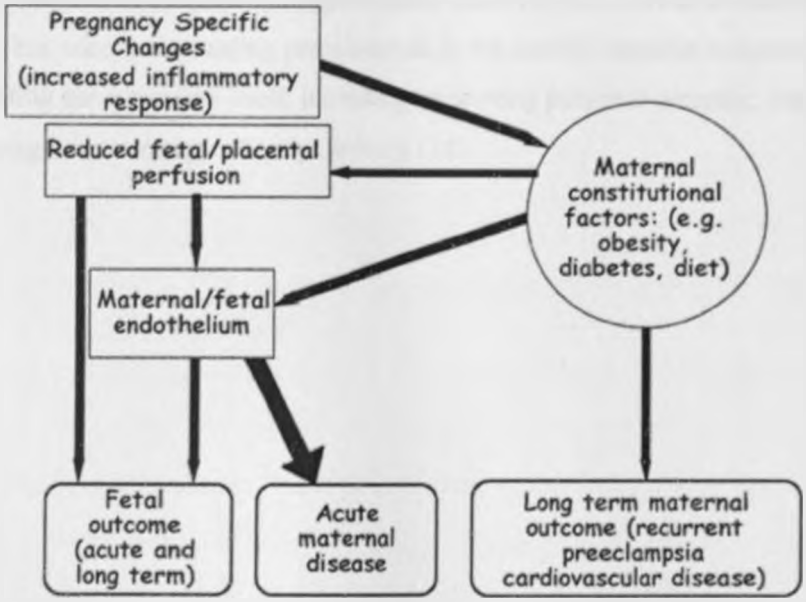


Figure 1. Maternal fetal/placental interactions in preeclampsia (25)

The Linkages of Stages 1 and 2

A key question in the 2 stage model of preeclampsia is: what is the linkage of the 2 stages?

This is the 'holy grail' of preeclampsia research because identifying such linkage would provide a target therapy common to all cases of preeclampsia regardless of predisposing factors. The search for unique factors present in the circulation of a woman with a preeclampsia, 'substance X' occupied at least a

generation of investigators (39). Some of the proposed products include;(i) microvillus particles, likely the products of synctiotrophoblast apoptosis (found to be present in excess in women with preeclampsia), (ii) several cytokines and other inflammatory markers (that could alter endothelial function); (iii) Placental estrogen, progesterone and human placental lactogen. (40) (iv) activating auto- antibodies to angiotensin subtype 1 receptor (AT1) and recently (v) soluble receptor for angiogenic factors endothelial growth factor and placental growth factor (S-Flt) (41). Oxidative stress is considered prime candidate for linkage of the 2 stages.

Clinical Implications

The fact that stage 1 of the preeclampsia, including abnormal placental bed vascular remodeling , is present in setting other than preeclampsia indicates that understanding this phenomenon has importance beyond preeclampsia. As we learn more about preeclampsia, it is increasingly evident that it is not merely hypertension unmasked by pregnancy. Hypertension seems to be a marker of the disorder rather than a causal factor .Thus success in treating preeclampsia is not merely aimed at reducing blood pressure but rather ameliorating the syndrome itself, including improving perinatal outcome, the major complication of the current management strategy of early delivery (34).

1.2.2 Maternal Thyroid Physiology

A Regulations of the thyroid in normal pregnancy

Table 2: Factors affecting thyroid physiology during normal pregnancy (10)

Physiologic Change	Thyroid-related consequences
Increased renal iodine clearance	Fall in plasma iodine and increased iodine requirement.
Decreased plasma iodine and placental transport to the fetus	In iodine deficient women, decreased T4, increased TSH, and goiter formation
Increased O2 consumption by feto-placental unit, gravid uterus and mother	Increased basal metabolic rate
First-trimester increase in hCG	Increased free T4 and T3 Decreased basal TSH (partial blunting of the pituitary-thyroid axis)
Increased serum TBG	Increased total T4 and T3
Increased plasma volume	Increased T4 and T3 pool size
Inner-ring deiodination of T4 and T3 by placenta	Accelerated rates of T4 and T3 degradation and production

During pregnancy, important changes in thyroidal status occur that are due to three modifications in the regulation of the thyroid hormones. First pregnancy induces marked increase in circulating levels of the major thyroxine transport protein (thyroid binding globulin) in response to high estrogen levels. Second, several thyroidal stimulatory factors of placental origin are produced in excess. Third, pregnancy is accompanied by a decreased availability of iodine for the maternal thyroid. This finding occurs because of increased renal clearance (due to increased glomerular filtration rate) and exertion that results in a relative iodine deficiency state (1)

Beginning early in the first trimester, TBG increases reaches its peak at about 20 weeks and stabilizes at approximately double the baseline for the remainder of pregnancy. TT4 increases sharply beginning at between 6 and 9 weeks and plateaus at 18 weeks. FT4 levels rises slightly and peaks along with human chorionic gonadotropin (hCG) levels then they return to normal. The rise in to TT3 is more pronounced up to 18 weeks and there after its plateaus (1). Thyroid releasing hormone (TRH) levels are not increased during normal pregnancy but this neurotransmitter does cross the placenta and may stimulate pituitary to

secrete TSH (42, 43). Interestingly, the secretion of TT4 and TT3 is not similar for all pregnant women. Approximately one third of women experience relative hypothyroxinemia, preferential T3 secretion and higher albeit normal serum TSH levels (44). As result of structural similarity between hCG and TSH, hCG has intrinsic thyrotrophic activity and thus high serum levels cause thyroid stimulation. Indeed TSH levels decrease in more than 80% of pregnant women, even though levels are in the normal range for non-pregnant women. (1)

B: Thyroid function parameters in Normal pregnancy

Presently there is controversy as to what type of thyroid hormone measurement represents the most reliable test to differentiate normal thyroid function from abnormalities associated with subtle thyroid dysfunction in pregnancy. The difficulties arise from 3 main facts: a) determination of TT4 have progressively gone out of fashion and when still used reference range for normal TT4 needs to be adapted to the pregnant state; b) the use of FT4 determinations encounters technical difficulties (due to the assays used) that may hinder interpretation of results; and c) there are changes in the normal pattern of serum TSH during pregnancy that requires correct interpretation of the data (10).

Serum TSH levels are influenced by circulating hCG concentrations, particularly (but not only) near the end of the 1st trimester. Thus serum TSH values decrease during first trimester and in approximately one fifth of healthy pregnant women, TSH value may be transiently lowered to subnormal levels at this time of gestation. By using the classical non-pregnant reference range of serum TSH (0.4 mIU/L - 4.0mIU/L) one might misdiagnose as normal women who have a slight TSH elevation and conversely, wrongly suspect hypothyroidism in normal women who simply have blunted serum TSH. Thus trimester and method specific reference interval should be used when reporting thyroid test values for pregnant patients (44).In this study manufacturer's (method) and trimester specific reference ranges along with sensitive and stable assays is used.

C: Thyroid dysfunctions in pregnancy:

1: Primary Hypothyroidism

The prevalence of hypothyroidism in pregnancy in western countries is estimated to be 0.3- 0.5% for overt hypothyroidism (OH) and 2 - 3% for subclinical hypothyroidism (SCH) (44). On a worldwide basis the most important cause of maternal thyroid deficiency, which is known to affect over 1.2 billion is iodine deficiency. Thyroid antibodies are found in 5 – 15% of normal women in the child bearing age and when iodine nutrition status is adequate, the main cause of hypothyroidism during pregnancy is chronic autoimmune thyroiditis (45).

Because many women remain asymptomatic, particular attention is required from obstetrical care providers for this condition to be diagnosed and to evaluate more systematically thyroid function when women attend the prenatal clinic for the first time. A serum TSH elevation(>5mIU/L) suggests primary hypothyroidism and measurement of FT4 further distinguish between OH and SCH, depending on whether free T4 is normal or clearly below normal for gestational age . Determination of thyroid antibodies, especially anti- thyroperoxidase (TPO-Ab) and anti-thyroglobulin (TG -Ab) antibodies, confirms autoimmune origin of the disorder (46).

Repercussions of hypothyroidism on the outcome of pregnancy.

Various studies have shown the consequences of hypothyroidism (OH or SCH) on both the maternal and fetal morbidity and mortality. Some of the repercussions include:

(i) Maternal complications such as anemia (47), postpartum hemorrhage, preeclampsia (ranging from 15%-44% in various studies) and abruptio placenta (47, 48).The repercussions are worse with OH.

(ii) Fetal and neonatal complications such as increased fetal distress in labor, prematurity and low birth weight (47, 48, 49, 50, 51) and increase in breech presentation and thus cesarean section (51). Also noted are impaired intra-uterine growth retardation (51) and congenital malformation (48, 49). In relation to above intrauterine fetal death (48, 49) and perinatal death (52, 53) are also increased in hypothyroidism.

Role of Maternal Hypothyroidism on the Psycho-neurological outcome in Pregnancy

Because of the heterogeneity of what is commonly referred to as gestational hypothyroidism, different clinical conditions must be considered. Thyroid insufficiency varies widely with regard to time of onset (first trimester versus later), degree of severity (SCH versus OH), progressive aggravation with gestation time (depending on the cause), and adequacy of treatment .Several studies have shown that there is a significantly increased risk of impairment in neuropsychological development indices, Intelligence Quotient (IQ) scores and school learning abilities in the offspring of untreated hypothyroid mother. Hardow et al in the large scale prospective study concluded that undisclosed (and hence untreated) hypothyroidism occurring during the first half of pregnancy (and presumably prolonged thereafter) was associated with a risk of poorer outcome of the pregnancy and 3-fold increased predisposition for having learning disabilities later in life. (54)

2: Primary Hyperthyroidism in Pregnancy

Hyperthyroidism during pregnancy is relatively uncommon, with a prevalence estimated to be between 0.1% and 1 % (0.4% Clinical and 0.6% subclinical) (44). In women of childbearing age the most common cause are hyperthyroidism is Graves' disease , as this etiology accounts for 85% of clinical hyperthyroidism in pregnancy. Another cause of hyperthyroidism result directly from the stimulatory action of hCG on the thyroid and this etiology is associated with transient hyperthyroidism in the first half of gestation (10).

Virtually all patients with significant symptoms have a serum TSH < 0.1 mIU/L as well as concurrent elevations in serum FT4 and FT3. (10) However interpretations of thyroid functions must take into account the hCG-mediated decrease in serum TSH that occurs during pregnancy. Near the end of first trimester, at the time of peak hCG values, serum TSH levels may be transiently lowered to value below 0.4 mIU/L in about 20% of euthyroid women. Thus, the degree and duration of TSH activity (mainly but not only) in first trimester must be considered in making differential diagnosis (55).

Table 3: Adverse pregnancy outcome and lack of control of maternal hyperthyroidism. (10)

Table 3: Adverse pregnancy outcome and lack of control of maternal hyperthyroidism				
	Poor control	Less than adequate control	Adequate control	Ref No
Preeclampsia	14-22%		7%	42
CHF *	60%		3%	47
Thyroid storm	21%		2%	45
Preterm delivery	88%	25%	8%	47
LBW **	23%		10%	45

Foot-note to Table 3: * CHF: congestive heart failure; ** LBW: low birth weight (< 2,500 g)

3: Screening for Thyroid dysfunction during pregnancy

There are no recommended universal screenings for thyroid dysfunction in women before or during pregnancy. Overall benefits of screening for thyroid dysfunction (primarily hypothyroidism) have not yet been universally justified by current evidence based medicine. Recent international guidelines have recommended "aggressive" case finding among the following groups of women who are at high risk for

hypothyroidism, preferably prior to pregnancy or in early gestation: history of thyroid antibodies (when known), clinical sign of thyroid dysfunction, type 1 diabetes mellitus, autoimmune disorders, prior history of head and neck irradiation and history of miscarriage or preterm delivery (10).

1.2.3: Thyroid Hormonal Dysfunction and Preeclampsia/eclampsia

A: Studies on thyroid hormonal dysfunction and preeclampsia/eclampsia

Various findings on thyroid dysfunctions and preeclampsia have been published. A report of 27 women with severe preeclampsia from Jordan in 2003 found no significant differences in the levels of FT4, FT3 and TSH in preeclamptic patients and various healthy controls in different gestational age subgroups (12). However the bulk of other studies have shown different results. Basbuq et al in their study in Turkey reported that moderate decreases in thyroid hormones correlated with severity of preeclampsia or eclampsia and high levels of endothelin. They concluded that the changes in results of thyroid hormones induced by preeclampsia or eclampsia might be a consequence of the dysfunction of the hypothalamic pituitary axis secondary to the disease itself (13).

In an Indian study the mean TSH titers in preeclamptic pregnancy were noted to be 3.8±0.53 mIU/L while in normal pregnancy, it was 2.3± 0.24 mIU/L (14). A subsequent study also in India found only TSH values significantly altered by preeclampsia (4.6± 3.64 mIU/L in the study group with preeclampsia compared to 2.5±2.01 mIU/L in normotensive controls). The study also showed that odds ratio corresponding to TSH levels >5 mIU/L in the study group compared to the control group was 4.85. Thus TSH was found to be a strong associating factor for preeclampsia (11).

Bumer et al concluded from the Amsterdam study that women with hypertensive disorders of pregnancy may have transiently lower FT4 levels without evidence of thyroid disorder (15).

The mechanism of higher incidence of biochemical hypothyroidism in preeclamptic women compared with normotensive pregnant women is not well understood. Mild alterations in thyroid hormones might occur due to non-thyroidal illness acting as a stress factor as well as due to decreased plasma albumin concentrations in these patients (56). A high level of endothelin in preeclampsia has been implicated (13). Endothelial cell dysfunction plays an important role in the pathogenesis of preeclampsia. Nitric oxide, a vasodilator from the endothelial cells regulates secretion of thyroid hormones by modulating regional blood flow. Animal studies showed that release of nitric oxide is altered in hypothyroidism (56). Reduced serum concentration of thyroxine binding globulin (TBG), T3 and T4 may also be explained by faulty estrogen production due to placental dysfunction in preeclamptic women (45).

The most consistent finding from different studies is link between biochemical hypothyroidism and preeclampsia.

B: Possible link between thyroid dysfunction and preeclampsia

Preeclampsia is a complex syndrome in which endothelial damage leads to a microangiopathic disease with hypertension just being one of the manifestations(34).There is increasing evidence that lipids may play a role in the modification of endothelial structure and function (35). Ndiang'ui showed that preeclamptic patients had higher serum homocysteine and an artherogenic lipid profile (both markers of endothelial dysfunction) compared to normotensive controls (57).

Subclinical hypothyroidism, or mild thyroid failure, was shown to be an independent risk factor for both myocardial infarction and radiologically-visible aortic atherosclerosis in a recent study of Dutch women over 55 years of age (18). This effect was independent of body mass index, total and HDL cholesterol, blood pressure and smoking status. The attributable risk for subclinical hypothyroidism was comparable to that for each of the major risk factors, hypercholesterolemia, hypertension, smoking and diabetes mellitus. The association was slightly stronger when subclinical hypothyroidism was associated with TPOAb, but thyroid autoimmunity itself was not an independent risk factor.

The finding of impaired flow-mediated, endothelium-dependent vasodilatation even in subjects with borderline hypothyroidism or high-normal serum TSH values is of potential importance. Baseline artery diameter and forearm flow were comparable, but flow mediated vasodilatation during the period of reactive hyperemia was significantly impaired even in the group with serum TSH of 2-4 mIU/L, compared with the group with serum TSH 0.4-2 mIU/L. The difference could not be attributed to a difference in maximal nitrate-induced vasodilatation, age, sex, hypertension, diabetes, smoking, serum cholesterol, or levels of total T3 and T4(19).This finding suggests that even minor deviation from an individual's pituitary-thyroid set point may be associated with alteration in vasodilatory response. There is no known direct action of TSH that would account for this effect.

From echocardiographic studies, there is evidence that mild thyroid failure can significantly increase systemic vascular resistance and impair cardiac systolic and diastolic function, as demonstrated by decreased flow velocity across the aortic and mitral valves. These changes, which were associated with reduced cardiorespiratory work capacity during maximal exercise, were reversed by T4 treatment sufficient to normalize serum TSH. Impairment of both diastolic and systolic function was demonstrable by echocardiography in a subclinically hypothyroid group of patients with TSH in the range 4-12 mIU/L

(17). Thyroxine treatment sufficient to normalize TSH to a mean of 1.3 mIU/L for 6 months was associated with improvement in myocardial contractility (58).

The Colorado study of over 25,000 subjects showed a continuous graded increase in serum cholesterol over a range of serum TSH values from <0.3 to >60 mIU/L (16). A recent meta-analysis suggests that T4 treatment of subjects with mild thyroid failure does lower the mean total and LDL cholesterol, and is without effect on HDL cholesterol or triglyceride (59). Meier et al (60) reported that the decrease in LDL cholesterol was more pronounced with higher initial TSH levels >12 mIU/L or with elevated baseline LDL concentration.

Thus TSH elevation may directly cause endothelial dysfunction which is a core pathogenetic mechanism in preeclampsia and eclampsia.

2.0 JUSTIFICATION, RESEARCH QUESTIONS , HYPOTHESIS AND OBJECTIVES

2.1 Justification

Several studies have associated thyroid dysfunction with preeclampsia. Kumar et al in a study done in India found out that preeclamptic women had high incidence of biochemical hypothyroidism compared with normotensive pregnant women (11). It has also been postulated that modest decreases in thyroid hormones with concomitant increases in TSH levels, in maternal serum correlated with severity of preeclampsia or eclampsia and high levels of endothelin (13). It has also been observed that preeclamptic and eclamptic women with higher TSH levels along with lower thyroid hormones (TH) were more likely to have Small for Gestational Age (SGA) newborns (32). Diseases of the thyroid gland itself are a predisposing factor for the development of preeclampsia. If the levels of TSH are above 5 mIU/L, then there is 4.8 times higher risk at the development of preeclampsia (11). This high –risk potential marker of preeclampsia needed a further investigation.

A multicentric study may ensure the association and mechanism of thyroid abnormalities in preeclamptic woman in terms of the geographical variation. No such study has been reported in Africa where the prevalence for preeclampsia may be as high as 18% in some settings (61). This study aimed to bridge the gap in geographical variation of the preeclampsia-thyroid dysfunction studies by showing the African and most especially the Kenyan perspective.

2.2 Hypothesis

There is no difference in maternal thyroid hormonal status between preeclamptic and healthy normotensive pregnant women.

2.3 Objectives

2.3.1 Broad Objective

To determine the maternal thyroid status of patients with preeclampsia and eclampsia compared to healthy normotensive matched controls.

2.3.2 Specific Objectives

a) To determine the thyroid hormonal levels in pregnant women with preeclampsia and eclampsia.

b) To determine the thyroid hormonal levels in healthy normotensive pregnant women.

c) To compare the thyroid hormonal levels between pregnant women with preeclampsia and eclampsia; and healthy normotensive mothers.

d) To determine estimated relative risk (odds ratio) of developing preeclampsia or eclampsia when thyroid dysfunction is present.

3.0 METHODOLOGY

Study design

This was a matched case -control study.

Study site

Antenatal clinic, antenatal wards and Labor ward at KNH.

Study Population

Pregnant woman with preeclampsia or eclampsia on follow up or admitted and equal age (± 1 year) and gestation (± 1 week) matched healthy normotensive women.

Cases were defined as follows:

1. Preeclampsia: -

   a) Mild: BP ≥ 140/90 mmHg on ≥ occasions at least 6 hours apart and proteinuria of +1 on dipstick

   b) Severe: BP ≥ 160/110mmHg and proteinuria ≥2+ on dipstick.

2. Eclampsia: convulsions or coma in a preeclamptic woman.

Controls were defined as follows:

Normotensive (BP <140/90 mmHg with no proteinuria) pregnant women age (± 1 year) and gestation (± 1 week) matched with selected cases.

Case Selection

1. Inclusion criteria

(a) Preeclamptic or eclamptic patients

- Diagnosed cases of preeclampsia and eclampsia (as per above criteria).
- No previous history of thyroid disease during pregnancy and postpartum period.
- No previous history of a baby with malformation.
- Informed consent (from the patient or authorized relative).

(b) Healthy normotensive woman.

- Age (± 1year) and gestation (± 1 week) matching with the selected preeclamptic or eclamptic woman
- Attending the ANC or admitted during the study period.
- Informed consent.

2. Exclusion Criteria (for both) included:

- History of any metabolic disorder (e.g. diabetes and any other endocrinopathies) before or during pregnancy.
- History of intake of any medication(both prescribed and non-prescribed) that might affect thyroid function(especially steroids, lithium, propranolol, heparin, phenytoin, tegretol, furosemide)
- History of renal disease
- History of hypertension not associated with pregnancy.
- Declined consent.

Definition of dysthyroidism

Maternal thyroidal dysfunction were taken as values of serum TSH, free T4 and free T3 falling outside the reagent manufacturer's and trimester specific reference ranges (appendices vi, vii and viii). Comparisons with values obtained from studies done on thyroid hormone levels in healthy pregnant levels were also applied.

Sample size

Kumar et al in an Indian study obtained a mean TSH of 4.6±3.64 mIU/L for preeclamptic compared to 2.5±2.01 mIU/L for normotensive controls (11). Using OpenEpi program and formula for comparing means:

$$n = \frac{(16)\sigma^2}{\Delta^2} + 1$$

n represents the required sample size *per group*

Δ^2 represents the expected mean difference (a difference worth detecting) =4.41

σ^2 represents the standard deviation of the variable as estimated for paired samples=9.2095

The following calculation is obtained as shown in table 4.

Table 4: sample size calculation using Mean Difference

Confidence Interval (2-sided)		**95%**	
Power		80%	
Ratio of sample size (controls/cases)		*1*	
	cases	**controls**	**Mean difference**
Mean	*4.6*	*2.5*	2.1(Δ)
Standard deviation	*3.64*	*2.01*	
Variance	13.2496		4.0401
Sample size of cases		*31*	
Sample size of controls		*31*	
Total sample size		*62*	

Approximated to 35. thus sample size of 35 (for each group) was used.

Sampling Technique

Cases

All consecutively diagnosed preeclampsia or eclampsia patients were assessed for eligibility and recruited until the desired number was achieved.

Controls

Age (± 1 year) and gestation (± 1 week) matched normotensive controls (to the selected cases) were concurrently recruited within a week of case identification.

Study Period

The study was carried out from February to April 2010 both months inclusive.

Recruitment.

The Principal investigator (PI) recruited participants for the study. Files for the patients and healthy controls were perused in the morning of the clinic or in the ward to assess eligibility for recruitment into the study.

Once sampled, screening of the eligible participants was done using Screening proforma (appendix i and ii). The purpose of the study, the benefits and risks of participations were explained to the participants and consent obtained (appendix iv and v). A questionnaire was administered to eligible patients (appendix iii). A sticker was put on the file to avoid duplication in recruitment.

Laboratory

Specimen Collection:

Five milliliters of blood was aseptically drawn from antecubital vein into a plain bottle from the participants after acquisition of consent. Samples were immediately put in a cooler box and transported to the Immunology Laboratory, University of Nairobi.

Separation and Storage

Blood in the labeled plain vacutainer was left to clot and serum separated by centrifugation then pipetted into cryovials for refrigeration at -20^{0}C in the laboratory. The cryovials were labeled with study numbers for identification and analyzed as a batch every fortnight.

Specimen Analysis

1. Serum FT4/FT3
   Competitive enzyme immunoassay using semi-automated ELISA(Enzyme Linked Immunosorbent Assay) micro-well reader was used as per appendix vii and viii (PATHOZYME® ***FREE THYROXINE*** and PATHOZYME® ***FREE TRIIODOTHYRONINE*** both from OMEGA DIAGNOSTICS UK were used respectively).Expected reference values for pregnant women of FT4 and FT3 were8 – 22 pg/ml and 1.4-4.2 pg/ml respectively.

2. Serum TSH:

The ultrasensitive TSH test using ***PATHOZYME ULTRASENSITIVE TSH*** from OMEGA DIAGNOSTICS (UK) based on a solid phase ELISA was used. Absorbance of calibrators, specimens and controls were determined using semi-automated ELISA microwell reader as per appendix vi. The expected reference value for 2nd and 3rd trimester was 0.5-5μIU/ml.

Quality Control

All aspects of quality control were adhered to. Standard operating procedures were adhered to through specimen collection, identification, separation, storage and interpretation. Repeated thawing and freezing was avoided. All manufacturers' instructions were strictly followed and internal quality control was run in each assay. The optical densities (OD) for all the calibrators and the controls were within the specified range. Post analytical measures including data interpretation were done as per manufacturers (method specific) and gestation specific reference range. The analysis was done in a laboratory that actively participates in regular external quality assurance.

Data handling and analysis

All data obtained from the study was entered into a Microsoft access database version 2010 .Where discrepancies were found manual verification and corrections were performed. The data was analyzed with statistical package for social sciences (SPSS) version 18.0 and OpenEpi version 2.3. All the quantitative parameters were expressed as a mean with standard deviation in both groups. To test for differences in the mean values between the two groups for various quantitative parameters student t-test was applied when the data followed normal approximation. Differences in the proportions between different categorical variables were tested through Chi-square test of significance. Statistical significance was taken as $p < 0.05$. To assess the association between TSH and preeclampsia odds ratio along with 95% confidence interval was estimated for TSH levels. To understand relationship between two quantitatively measured variables simple correlations coefficient were estimated and tested for its significance using the t-test.

Ethical considerations

1. The study was undertaken after approval by the Department of Human Pathology University of Nairobi and the KNH Scientific & Ethical Review Committee.
2. Subjects gave an informed consent before being recruited in the study (Appendix v) and the purpose of the study was fully explained to the participants.

3. Study subjects information was kept confidential, the names only being included in the questionnaire to assist in the delivery of test results. The collected specimens were only used for the purpose of the study.

4. The results especially the abnormal were relayed to the clinicians to help in management.

6.0 RESULTS

Forty five preeclamptics and eclamptics were screened ; and 35 were recruited. Major reason for non-recruitment included: administration of dexamethasone in preparation of early delivery(seven cases), superimposed preeclampsia(two cases) and declined consent (one case).Thirty seven controls were screened; 35 recruited , and two declined consent. Thus seventy clients were recruited for the study, 35 preeclamptics and eclamptics (subjects) and an equal number of normotensive pregnant mothers (controls).

Table 5: The Age (years), Gestation (weeks) and blood pressure (mmHg) of the study participants.

Variable	**Subjects (n=35)**			**Controls(n=35)**			
	Mean(SD)	**Median**	**Range**	**Mean(SD)**	**Median**	**Range**	**P value**
Age(years)	28.7(4.6)	28.0	21-36	28.1(3.6)	28.0	22-35	0.565
Gestation(weeks)	32.1(5.1)	33.0	20-38	31.4(4.7)	33.0	20-37	0.564
Systolic BP (mmHg)	161(19.7)	160	140-225	115(8.5)	110	100-130	<0.001
Diastolic BP (mmHg)	104(11.8)	100	90-128	69(7.9)	70	60-85	<0.001

The mean age among the studied population was 28.7 years with SD of 4.6 whereas the control group had a mean age of 28.1 years with SD of 3.6. There was no significant statistical difference between the two groups (p value=0.565).

The mean gestation among the studied population was 32.1 weeks with SD of 5.1 compared to 31.4 weeks with SD of 4.7 in the control group. There was no significant statistical difference between the two means (p=0.564).This shows that the age and gestation matching between the subjects and controls had been achieved. The subjects, as expected, had higher blood pressure compared to controls (p<0.001) as shown in table 5.

Table 6: Parity distribution in the subjects and control group.

Parity			Study group		Total
			Subjects	Control	
	0	Count	21	13	34
		% within Study group	60.0	37.1	48.6
	1-2	Count	11	22	33
		% within Study group	31.4	62.9	47.1
	3+	Count	3	0	3
		% within Study group	8.6	.0	4.3
Total		Count	35	35	70
		% within Study group	100.0	100.0	100.0

Primigravidae comprised 60% of the subjects compared to 37.1% of the controls as shown in table 6. There was a statistical significance between the two groups where equal variance was assumed (p=0.014). First pregnancy increased the risk for developing preeclampsia (OR 2.54, 95% CI 1.02-6.61 p=0.031)

Table 7: Family history of Preeclampsia among the pregnant women.

Family History			Study group		Total
			Subjects	Control	
	No	Count	24	33	57
		% within Study group	68.6	94.3	81.4
	Yes 1st degree relative	Count	11	2	13
		% within Study group	31.4	5.7	18.6
Total		Count	35	35	70
		% within Study group	100.0	100.0	100.0

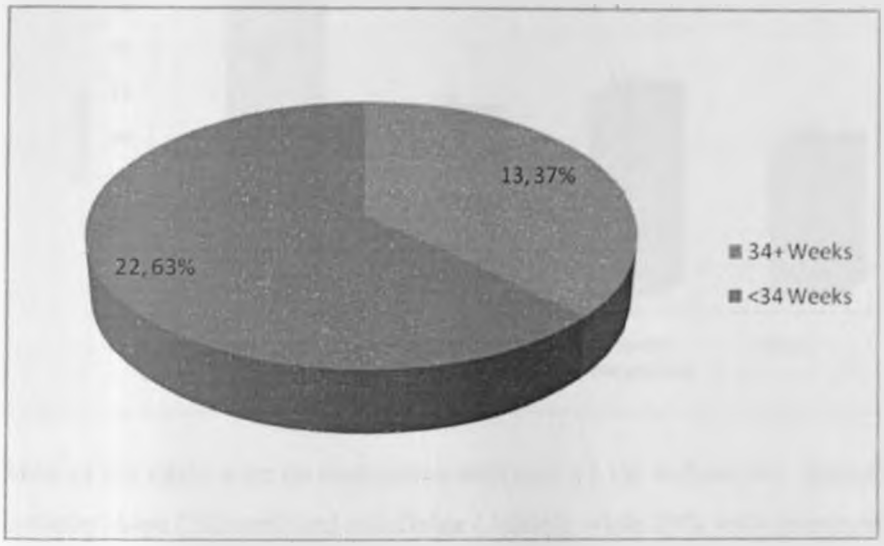
Those with negative family history comprised 81.4 %(n=57) of the whole study group as shown in table 7. However 31.4% of subjects compared to 5.7% had positive family history of hypertensive disorder in pregnancy. There was a significant statistical difference between the two groups (p=0.006). A family history of preeclampsia in a first degree relative was associated with an increase in risk (OR 7.6, 95% CI 1.53-37.29, p=0.006)

Table 8: Recurrence of preeclampsia among the study group.

Valid		Frequency	Percent	Valid Percent	Cumulative Percent
	N/A	21	60.0	60.0	60.0
	No	6	17.1	17.1	77.1
	Yes	8	22.9	22.9	100.0
	Total	35	100.0	100.0	

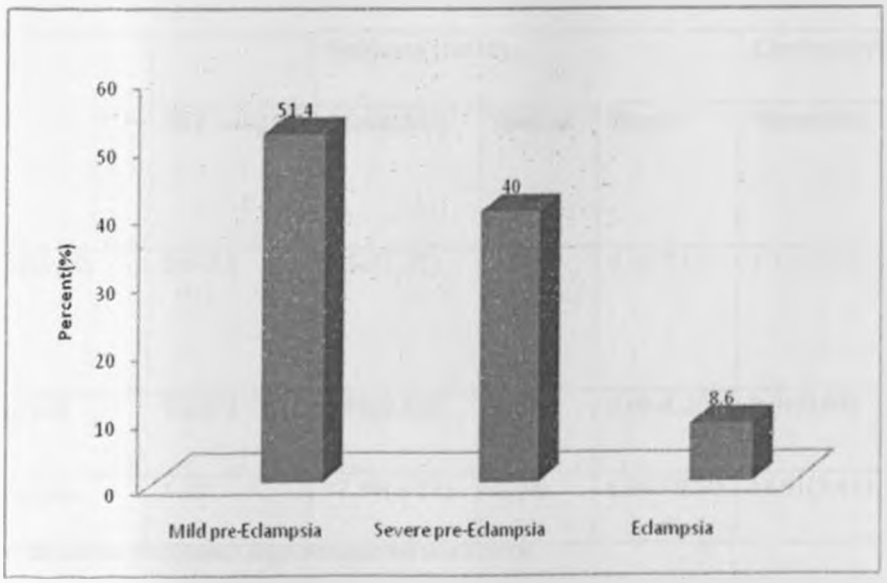
Twenty three percent of the women in the subject group had previous pregnancy complicated by preeclampsia compared to seventeen percent who had previous normal pregnancies as shown in table 8. One (2.85%) out of the 22 multiparas in the control group had previous pregnancy complicated by preeclampsia. Thus odds ratio of preeclampsia in women with a history of the disorder compared to women with no such history was 28.0 (p<0.001. 95% CI 2.90-270.5)

Figure 2: Gestation (weeks) at diagnosis of preeclampsia and eclampsia.



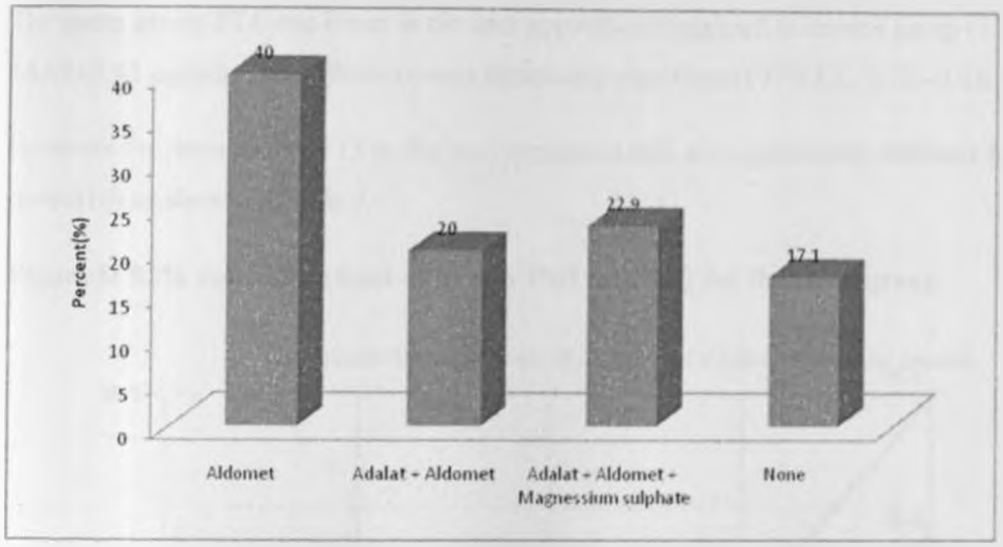
Almost two-thirds(63% n=22) of the cases had diagnosis made before 34 weeks .This shows the early occurrence of preeclampsia in our settings as shown in figure 2.

Figure 3: Classification of Hypertensive disorders in pregnancy among the subjects.



Almost half of the case group (48.6%, n=17) had severe preeclampsia and eclampsia while the rest (51.4%) had mild form of preeclampsia. Three mothers (8.6%) had eclampsia (defined as preeclampsia with coma or convulsion) (figure 3).

Figure 4: Medication among the preeclamptic and eclamptic women



Most of the cases were on medication with only 17.1% without any. About 20% of the cases were on both α-methyldopa (aldomet) and nifedipine (Adalat); while 23% with severe preeclampsia and eclampsia had magnesium sulfate added to the regimen. This concurs with the severity of the disease among the cases. Patients who were on medications that could interfere with thyroid functions (in vivo and in vitro) e.g. dexamethasone were excluded (Figure 4).

Table 9: Thyroid functions tests in study group

		Subjects (n=35)			Controls(n=35)			P value
	Ref range [b]	Mean(SD)	median	Range	Mean(SD)	Median	Range	
TSH(μIU/ml)	0.4-5.0	2.51(1.51)	1.7	0.40-7.00	1.53(0.92)	1.30	0.10-4.90	0.044
FT3(pg/ml)	1.4-4.2	1.94(0.80)	2.10	0.60-4.20	2.04(0.96)	1.80	1.2-4.80	0.618
FT4 (pg/ml)	8-22	12.39(3.27)	12.00	6.00-19.00	14.01(3.41)	14.50	7.00-21.0	0.045

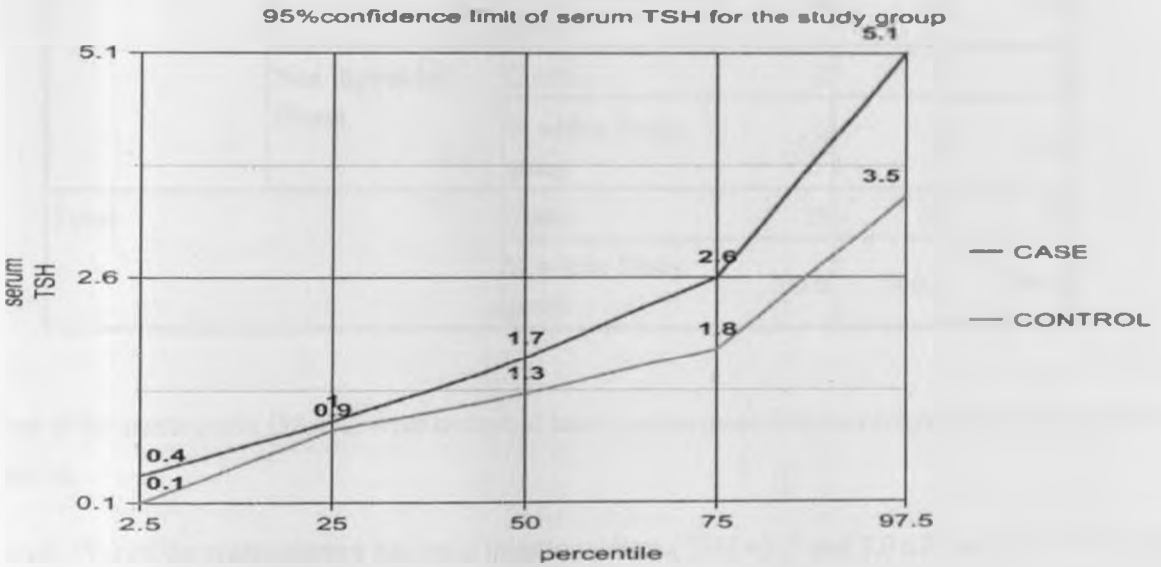
[b] the manufactures reference ranges for second and third semester

The mean values of thyroid hormones were within the normal laboratory (and manufacturers) reference ranges in both the groups. The mean values of serum TSH was higher (2.51±1.51 μIU/ml) in the case population compared to controls (1.53±0.92 μIU/ml). This revealed a statistical difference between the two means (95%CI, 0.02-1.20, p=0.044).

The mean serum FT4 was lower in the case population compared to control group (12.39±3.27 versus 14.01±3.41 pg/ml). This difference was statistically significant (95% CI, -3.22--0.36, p=0.045).

However the mean serum FT3 in the case population was not significantly different from the controls (p=0.618) as shown in Table 9.

Figure 5: 95% confidence limit of Serum TSH (μIU/ml) for the study group



The values of TSH corresponding to the first quartile in the study group and in controls were 1.0 and 0.9 while those corresponding to the third quartile were 2.6 and 1.8 respectively. The inter-quartile range for the study and control group was 1.6 and 0.9 respectively. Thus the difference in the two groups was normally distributed as shown in figure 5.

Table 10: Distribution of thyroid hormonal status among the study group

			Study group		Total
			Subject	Control	
Classification of Thyroid Status	Euthyroid	Count	30	34	64
		% within Study group	85.7	97.1	91.4
	1^0 Hypothyroidism	Count	3	0	3
		% within Study group	8.6	0	4.3
	1^0 Hyperthyroidism	Count	0	1	1
		% within Study group	0	2.9	1.4
	Non thyroidal illness	Count	2	0	2
		% within Study group	5.7	0	2.9
Total		Count	35	35	70
		% within Study group	100.0	100.0	100.0

Most of the participants (91.4%) were euthyroid based on the manufacturers reference ranges as shown in table 10.

Two (5.7%) of the preeclamptics had mild hypothyroidism (TSH =5 .1 and 7.0 µIU/ml with FT4 6.0 and 7.0 pg/ml respectively); while one had subclinical hypothyroidism with only elevated Serum TSH (TSH=5.1 µIU/ml) with normal FT4 and FT3 .One normotensive control had mild hyperthyroidism (TSH=0.1µIU/ml with FT3=4.8 pg/ml with normal FT4)

Two cases with severe preeclampsia had thyroid hormone levels consistent with non-thyroidal illness (Serum TSH and FT4 within reference range associated with depressed FT3=0.6pg/ml) as shown in table 10.

However the difference between the two groups in terms of thyroid status was not statistically significant (p=0.162).

Table 11: Distribution of normal and abnormal TSH levels in pregnant women

	TSH< 5.0 µIU/ml Number (%)	TSH > 5.0µIU/ml Number (%)	Total	Odds Ratio (95% confidence interval)
Control Group	35(100)	(0)(0)	35	1
Case Group	32(91.4)	3(8.6)	35	7.65 (p=0.0576, 95% CI 0.3803-153.7).
Total	67	3	70	

Out of 35 pregnant women in each group, 3 (8.6%) women in the preeclamptic study group and none (0%) in the control group had abnormal TSH level (> 5 µIU/ml).The difference between the two groups is found not to be statistically significant (p =0.0576) as shown in table 11.

Severity of preeclampsia and thyroid function tests was also investigated and results are shown below in Table 12

Table 12: Severity of preeclampsia and thyroid tests.

Group Ref value	**TSH** (µIU/ml) (0.5-5.0)	**FT4**(pg/ml) (8-22)	**FT3**(pg/ml) (1.4-4.2)
Mild preeclampsia(n=18)	2.20±1.68	11.97±3.17	2.02±0.46
Severe preeclampsia(n=17)	2.08±1.36	12.82±3.40	1.85±1.06
Control(n=35)	1.53±0.92	14.01±3.41	2.04±0.96
P value(mild preeclampsia versus controls)	0.065	0.039*	0.934
P value(severe preeclampsia versus controls)	0.091	0.243	0.513
P value for ONE WAY ANOVA	0.130	0.103	0.752

*-significant t-test

With exception of mean FT4 between mild preeclamptics and controls which was significant (p=0.039) the rest of the correlations did not yield any statistical significance (p>0.05) as shown in table 12. The one way ANOVA did not show any significance among the 3 groups (p>0.05) in terms of serum TSH, FT4 and FT3. Thus there was no correlation between the thyroid tests and severity of preeclampsia.

Table 13: Severity of preeclampsia and raised serum TSH levels (>3.5µIU/ml).

			TSH >3.5		Total
			Yes	No	
Preeclampsia	**Mild**	Count	1	17	18
		% within TSH >3.5	16.7	58.6	51.4
	Severe preeclampsia + Eclampsia	Count	5	12	17
		% within TSH >3.5	83.3	41.4	48.6
Total		Count	6	29	35
		% within TSH >3.5	100.0	100.0	100.0

Six subjects had serum TSH>3.5µIU/ml; of which 5(83.3%) had severe preeclampsia or eclampsia and one had mild preeclampsia as shown in table 13. However the association was not statistically significant (p=0.0576).Thus raised serum TSH (>3.5 µIU/ml) did not correlate with severity of preeclampsia.

7.0 DISCUSSION

The results in this study revealed that the mean serum TSH concentration was significantly higher when preeclamptics were compared with normotensive pregnant mothers (2.51±1.51 versus 1.53±0.92 μIU/ml, p=0.047).This is similar to several studies which demonstrated significantly higher serum TSH in preeclamptics compared to normotensive mothers (11, 13, 14, 62). Khaliq et al in India had noted a mean TSH titers in preeclamptic pregnancy to be 3.8±0.53 μIU/ml while it was 2.3±0.24μIU/ml in normotensive pregnancy (p=0.01) (14). A subsequent study in India found TSH values significantly altered by preeclampsia (4.6±3.64 in the study group compared with 2.5±2.01μIU/ml in normotensive controls) (11). Mean serum TSH in this study was significantly lower than in the above two studies. This may be due to population differences in serum TSH reference levels which may arise from nutritional and genetical difference or inter-assay variability. However, other studies found no significant difference in serum TSH levels between preeclamptics and normotensive mothers. A report of 27 women with severe pre-eclampsia from Jordan found that there was no significant difference in the levels of FT4, FT3 and TSH between the preeclamptic patients and healthy controls in the various gestational age subgroups (12). Similar results were obtained by a recent study in Iran consisting of 314 pregnant women (132 mothers with preeclampsia and age- and- gestation matched 182 normotensive controls).The mean values of thyroid hormones were within the normal laboratory reference ranges in both groups. The mean TSH levels were not significantly higher in pre-eclamptic group as compared to controls (p>0.05) (63).

In this study out of 35 pregnant women in each group, 3 (8.6%) women in the preeclamptic study group and none (0%) in the control group had abnormal TSH levels (> 5 μIU/ml).This difference between the two groups was found not to be statistically significant (p =0.0576). The findings supported the reports that pre-eclamptic women had not a higher incidence of biochemical hypothyroidism compared with normotensive pregnant women. In the Iranian report, out of 314 pregnant women studied, 8(6.06%) women in the pre-eclamptic study group and 8(4.39%) in the control group had abnormal TSH levels (>5 mIU/ml).The difference between the two groups was not found to be statistically significant (p>0.05) (63).This is significantly different from two other reports (11, 13).

The cause of higher serum TSH levels in preeclamptics has not been fully delineated. Basbuq et al found out that increases in TSH levels in maternal serum correlated with severity of pre-eclampsia or eclampsia and high levels of endothelin. The endothelial cell dysfunction plays an important role in the pathogenesis of preeclampsia. Nitric oxide, a vasodilator released from the endothelial cells, regulates secretion of thyroid hormones by modulating regional blood flow in an animal study (13). The clinical syndrome of pre-eclampsia has been hypothesized to result from excessive release of anti-angiogenic proteins—most

notably soluble fms-like tyrosine kinase 1—from the placenta into maternal blood, resulting in an anti-angiogenic state with low levels of free placental growth factor and free vascular endothelial growth factor(20). Administration of vascular endothelial growth factor inhibitors such as soluble fms-like tyrosine kinase 1 to rodents induces hypertension, proteinuria, and glomerular endotheliosis, the hallmarks of pre-eclampsia. In mice, two weeks' exposure to exogenous soluble fms-like tyrosine kinase 1 or to other vascular endothelial growth factor inhibitors resulted in a reduction of thyroid tissue capillary density by two thirds and increased thyroid stimulating hormone concentration (21). Together with reports of hypothyroidism in patients with cancer treated with vascular endothelial growth factor receptor inhibitors, the evidence suggests that high levels of exposure to soluble fms-like tyrosine kinase 1 as in pre-eclampsia may be associated with increased risk for reduced thyroid function during and after pregnancy (22).

Serum TSH values are influenced by the thyrotrophic activity of elevated circulating hCG concentrations, particularly (but not only) near the end of the 1st trimester. Thus, serum TSH values decrease during the first trimester in response to hCG elevation and, in approximately one fifth of healthy pregnant women, serum TSH values may be transiently lowered to subnormal values at this time of gestation (44). By using the classical non pregnant reference range for serum TSH (0.4 mIU/L for the lower limit and 4.0 mIU/L for the upper limit), one might therefore misdiagnose as "normal", women who already have a slight TSH elevation and, conversely, wrongly suspect hyperthyroidism in normal women who simply have a transiently blunted serum TSH. During the remaining period of pregnancy, serum TSH returns progressively to the normal range. As it was the case for free thyroid hormone measurements, it has recently been proposed to use "trimester-specific" reference ranges for serum TSH levels during pregnancy (44). Dashe et al have recently published a proposed "normogram" for serum TSH changes during pregnancy (64). The authors showed that 28% of singleton pregnancies with a serum TSH greater than 2 standard deviations above the mean would not have been identified when using the non-pregnant serum TSH range.

In this study the serum TSH in the normotensive healthy controls approximates the above finding with 2.5 percentile, median and 97.5 percentile as 0.1, 1.3 and 3.5 mIU/L respectively. It has been shown that the lower normal limit of serum TSH decreases to 0.03 mIU/L in 1st and 2nd trimesters, and is still reduced to 0.13 mIU/L in the 3rd trimester. Conversely, serum TSH levels above 2.3 mIU/L (1st trimester) and 3.1-3.5 mIU/L (2nd and 3rd trimesters) may already be indicative of a slight thyroid hypofunction (65). If the upper limit for serum TSH was reduced to 3.5μIU/ml for this study, 6 (17.1%) of the preeclamptics would have abnormal serum TSH compared to one (2.9%) normotensive control. However, this difference was still not statistically significant (p=0.0589).

The ideal situation would be to have reference ranges derived from well selected healthy pregnant mothers to be used for our geographical settings .However it is difficult to reliably exclude all individuals with thyroid dysfunction when determining the TSH reference range; given the high prevalence of thyroid dysfunction (especially subclinical hypothyroidism) in the general population exacerbated by the sensitivity and/or specificity limitations of current TPOAb tests. Although there are number of possible reasons for the persistent 'skew' in the TSH upper limit ,the dominant cause is likely to be the inclusion of TPOAb-negative individuals with occult autoimmune thyroid dysfunction that is not detected as a humoral response (i.e. TPOAb) (66). The need for rigorous exclusion of thyroid antibody-positive individuals is reinforced by the new National Association of Clinical Biochemists (NACB) consensus guideline number 22 that states, "TSH reference intervals should be established from the 95 % confidence limits of the log-transformed values of at least 120 rigorously screened normal euthyroid volunteers who have; (a) No detectable thyroid autoantibodies, TPOAb or TgAb (measured by sensitive immunoassay); (b) No personal or family history of thyroid dysfunction; (c) No visible or palpable goiter and, (c) Who are taking no medications except estrogen" (67).

The mean FT4 was significantly lower in the preeclamptics compared to control group (12.4±3.27 versus 14.0±3.41pg/ml, p=0.045). This corroborates two other studies that had showed reduced serum FT4 in the preeclamptics. Basbug et al in their study in Turkey reported moderate decrease in thyroid hormones which correlated with severity of preeclampsia (13). Bumer et al concluded from the Amsterdam study that women with hypertensive disorders in pregnancy may have transiently lowered FT4 without evidence of thyroid disorder (15). However other reports have disputed these findings (11, 63) with the mean FT4 levels not being significantly different between the two groups. The explanation being forwarded for the reduction in FT4 in preeclamptics is similar to that of higher serum TSH (13, 20, 21, 22). There is still need for more studies in this area.

There was no difference in the levels of FT3 when preeclamptic patients were compared to healthy controls (1.9±080 versus 2.0±.096 pg/ml, p=0.618). This is in agreement with other studies where there was no significant difference in FT3 between preeclamptic patients and healthy pregnant mothers (12, 14. 62,63). It is not clear why only FT4 levels and not FT3 were significantly different but it could be due to deranged peripheral metabolism of thyroid hormones in preeclamptics. TT4, TT3 and serum albumin were not measured in these study women.

When the manufacturers' reference ranges for 2nd and 3rd trimesters were used 91.4 %(n=70) of the participants were euthyroid. three preeclamptics (8.6%) had hypothyroid. One normotensive control had mild hyperthyroidism. Thyroid antibodies (TG-Ab and TPO-Ab) were not measured and this could have

led to inclusion of pregnant mothers with early thyroid disease in the control group despite rigorous screening. In women of childbearing age the most common cause of hyperthyroidism is Graves' disease, as this etiology accounts for 85% of clinical hyperthyroidism in pregnancy but it could also be due to persistent gestational thyrotoxicosis. Patients with Graves' disease usually have positive thyroid antibodies (TG-Ab and TPO-Ab) (10) and, therefore, antibody presence could have been used to identify the cause of the hyperthyroidism that was discovered in one pregnant mother belonging to the control group.

Two of the preeclamptics (5.6%) had thyroid hormone profile highly suggestive of non-thyroidal illness (depressed FT3). During preeclampsia, there is involvement of the liver and kidney that may lead to decreased peripheral conversion of T4 to T3, hence decreasing the T3 levels. Also, "low T3 syndrome" (reduced T3 with normal serum TSH and FT4) has been reported in preeclampsia (68). Modest changes in results of thyroid function tests in preeclamptic and eclamptic women might indicate hypothalamic-pituitary dysfunction secondary to disease stress. Nonthyroidal illness is frequently accompanied by changes in circulating thyroid hormone concentrations. The changes in thyroid indices in preeclampsia might not indicate a form of hypothyroidism because they are very modest, and transient increases or decreases in serum TSH concentrations are often observed with nonthyroidal illness(56).However in this study no significant difference was found between the thyroid hormonal status in the preeclamptics and normotensive pregnant mothers (p=0.162). This also confirmed by various reports (12, 62, 63).

Proponents of association between biochemical hypothyroidism and preeclampsia argue that studies regarding normal thyroid function test may be due to the fact that the blood samples were taken just at the time of diagnosis of preeclampsia and that it is possible that low titers of T3 and T4 along with high TSH levels would be observed at a later stage of pre-eclampsia (i.e. with severe disease and low plasma albumin levels) (11, 14). In this study thyroid hormones were measured both at time of diagnosis and at advanced stages of the disease and still the results only demonstrates mild serum TSH increment in preeclamptics with no concomitant changes in other parameters. This study also differs from others in that 83% of the preeclamptics and eclamptics had been started on anti-hypertensive and anti-convulsants (mainly magnesium sulfate) before blood was drawn for thyroid hormones estimation. The effect of the drugs on preeclampsia and hence thyroid hormone alterations may not be obvious but the drugs in question have no known direct or indirect effect on thyroid hormones. It is for this reason that cases started on dexamethasone (which suppresses TSH secretion) were excluded from the study.

In this study, raised serum TSH was not found to be a risk factor for the occurrence of preeclampsia. There is still a raging debate regarding the thyroid functional changes in pre-eclampsia. Alterations in

thyroid gland function have been correlated with the severity of preeclampsia by some and totally rejected by others as shown above. More studies are needed to address this topic.

In this study other risk factors for preeclampsia were also investigated and the results are similar to other studies including well controlled double blind cohort studies.

Past obstetrical history of preeclampsia is a strong risk factor for preeclampsia in a future pregnancy. A systematic review of controlled studies reported that the relative risk of preeclampsia in women with a history of the disorder compared to women with no such history was 7.19 (95% CI 5.85-8.83) (69). Women with early, severe preeclampsia are at greatest risk of recurrence, rates of 25 to 65 percent have been reported (69, 70). In women who had mild preeclampsia during the first pregnancy, the incidence of preeclampsia in a second pregnancy is 5 to 7 percent, compared to less than 1 percent in women who had a normotensive first pregnancy (71, 72). Thus odds ratio of preeclampsia in women with a history of the disorder compared to women with no such history was 28.0 ($p<0.001$, 95% CI 2.90-270.5) in this study.

First pregnancy increases the risk for developing preeclampsia (RR 2.91, 95% CI 1.28-6.61) (73). It is unclear why the primigravid state is such an important predisposing factor. First pregnancy increased the risk for developing preeclampsia (OR 2.54, 95% CI 1.02-6.61 P=0.031) in this study.

A family history of preeclampsia in a first degree relative is associated with an increase in risk (RR 2.90, 95% CI 1.70-4.93) (73), suggesting a heritable mechanism in some cases (73). The father of the baby may contribute to the increased risk, as the paternal contribution to fetal genes may have a role in defective placentation and subsequent preeclampsia. A family history of preeclampsia in a first degree relative was associated with an increase in risk (OR 7.6, 95% CI 1.53-37.29, p=0.006) in this study.

Also in our study preeclampsia especially appeared at earlier gestation compared, for instance, by studies from United States. The disease is mild in 75 percent of cases in the United States, and severe in 25 percent (74). Ten percent of preeclampsia occurs in pregnancies less than 34 weeks of gestation. This does not agree with the findings of this study where 42.9% had severe disease and 62.9% of preeclampsia occurred in pregnancies less than 34 weeks. This reveals a heavier burden of the disease with possible higher mortality and morbidity. There could be possible regional, ethnic and socioeconomic consideration responsible for the differences. The time of diagnosis compares well with other local data (5, 6, 9, and 57).

8.0: CONCLUSION, RECOMMENDATIONS AND LIMITATIONS

Conclusions

1. Mean thyroid hormones namely serum TSH and FT4 are significantly different between preeclamptics and normotensive, non-proteinuric pregnant mothers but the levels are within the reference range.
2. Mean serum FT3 is not significantly different between the two groups.
3. There is no correlation between raised serum TSH and preeclampsia thus biochemical hypothyroidism may not be associated with preeclampsia.
4. There is no significant difference in the maternal thyroid hormonal status between preeclamptics and normotensive mothers.

Recommendation

The clinical significance and explanation of the mild thyroid alterations (increased serum TSH and lower FT4) in preeclampsia and eclampsia should be evaluated more, probably in large well controlled studies.

Limitation

Single point evaluation of the thyroid hormones may give erroneous picture as the mothers were also not followed up to delivery period (possibility of postpartum thyroiditis). Thyroid antibodies were not assayed in this study.

Appendix I: Screening Proforma for Preeclampsia eclampsia study group

MATERNAL THYROID HORMONAL STATUS IN PREECLAMPSIA AND ECLAMPSIA PATIENTS AT KENYATTA NATIONAL HOSPITAL

Age (years)	☐☐
Parity	☐☐ + ☐☐
Gestation (weeks)	☐☐

DIAGNOSTIC CRITERIA (Tick appropriate)	YES	NO
a) Highest recorded BP (i) Systolic ≥ 140mmHg		
(ii) Diastolic ≥90mmHg		
b) Highest recorded proteinuria ≥+1		
NO HISTORY OF:		
a) Thyroid disease throughout pregnancy or postpartum period		
b) Metabolic disorder before or during pregnancy		
c) Hypertension not associated with pregnancy		
d) Baby with malformation		
e) Renal disease		
Current Medication		
NOT on Steroids, lithium, propranolol, heparin, phenytoin ,carbamazepine or furosemide(prescribed drugs)		
Eligibility		
1. Nature of the study fully communicated		
2. Are you willing to participate in this study?		

If answers to ALL questions are YES, recruit as **PREECLAMPSIA**, and if in **COMA OR WITH CONVULSIONS** recruit as **ECLAMPSIA** and issue Study Number. If NO, do NOT recruit.

FOR OFFICAL USE:

RECRUITED (encircle) YES NO

STUDY NUMBER: | | | |

Once recruited, proceed to Study Questionnaire (Appendix III).

Appendix II: Screening Proforma for normotensive control group

MATERNAL THYROID HORMONAL STATUS IN PREECLAMPSIA AND ECLAMPSIA PATIENTS AT KENYATTA NATIONAL HOSPITAL

Parity ☐☐ ☐☐		
MATCHED CHARACTERISTICS WITH THE CASE SELECTED	**YES**	**NO**
a) **Age (± 1 year)** ☐☐		
b) **Gestation (± 1 week** ☐☐		
DIAGNOSTIC CRITERIA		
a) Highest recorded BP (i) Systolic < 140mmHg		
(ii) Diastolic < 90mmHg		
b) Highest recorded proteinuria ..... Nil		

NO HISTORY OF:	YES	**NO**
a) Thyroid disease throughout pregnancy or postpartum period		
b) Metabolic disorder before or during pregnancy		
c) Hypertension not associated with pregnancy		
d) Baby with malformation		
e) Renal disease		
Current Medication		
NOT on Steroids, lithium, propranolol, heparin, phenytoin ,carbamazepine or furosemide(prescribed drugs)		

Eligibility		
1. Nature of the study fully communicated 2. Are you willing to participate in this study?		

If answers to ALL questions are YES, recruit as **NORMOTENSIVE CONTROL** for the **SELECTED CASE** and issue Study Number. If NO, do NOT Recruit.

FOR OFFICAL USE:

RECRUITED (encircle) YES NO

STUDY NUMBER: | | | |

Once recruited, proceed to Study Questionnaire (Appendix III).

Appendix III: Study questionnaire for case and matched control

MATERNAL THYROID HORMONAL STATUS IN PREECLAMPSIA AND ECLAMPSIA PATIENTS IN KNH

Date ☐☐/☐☐/☐☐

dd / mm / yy

A. Socio-demographic data			
		Case	Matched Control
1.	Name		
2.	Age	☐☐	☐☐
3.	Hospital Number	☐☐☐☐☐☐	☐☐☐☐☐☐
4	Study number	☐☐☐	☐☐☐

B. Medical History			
		Case	Matched Control
1.	Diagnosis:	(tick one)	(tick one)
	Preeclampsia		
	Eclampsia		
	Healthy normotensive		
2	Medications currently on:		
	Anti-hypertensive (Adalat, aldomet,) magnesium sulfate.		
	Others (specify)		

C. Family History			
		Case	Matched Control
1.	Any relative diagnosed with hypertensive disorders in pregnancy? YES/NO		
2.	If yes, relation to you is…… 1° degree…….. [Mother, sister] 2° degree…….. [Aunt, cousin]		

D. Obstetric History			
		Case	Matched Control
1	Parity		
2.	Current gestation		
3.	Gestation at the time of diagnosis (Preeclampsia/eclampsia)		
4.	Highest recorded BP (from the patients file) a. Systolic (mmHg) b. Diastolic (mmHg)		
5.	Highest recorded proteinuria a. nil b. +1		

	c. ≥ +2		
6.	Previous pregnancy complicated by hypertension? YES/NO		
7.	Have you experienced any of the following symptoms during this pregnancy?(YES/NO) a. Persistent severe headache b. Visual disturbances c. Upper abdominal pain		

E. Physical Examinations (Current)			
		Case	Matched Control
3.	BP reading a. Systolic b. diastolic		
4.	Fundal height		

F. Laboratory results			
1.	Serum TSH (µIU/mL)		
2.	Serum FT4 (pg/ml)		
3.	Serum FT3 (pg/ml)		

G. CLASSIFICATION OF RESULTS (Tick one)			
1.	Euthyroid		
2.	Primary hypothyroidism		
3.	Secondary hypothyroidism		
4.	Primary hyperthyroidism		
5.	Secondary hyperthyroidism		
6.	Non thyroidal illness		

Appendix IV: Study explanation for Participants

Introduction and Objectives of the study

I am Dr Obiero RO., a master's student in Human Pathology at the University of Nairobi and I am conducting a Study on the thyroid gland. Thyroid gland produces thyroid hormones that are essential for normal growth and development and have many effects on metabolic processes. In a number of disease processes, abnormalities of the thyroid gland arise with serious effects both on the mother and unborn child. My interest is in 3 compounds: Free T3, free T4, and TSH. This Study aims to:

1. To compare the maternal thyroid hormonal status of patients with preeclampsia/eclampsia with that of healthy normotensive pregnant women.
2. To estimate the measures of disease association between preeclampsia/eclampsia and thyroid dysfunction.

Benefits and risks of the Study to you

By participating in it you will benefit by:

1. Having examination and lab tests done on you at no additional cost.
2. A report of your thyroid function Status being sent to your physician.
3. Receive appropriate advice and interventional measures to stop or reverse progression of thyroid dysfunction.

Risk: 5 ml of blood will be drawn from the antecubital vein. The prick will be painful with remote risk of thrombophlebitis.

If you Consent to participate, you will:

- Sign a Consent form (Appendix v)
- Answer a number of questions Contained in the screening and study questionnaire (Appendices i and ii)
- Undergo physical exam.

Participation is voluntary and you can withdraw at any time. Refusal to participate does not prevent you from accessing medical services. Any information given to us will remain confidential. You may ask me questions regarding the study now or any time during the study.

If you have any question relating to the study, kindly Contact:

1. Dr Obiero R.O : 0750735103/0720735103(PI)
2. The Secretary to the Ethical Research Committee. KNH Tel No. 272260 Ext. 44102

Appendix V: consent form for participants

MATERNAL THYROID HORMONAL STATUS IN PREECLAMPSIA AND ECLAMPSIA PATIENTS AT KNH.

I.....
.....after reading and being explained to on the study purpose by Dr. Obiero R.O. ,do hereby give informed consent to the participants in the evaluation of MATERNAL THYROID HORMONAL STATUS IN PREECLAMPSIA AND ECLAMPSIA PATIENTS AT KNH.

I am aware that I can withdraw from the study without any benefits or quality of management of my medical condition being interfered with.

Signed:.....

Thumbprint: ..... Date:
.....

Signature of the PI (Dr Obiero).....

Witness: .....
Date:.....

Appendix VI: METHODOLOGY FOR SERUM TSH

PATHOZYME ULTRASENSITIVE TSH *is an Enzyme Immunoassay (EIA) for the quantitative determination of Thyroid Stimulating Hormone (TSH) in human serum.*

Principle

Specific mouse monoclonal anti-TSH antibodies are prepared, purified and coated onto microtitration wells. Test sera are applied and goat anti-TSH antibody labeled with Horseradish Peroxidase enzyme (Conjugate) is added. If human TSH is present in the sample it will combine with the antibody on the well and the enzyme Conjugate, resulting in the TSH molecule being sandwiched between the solid phase and the enzyme linked antibodies. After incubation the wells are washed to remove unbound labeled antibodies. On addition of the Substrate (TMB), a color will develop only in those wells in which the enzyme Conjugate is present, indicating the presence of TSH. The enzyme reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

Specimen collection and preparation

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required. Do not use haemolysed, contaminated or lipemic serum for testing as this will adversely affect the results. Serum may be stored at 2°C to 8oC for up to 48 hours prior to testing. If longer storage is required, store at –20°C for up to 1 year. Thawed samples must be mixed prior to testing. Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Quick reference test procedure

1. Dispense 100µl of test serum or Standards and 100µl Anti-TSH Conjugate into each well. Gently mix for 30 seconds.

2. Incubate for 120 minutes at room temperature (20°C to 25°C) shaking at 175 +/- 25 RPM.

3. Discard well contents and wash 5 times with wash buffer.

4. Add 100µl of Substrate Solution to each well. Gently shake for 5 seconds.

5. Incubate in the dark for 20 minutes at room temperature (20oC to 25oC).

6. Add 100µl Stop Solution to each well.

7. Read the Optical Densities immediately (no later than 10 minutes) using a micro plate reader with a 450nm filter.

Calculation of results

Calculate the mean absorbance value (A450) for each set of Standards and specimens. Construct a standard curve by plotting the mean absorbance obtained from each Standard against its concentration in µlU/ml on graph paper, with absorbance values on the Y-axis and concentrations on the X-axis. Use the

mean absorbance values for each specimen to determine the corresponding concentration of TSH in µIU/ml from the standard curve. If levels of Calibrators or users known samples do not give expected results, test results must be considered invalid. If using a software package choose quadratic regression curve fit.

Performance characteristics

The graph produced by the calibrators should be Hyperbolic in shape with the OD 450 of the calibrators proportional to their concentration. The OD of calibrator A should be less than 0.75 and the OD of calibrator F for the assay greater than 1.5 results to be valid.

Normal values for adults between the ages of 21 and 54 years are 0.4 to 4.2µIU/ml rising to 0.5 to 8.9 µIU/ml between the ages of 55 and 87. During Pregnancy the normal ranges are as follows: 1st Trimester 0.3 to 4.5µIU/ml, 2nd Trimester 0.5 to 4.6µIU/ml and 3rd Trimester 0.5 to 5.0µIU/ml. The minimum detectable concentration of TSH by **PATHOZYME ULTRASENSITIVE TSH** is estimated to be 0.05µIU/ml.

Concentrations of 1,000µIU/ml have been observed using **PATHOZYME TSH** with no prozone (Hook) effect.

Appendix VII: METHODOLOGY FOR SERUM FREE T4.

PATHOZYME® FREE THYROXINE *Enzyme Immunoassay for the quantitative determination of fT4 in human serum.*

Principle

The fT4 test is a solid phase competitive enzyme immunoassay. Patient serum samples, standards, and Thyroxine-Enzyme Conjugate are added to wells coated with monoclonal T4 antibody. After incubation at room temperature, the wells are washed to remove unbound T4 conjugate. On the addition of Substrate (TMB), a color develops only in those wells in which enzyme are present, indicating a lack of fT4. The reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled fT4 in the sample.

Specimen collection and preparation

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required. Do not use haemolysed, contaminated or lipemic serum for testing as this will adversely affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at –20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Quick reference test procedure

1. Dispense 50µl of Standards or test serum into each well.

2. Dispense 100µl of Thyroxine Enzyme Conjugate into each well and mix thoroughly for 30 seconds.

3. Incubate for 60 minutes at room temperature (20°C to 25°C).

4. Discard well contents and wash 5 times with distilled water.

5. Add 100µl of Substrate solution to each well. Gently shake for 5 seconds.

6. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).

7. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.

8. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450 nm filter.

Calculation of results

Calculate the mean absorbance value (A_{450}) for each set of Reference Standards and samples. Construct a standard curve by plotting the mean absorbance obtained for each Reference Standard against its concentration in pg/ml on graph paper, with absorbance values on the Y-axis and concentrations on the X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of fT4 in pg/ml from the standard curve.

If levels of Calibrators or users known samples do not give expected results, test results must be considered invalid. If using a software package choose a polygon with data extrapolation curve fit.

Performance characteristics

The graph produced by the Calibrators should be Hyperbolic in shape with the OD450 of the Calibrators inversely proportional to their concentration. The OD of Calibrator A should be greater than 1.5 and the OD of Calibrator F should be less than 0.75 for the assay results to be valid.

Based on random selected out-patient clinical laboratory samples, the normal range of fT4 is 8-20 pg/ml (adult non-pregnant) and 8-22pg/ml (pregnancy). The minimum detectable concentration of fT4 by **PATHOZYME FREE T4** is estimated to be 0.5 pg/ml.

Appendix VIII: METHODOLOGY FOR SERUM FT3

PATHOZYME® FREE TRIIODOTHYRONINE *Ref OD457 Enzyme Immunoassay for the quantitative determination of fT3 in human serum.*

Principle

The fT3 test is a solid phase competitive enzyme immunoassay. Patient serum samples, standards, and T3-Enzyme Conjugate are added to wells coated with monoclonal T3 antibody. FT3 in the specimen and the T3 labeled conjugate compete for available binding sites on the antibody. After incubation at room temperature, the wells are washed with distilled water to remove unbound T3 conjugate. On addition of the Substrate, a color develops only in those wells in which enzyme are present, indicating a lack of serum fT3. The reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance measured at 450 nm.

Specimen collection and preparation

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required. Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at –20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Quick reference test procedure

1. Dispense 50µl of Standards or test serum into each well.

2. Dispense 100µl of Conjugate into each well and mix thoroughly for 30 seconds.

3. Incubate for 60 minutes at room temperature (20°C to 25°C).

4. Discard well contents and wash 5 times with distilled water.

5. Add 100µl of Substrate solution to each well. Gently shake for 5 seconds.

6. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).

7. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.

8. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450 nm filter

Expected values and sensitivity

The graph produced by the Calibrators should be Hyperbolic in shape with the OD450 of the Calibrators inversely proportional to their concentration. The OD of Calibrator A should be greater than 1.5 and the

Calibrator F should be less than 0.75 for the assay results to be valid. Based on random selected tient clinical laboratory samples, the normal range of fT3 is 1.3 - 4.2 pg/ml (non-pregnant) and 1.4- /ml (pregnancy). The minimum detectable concentration of fT3 by PATHOZYME FREE T3 is ted to be 0.05 pg/ml.

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21st January 2010

Dr. Obiero Raphael Okoth
Department of Human Pathology
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Dear Dr. Okoth

RESEARCH PROPOSAL: "MATERNAL THYROID HORMONAL STATUS IN PREECLAMPSIA AND ECLAMPSIA AT KENYATTA N. HOSPITAL." (P334/12/2009)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and approved your above cited research proposal for the period 21st January 2010 – 20th January, 2011.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

DR. L. MUCHIRI
AG. SECRETARY, KNH/UON-ERC

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