STUDY ON RELATIONSHIP BETWEEN THYROID HORMONES AND GLUCOSE HOMEOSTASIS AMONG POST MENOPAUSAL DIABETIC WOMEN

Thesis submitted to Faculty of Medicine
BLDE (Deemed to be University), Vijayapura, Karnataka, India.
For the Award of the Degree of

Doctor of Philosophy (Medical)
Subject: Physiology

CH. KALASHILPA

PhD Research scholar

Department of Physiology
Shri B.M.Patil Medical College, Hospital & Research Centre
BLDE (Deemed to be University)
Vijayapura, Karnataka

AUGUST - 2018

DECLARATION

I hereby declare that the thesis entitled **"Study on relationship between thyroid hormones and glucose homeostasis among post menopausal diabetic women**" is a bonafide and genuine research work carried by me under the guidance of Dr.Sumangala.M.Patil, Professor, Department of Physiology, BLDE (Deemed to be University), Shri B.M. Patil Medical College, Hospital & Research center, Vijayapur, Karnataka, India. No part of this thesis has been formed the basis for the award of any degree or fellowship previously.

Date:

Place: Vijayapur

Signature of the candidate

Ch. Kalashilpa
PhD Research Scholar,
Reg No. 14PHD004
Department of Physiology,
Shri B.M Patil Medical College,
Hospital & Research Center,
BLDE (Deemed to be University),
Karnataka, INDIA.

BLDE (DEEMED TO BE UNIVERSITY)

Vijayapura, Karnataka, India

Certificate

This is to certify that this thesis entitled **"*Study on relationship between thyroid hormones and glucose homeostasis among post menopausal diabetic women*"** is a bonafide work of Ms.Ch.Kalashilpa and was carried out under our supervision and guidance in the Department of Physiology, Shri B.M. Patil Medical College, Hospital & Research Centre, Vijayapura, Karnataka, India.

Dr. Sumangala M. Patil
Guide
Professor, Department of Physiology
Shri B.M.Patil Medical College, Hospital & Research Centre
Vijayapura, Karnataka

Dr Manjunatha R. Aithala
Professor & Head
Department of Physiology
Shri B.M.Patil Medical College,
Hospital & Research Centre,
Vijayapura, Karnataka

Dr. S.P. Guggarigowdar
Principal
Shri B.M.Patil Medical College,
Hospital & Research Centre,
Vijayapura, Karnataka

ACKNOWLEDGEMENT

I sincerely take an opportunity to acknowledge gratitude to all the people including study participants without whom this thesis would not have been possible.

I express my sincere gratitude to my guide, **Dr.Sumangala.M.Patil**, Professor, Department of Physiology,BLDE(Deemed to be University) Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapura for her constant support, invaluable suggestions and guidance. Her full faith and trust in me have contributed immensely in successfully completing this journey. She is easily approachable and venerable teacher. She has always made herself available to clarify my doubts despite her busy schedules and I consider it as a great opportunity to do my research under her guidance and to learn from her research expertise.

I would like to extend my sincere gratitude to **Prof Dr. Kusal K. Das**, Laboratory of vascular physiology and medicine, Department of physiology, BLDE(Deemed to be University) Shri B. M. Patil Medical College, Hospital and Research Centre, for his ever great support and guidelines during the period of my research work. He is a source of inspiration and knowledge to me. I am extremely grateful to you sir.

I would like to extend my sincere gratitude to **Dr. Manjunatha Aithala** Professor & Head. Department of Physiology,BLDE(Deemed to be University) Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapura, for his constant support, care, and encouragement he has given for completion of my research work.

I would like to extend my sincere gratitude to Dr. M S Biradar, Vice Chancellor, BLDE(Deemed to be University), Dr. S P Guggarigoudar ,Principal & Dr. Tejashwini Vallabha, Vice Principal, BLDE(Deemed to be University), Dr J.G Ambekar(Registrar) Shri B M Patil Medical College, Hospital & Research Centre, Vijayapura for their valuable support in completion of my PhD work.

I would like express my sincere gratitude to Dr.Basavaraj Devarnavadgi, Prof &Head. Department of Biochemistry for providing the facilities to work in the central biochemistry laboratory and I would like to thank Dr. Sanjay Walvekar, Lecturer Department of biochemistry, for his constant support and suggestions during my research work in the laboratory.

I am grateful to acknowledge my sincere thanks to Dr. Shailaja Patil, Professor & Head, Department of Community Medicine, Dr. Nilima Dongre, Associate Professor, Department of Biochemistry, Mr.Mohd Shannawaz, Lecturer and Mrs Vijaya Sorgavi, Lecturer, Department of Community Medicine for their constant help & suggestions during completion of this work. I would like to extend my sincere thanks to all the members of PhD committee for their valuable suggestions and constant monitoring of my research work.

I would like to extend my thanks to technicians of biochemistry lab for their constant support during the period of my research work.

My special thanks are extended to the all the staff of BLDE University for providing academic facilities & to the staff of library for providing library facilities.

Finally, I would like to thank all the members of my family for their constant support and encouragement for completion of my research work.

Ch.Kalashilpa

DEDICATION

I dedicate this thesis to
My beloved parents

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ADH	Antidiuretic Hormone
ACTH	Adrenocorticotropic Hormone
Acetyl- CoA	Acetyl Coenzyme A
ANP	Atrial natriuretic peptide
ANOVA	Analysis of variance
BSA	Body surface area
BMI	Body Mass Index
BP	Blood pressure
BNP	Brain natriuretic peptide
bpm	Beats per minute
β	Beta
CRH	Corticotropin- releasing hormone
CNP	C-type Natriuretic pepetide
CBC	Complete blood picture count
CLIA	Chemiluminescence immunoassay
CNS	Central nervous system
CVS	Cardiovascular system
Co_2	Carbondioxide
cm	Centimeter
DEA	Diethanolamine
DIT	Diiodotyrosine
DBP	Diastolic blood pressure
DM	Diabetes mellitus
E_1	Estrone
E_2	Estradiol
E_3	Estriol
EDTA	Ethylenediaminetetraacetic acid
ELFA	Enzyme-linked fluorescence assay
ER	Estrogen receptor
FSH	Follicle stimulating hormone
FBS	Fasting blood sugar

fl	Femtolitre
GnRH	Gonadotropin-releasing hormone.
GIT	Gastro intestinal tract
GH	Growth Hormone
GLUT-4	Insulin responsive glucose transporter
g/dl	Gram per decilitre
gm %	Gram percentage
HCG	Human chorionic gonadotropin
HPO axis	Hypothalamic-Pituitary-Ovarian *axis*
HRP	Horseradish peroxidase
HMP	Hexose MonoPhosphate
Hb	Haemoglobin
HbA1c	Glycosylated Haemoglobin
HDL	High-density lipoproteins
H_2O	Water
H_2O_2	Hydrogen peroxide
IU/L	International Units per liter
IL 6	Interleukin 6
kg	Kilogram
kg/m^2	Kilogram per square meter
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
LPL	Lipoprotein lipase
LAS	Large artery stiffness
MIT	Monoiodotyrosine
MAP	Mean arterial pressure
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular haemoglobin concentration
mIU/ml	Milli international units per liter
mμIU/ml	Milli micron international units per milli liter
mU/L	MilliUnits per liter

mmol/l	Millimoles per liter
ml	Milliliter
mmHg	Millimeters of mercury
mm^3	Cubic millimeter
NEFA	Non-esterified fatty acids
NADPH	Nicotinamide adenine dinucleotide phosphate
NHANES	The National health and nutrition examination survey
ng/mL	Nanogram per liter
ng/dl	Nanogram per deciliter
nmol/l	Nanomoles per liter
nmol/ml	Nanomoles per milli leter
P_4	Progesterone
PIF	Prolactin inhibitory factor
PVR	Peripheral vascular resistance
PCV	Packed cell volume
PR	Pulse rate
PP	Pulse pressure
pg	Picogram
pg/mL	Picogram per millilitre
rt_3	Reverse T_3
RFV	Relative fluorescence value
RLU	Relative light units
RVS	Renal vascular resistance
RBC	Red blood cells
SHBG	Sex hormone binding globulin
SBP	Systolic blood pressure
SPR	Solid Phase Receptacle
SD	Standard deviation
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
T_3	Triiodothyronine
T_4	Thyroxine

TR	Thyroid hormone receptor
TNF-α	Tumour necrosis factor
TCA cycle	Tricarboxylic acid cycle
TTAB	Tetradecyltrimethylammonium bromide
U/L	Units per liter
WBC	White blood corpuscles
WHO	World health organization

ABSTRACT

Aim and Objectives: We aimed to establish the link between thyroid hormones and glucose homeostasis in pre and post menopausal non-diabetic women as well as in pre and post menopausal diabetic women and to compare the relation between thyroid hormones and glucose homeostasis with pre and post menopausal non-diabetic and diabetic women.

Materials and Methods: It was a cross sectional study. Total 200 women, among them 50 premenopausal non-diabetics and 50 premenopausal diabetics aged between 25-45 years, 50 post menopausal non-diabetics and 50 post menopausal diabetics aged between 46-65 years were selected for the present study. Anthropometric and Physiological parameters were recorded. Haematological parameters which includes Complete blood picture count, Blood glucose profile includes Fasting Blood sugar and Glycosylated haemoglobin and thyroid profile which includes TSH, T_3, T_4 and Reproductive profile includes Luteinizing hormone, Follicle stimulating hormone, Estrogen and Progesterone hormonal analysis were done by using standard procedures. Statistical analysis was done using One Way Anova followed by 'Post Hoc t tests' were done using SPSS software version 16.

Results: We found there is an increase in FBS of pre menopausal diabetics but HbA1c is same in both pre and post menopausal diabetics. There is an increase in T_3 and T_4 levels and decrease in TSH levels in post menopausal diabetics. LH, FSH and E_2 and P_4 showed to be decreased in post menopausal diabetics.

Conclusion: These findings suggest that current thyroid disorders deteriorate the glucose metabolism in post menopausal women suffering from diabetes mellitus. So monitoring of thyroid profile in post menopausal diabetic women would be a better therapy in future.

Key words: Thyroid hormones, glucose homeostasis, post menopausal diabetic, post menopausal non-diabetic, pre menopausal diabetic, pre menopausal non-diabetic.

CHAPTER 1
INTRODUCTION

1.1. Introduction:

Endocrine disorders affect important organs of the body which leads to many diseases. Among them, thyroid and diabetes are the two major endocrine disorders usually seen with increasing frequency in women. There is mutual influence between thyroid and diabetes as, thyroid regulates the carbohydrate metabolism and pancreatic function where, diabetes affects thyroid at the hypothalamic level, at the peripheral conversion of T_4 to T_3 in the tissues[1].

Insulin resistance, physical inactivity, advanced age, obesity, hormonal imbalance etc are the main causes for the development of many diseases including diabetes and thyroid[2].

There is an increased frequency of thyroid dysfunction in diabetic women as age advances compared to non-diabetics. There were several reports documented higher than normal prevalence of thyroid disorders in women suffering from diabetes[3]. Some Indian and other international studies reported that the prevalence rate of thyroid disorder in diabetic women ranges between 30-32% and they have shown that the thyroid disorders are more common in females particularly in elderly persons[3,4]. There is a high prevalence (31.2%) rate of thyroid dysfunction among diabetics particularly in women, in India[5].

The hypo and hyper activity of thyroid gland impacts metabolism of carbohydrates at the site of islets of langerhans and glucose using target tissues assuming important therapeutic and diagnostic questions. Thyroid and insulin both involved in the metabolism at cellular level and the excess or deficiency of any of them can cause the altered function of one among other. Most of the abnormalities of thyroid may co-exist and interact with diabetes[6].

"Menopause is defined as permanent cessation of menstruation resulting from the loss of ovarian follicular activity which is recognized to have occurred after 12 consecutive months of amenorrhea, for which there is no other pathological or physiological cause" [7]. This transition is gradual which takes place over a period of time from the reproductive to non-reproductive phase of life.The phase of transition from pre menopause to post menopause is often associated with the development of

metabolic syndrome features. "These risk factors may be due to the direct result of ovarian failure or an indirect result of the metabolic consequences of central fat redistribution with estrogen deficiency"[8-10].

Diabetes and thyroid are also associated with menopause. Like other disorders of metabolism, diabetes signifies with the midlife of the women corresponds with transition phase of menopause in women[11].

"Menopause is connected with hyper functioning of anterior lobe of pituitary gland. So there is a chance of increase in the products which are chemical in nature and the functions which are biological in nature may be altered. Among all these, the "diabetogenic" factor is increased and it may cause diabetes or provoke an already existing diabetes either by its action of antagonistic nature on insulin or cellular degeneration of islets of langerhans" [12].

Glucose homeostasis is brought by complex mechanism including intake of food, regulation of secretion of insulin and its action at the level of target tissue. The thyroid hormone regulation has been identified as an important determinant factor of glucose homeostasis[13-15].

In most of the non-diabetic women, menopause is also considered as an important and self-reliant risk factor for increasing fasting plasma glucose levels but glucose metabolism is affected by genetic and environmental factors[16].

The connection among thyroid disease and diabetes mellitus is described by complex interaction of interdependence[17]. There is a negative impact of thyroid on diabetes which usually difficult to diagnose the dysfunction of thyroid and it affects the patient's treatment also. These may results in poor glycaemic control which leads to either hypo or hyperthyroidism.

There is a complex interaction of insulin and iodothyronines on the metabolism of carbohydrates represents the iodothyronines are antagonists of insulin with high levels usually being diabetogenic, and the absence of hormone deprives the development of diabetes and hyperthyroidism which are metabolic diseases that may affects the carbohydrates level[18].

Thyroid hormones affect glucose homeostasis through different mechanisms. Hyperthyroidism induces hyperglycaemia has long been recognized[19]. It is well known that glycemic control is worsening in diabetic patients with hyperthyroidism and it has been shown to trigger diabetic ketoacidosis[20,21].

Glucose metabolism is also affected in the case of hypothyroidism as well via different mechanisms where, requirement of insulin decreases in diabetic hypothyroid patients by decreased rate of production of glucose in the liver [22].

In women, thyroid disorders are also associated with pre menopause and post menopause. Thyroid hormones perform a vital role in the normal functioning of reproductive system both through direct effects on the ovaries and by combining indirectly with sex hormone binding proteins. The thyroid dysfunction may lead to hypothyroidism or hyperthyroidism in diabetic patients, can also lead to menstrual irregularities and infertility[23].

Many studies have been done to know the influence of thyroid disorders in diabetes but there is insufficient evidence on the interaction between thyroid hormones and glucose homeostasis among post menopausal women. Therefore we have taken up this study.

REFERENCES

1. Hage M, Zantout MS, Azar ST. Thyroid disorders and diabetes mellitus. J Thyroid Res 2011; 2011:439463.
2. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Spertus JA. Diagnosis and management of the metabolic syndrome. Circulation 2005;112(17):2735-52.
3. Vikhe VB, Kanitkar SA, Tamakuwala KK, Gaikwad AN, Kalyan M, Agarwal RR. Thyroid dysfunction in patients with type 2 diabetes mellitus at tertiary care centre. Natl J Med Res. 2013;3(4):377-80.
4. Díez JJ, Sánchez P, Iglesias P. Prevalence of thyroid dysfunction in patients with type 2 diabetes. Exp Clin Endocrinol Diabetes 2011;119:201-7.
5. Demitrost L, Ranabir S. Thyroid dysfunction in type 2 diabetes mellitus: A retrospective study. Indian J Endocrinol Metab 2012;16 Suppl 2:S334-5.
6. Mukherjee S, Datta S, Datta P, Mukherjee AK, Maisnam I. A study of prevalence of primary hypothyroidism in recently diagnosed type 2 diabetes mellitus in a tertiary care hospital. Int J Sci Rep 2015 ; 1(2):105-12.
7. WHO Scientific Group. Research on the menopause in the 1990s. Report of a WHO Scientific Group. World Health Organ Tech Rep Ser 1996; 866:1-07.
8. Carr MC. The emergence of the metabolic syndrome with menopause. J Clin Endocrinol & Metab. 2003 ; 88(6):2404-11.
9. Fu CH, Yang CC, Lin CL, Kuo TB. Alteration of cardiovascular autonomic functions by vegetarian diets in postmenopausal women is related to LDL cholesterol levels.Chin J Physiol 2008 ; 51(2):100-5.
10. Pellizzer AM, Straznicky NE, Lim S, Kamen PW, Krum H. Reduced dietary fat intake increases parasympathetic activity in healthy premenopausal women. Clin Exp Pharmacol Physiol 1999;26(8):656-60.
11. Narayan KV, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. Jama 2003 ;290(14):1884-90.
12. Gitlow S, Kurschner DM. Estrogen, diabetes and the menopause. Arch Intern Med 1943;72(2):250-9.
13. Dimitriadis G, Baker B, Marsh H, Mandarino L, Rizza R, Bergman R, Haymond M, Gerich J. Effect of thyroid hormone excess on action, secretion,

and metabolism of insulin in humans. Am J Physiol 1985 May 1;248(5):593-601.

14. Weinstein SP, Watts J, Haber RS. Thyroid hormone increases muscle/fat glucose transporter gene expression in rat skeletal muscle. Endocrinology. 1991;129(1):455-64.
15. Torrance CJ, Devente JE, Jones JP, Dohm GL. Effects of thyroid hormone on GLUT4 glucose transporter gene expression and NIDDM in rats. Endocrinology. 1997;138(3):1204-14.
16. Barclay L, Medscape Medical News: Menopause 14, 2007, 404-407.
17. Palma CC, Pavesi M, Nogueira VG, Clemente EL, Pereira MD, Pereira LC, Pacheco FF, Braga TG, de Faria Bello L, Soares JO, dos Santos SC. Prevalence of thyroid dysfunction in patients with diabetes mellitus. Diabetol Metab Syndr 2013; 5(1):58.
18. Sathish R, Mohan V. Diabetes and thyroid diseases-A Review. Int J Diab Dev Ctries 2003; 23(4):120-3.
19. Maxon HR, Kreines KW, Goldsmith RE, Knowles HC. Long-term observations of glucose tolerance in thyrotoxic patients. Arch Intern Med 1975;135(11):1477-80.
20. Sola E, Morillas C, Garzon S, Gómez-Balaguer M, Hernandez-Mijares A. Association between diabetic ketoacidosis and thyrotoxicosis. Acta Diabetol 2002;39(4):235-7.
21. Bhattacharyya A, Wiles PG. Diabetic ketoacidosis precipitated by thyrotoxicosis. Postgrad Med J 1999;75(883):291-3.
22. Okajima F, Ui M. Metabolism of glucose in hyper-and hypo-thyroid rats in vivo. Glucose-turnover values and futile-cycle activities obtained with 14C- and 3H-labelled glucose. Biochem J 1979 ;182(2):565-75.
23. Poppe K, Glinoer D. Thyroid autoimmunity and hypothyroidism before and during pregnancy. Hum Reprod update 2003 ; 9(2):149-61.

CHAPTER 2

AIM AND OBJECTIVES

2.1. AIM AND OBJECTIVES OF THE STUDY:

Aim of the present study is to assess the relationship between thyroid hormones and glucose homeostasis in pre and post menopausal non-diabetic and diabetic women.

2.2. Primary Objectives:

- To establish a link between thyroid profile and glucose homeostasis in pre and post menopausal non-diabetic and diabetic women.
- To compare the relationship between thyroid profiles and glucose homeostasis with pre and post menopausal non-diabetic and diabetic women.

2.3. Secondary Objectives:

- To establish the association between thyroid profile (TSH, T_3, T_4) and reproductive hormones (LH, FSH, Estrogen and Progesterone) in pre and post menopausal non-diabetic and diabetic women.

2.4. HYPOTHESIS:

Thyroid hormone levels may be linked with menopause with special relation to diabetes. We also hypothesized that, such links may influence in differential regulation of glucose homeostasis.

CHAPTER 3

REVIEW OF LITERATURE

3.1. HORMONES:

Introduction:

Hormones are the secretary products of the ductless glands, which are released in catalytic amounts into blood stream and transported to specific target cells (or organs), where they elicit physiologic, morphologic and biochemical responses. The chemical messengers **(Figure 1)** which perform hormonal functions are defined as

1. **Endocrine hormones:** These include the chemical messengers whose function is the transmission of a molecular signal from a classic endocrinal cell through the blood stream to a distant organ.

2. **Neurocrine hormones:** Nervous communication involves the release of chemical messengers from nerve terminals, which may reach their target cells through different routes, includes neurotransmitters, neural signals, neurohormones or peptides.

3. **Paracrine hormones:** These are chemical messengers which after getting secreted by a cell are carried over short distance by diffusion through the interstitial spaces(extracellular fluid) to act on the neighbouring different cell types as or regulatory substance. For example, in islets of Langerhans somatostatin secreted by the delta cells acts on the alpha and beta cells.

4. **Autocrine hormones:** These refer to those chemical messengers which regulate the activity if neighboring similar type of cells. Examples of autocrine hormones are prostaglandins[1].

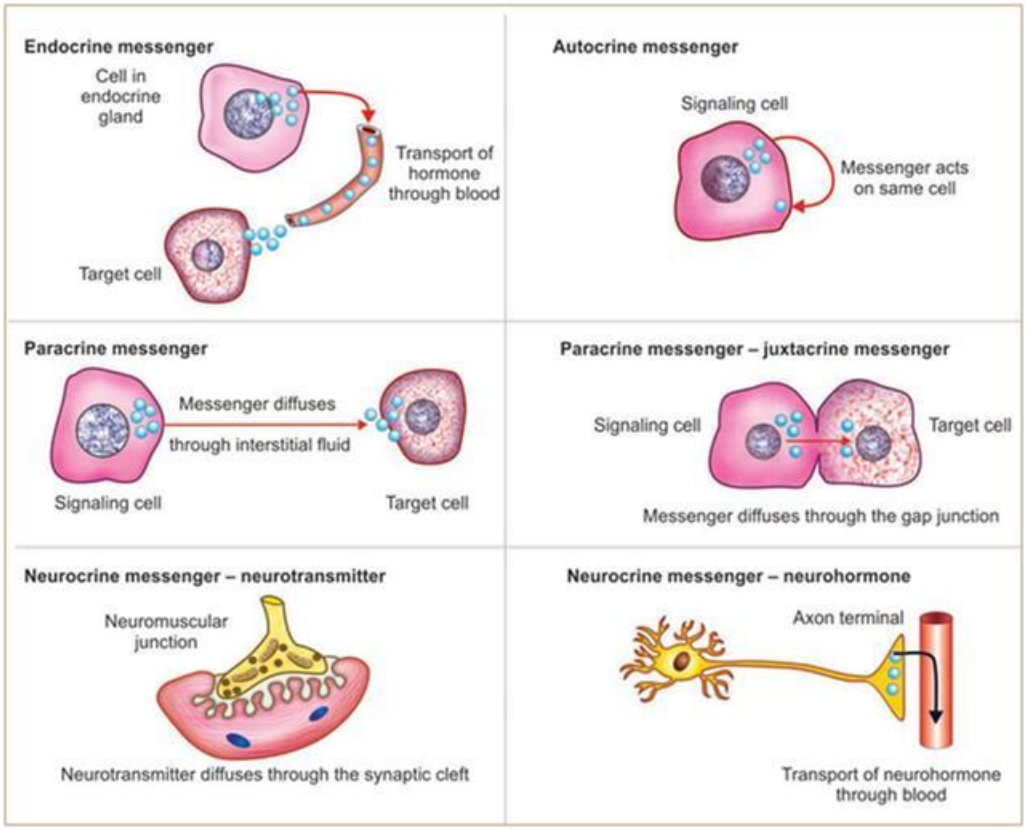


Figure 1: Chemical messengers

(Source: Sembulingam K, Sembulingam P. Essentials of medical physiology, 6th ed, 2012)

Endocrine glands:

These are the glands which synthesize and release the chemical hormones into the blood. These are also called as "Ductless glands" in which the hormones secreted by them are directly released into the blood without any duct. They play an important role in homeostasis and control of various other activities in the body through their hormones. Hormones are transmitted by the blood to target tissues or organs in different parts of the body where the actions are executed[2].

The major endocrine glands are:

- Hormones secreted by endocrine glands **(Table 1)**.
- Hormones secreted by gonads **(Table 2)**.
- Hormones secreted by other organs **(Table 3)**.
- Local hormones **(Table 4)**.

Table 1: Hormones secreted by major endocrine glands

<table>
<tr><td>Anterior Pituitary</td><td>1. Growth Hormone(GH)
2.Thyroid-Stimulating Hormone(TSH)
3.Adrenocorticotropic Hormone(ACTH)
4.Follicle Stimulating Hormone(FSH)
5. Luteinizing hormone(LH)
6. Prolactin</td><td rowspan="4">Adrenal Cortex</td><td>Mineralocorticoids
1.Aldosterone
2.11-deoxycorticosterone</td></tr>
<tr><td>Posterior Pituitary</td><td>1. Antidiuretic hormone(ADH)
2. Oxytocin</td><td>Glucocorticoids
1.Cortisol
2. Corticosterone</td></tr>
<tr><td>Thyroid gland</td><td>1. Thyroxine(T_4)
2. Tri-iodothyronine(T_3)
3. Calcitonin</td><td>Sex hormones
1.Androgens
2.Estrogen
3.Progesterone</td></tr>
<tr><td>Parathyroid gland</td><td>Parathormone</td><td rowspan="2">Adrenal Medulla</td><td rowspan="2">1. Catecholaimes
2.Adrenaline (Epinephrine)
3. Noradrenaline (Norepinephrine)
4. Dopamine</td></tr>
<tr><td>Pancreas - Islets of Langerhans</td><td>1. Insulin
2. Glucagon
3. Somatostatin
4. Pancreatic polypetide</td></tr>
</table>

Table 2: Hormones secreted by Gonads

Testis	1.Testosterone 2.Dihydrotestosterone 3.Androstenedione
Ovary	1.Estrogen 2.Progesterone

Table 3: Hormones secreted by other organs

Thymus	1. Thymosin 2. Thymin
Kidney	1. Erythropoietin 2. Thrombopoietin 3. Renin 4. 1,25-dihdroxycholecaliferol(calcitrol) 5. Prostaglandin
Heart	1. Atrial natriuretic peptide(ANP) 2. Brain natriuretic peptide(BNP)
Placenta	1. Human chorionic gonadotropin(HCG) 2. Human chrionic somatotropin 3. C-type natriuretic peptide

Table 4: Local Hormones

1.Prostagalndins	7. Serotonin
2. Thromboxanes	8. Histamine
3. Prostacyclin	9. Substance P
4. Leukotrienes	10. Heparin
5. Lipoxins	11. Bradykinin
6. Acetylcholine	12. Gastrointestinal hormones

Based on chemical nature, hormones are classified into three types **(Table 5).**

1. Steroid hormones: Hormones synthesized from cholesterol or its derivatives. They are secreted by adrenal cortex, gonads and placenta.

2. Protein hormones: These are large or small peptides secreted by pituitary gland, parathyroid gland, pancreas and placenta.

3. Tyrosine derivatives: Two types of hormones namely thyroid hormones or adrenal medullary hormones are derived from the amino acid tyrosine.

Table 5: classification of hormones depending upon their chemical nature

Steroids	**Proteins**	**Derivat**
Aldosterone	Growth hormone (GH)	Thyroxine(T_4)
11-deoxycorticosterone	Thyroid-stimulating hormone (TSH)	Tri-iodothyronine(T_3)
Cortisol	Adrenocorticotropic hormone(ACTH)	Adrenaline(Epinephrine)
Corticosterone	Follicle-stimulating hormone(FSH)	Noradrenaline (Norepinephrine)
Testosterone	Luteinizing hormone(LH)	Dopamine
Dihydrotestosterone	Prolactin	
Dehydroepiandrosterone	Antidiuretic hormone(ADH)	
Androstenedione	Oxytocin	
Estrogen	Parathromone	
Progesterone	Calcitonin	
	Insulin	
	Glucagon	
	Somatostatin	
	Pancreatic polypeptide	
	Humanchorionic gonadotropin(HCG)	
	Human chorionic somatomammotropin	

3.2. NEUROENDOCRINE MECHANISMS.

The neuroendocrine mechanisms are the basic factors in the reproductive cycle. A transducer concept has been evolved in which the specialized neural cells of the hypothalamus function as the final common pathway to guide the appropriate anterior pituitary hormonal response.

Hypothalamus and its secretions:

Hypothalamus plays an important role in the neuroendocrine regulation. It produces a series of specific releasing and inhibiting hormones which have got effect on the production on the specific pituitary hormones.

Gonadotropin Releasing Hormone (GnRH):

The GnRH is also named as luteinizing hormone releasing hormone (LHRH). GnRH is a decapeptide and is concerned with the release, synthesis and storage of both the gonadotropins (FSH and LH) from the anterior pituitary. The divergent patterns of FSH and LH in response to a single GnRH are due to modulating influence of the endocrine environment specially the feedback effects of steroids on anterior pituitary gland. GnRH is secreted by the arcuate nucleus of the hypothalamus in a pulsatile fashion. Developmentally these neurons originated from the olfactory area. The half life of GnRH is very short (2-4 minutes) [3].

Prolactin Inhibitory Factor (PIF):

Prolactin secretion from the anterior pituitary seems to be under chronic inhibition by PIF. Dopamine is the Physiological inhibitor.

Thyrotropin Releasing Hormone (TRH):

The hormone TRH, a tripeptide stimulates the release of not only the thyrotropin but also prolactin from the pituitary.

Corticotropin Releasing Hormone (CRH): It is a tetradecapeptide.

Other secretions are: Growth hormone releasing Hormone (GHRH) and melanocytic releasing factor[3].

3.3. Menarche:

The onset of first menstruation in life is called menarche. It may occur anywhere between 10 and 16 years, the peak time being 13 years. There is endometrial proliferation due to ovarian estrogen but when the level drops temporarily, the endometrium sheds and bleeding is visible. It denotes an intact hypothalamic pituitary-ovarian axis, functioning ovaries, presence of responsive

endometrium to the endogenous ovarian steroids and the presence of a patent uterovaginal canal. The first period is usually anovular. The ovulation may be irregular for a visible period following menarche and may take about 2 years for regular ovulation to occur[3].

3.4. Menstruation:

"Menstruation is the end point in the cascade of events starting at hypothalamus and ending in the uterus". The menstrual cycle is usually of 28 days, measured by the time between the first day of one period and the first day of the next. "The duration of bleeding is about 3-5 days and estimated blood loss is between 50 and 200ml. The regular cycle of 28 days is seen only in a small proportion of women". A deviation of 2 or 3 days from the 28-day rhythm depends on the H-P-O function, where as the amount of blood loss depends upon the uterine condition[4].

3.5. Menstrual cycle:

The reproductive system of adult women, unlike in men, exhibit regular changes during their reproductive phase. These cyclic changes include[5]:

1. Changes in ovaries- ovarian cycle.
2. Changes in uterine endometrium-uterine cycle.
3. Changes in cervical mucus.
4. Changes in vagina.
5. Changes in the breast.

Menstrual cycle is a collective term for all the complex cyclic changes in the female reproductive system. The average length of a menstrual cycle is 28 days but may vary from 20-45 days. By convention, the days of a menstrual cycle are counted starting with the first day of menstrual bleeding, since it is a conspicuous event. The two important events that occur during a menstrual cycle are:

1. Release of a single ovum, ensuring formation of a single embryo and
2. The endometrial changes necessary for implantation and growth of the embryo.

Phases of Menstrual cycle: It consists of three phases **(Figure 2)**.

1. Proliferative Phase (Follicular Phase):

The proliferative phase starts at the end of menstrual phase. Its duration is about 10 days. At the beginning of this phase most of the endometrium has been desquamated. Later, the endometrium starts healing and then proliferates rapidly from fifth to fourteenth day of menstrual cycle. This proliferation occurs under the influence of estrogen. There is growth of simple tubular glands and blood vessels. The endometrium increases in thickness to about 3-5mm. The endometrial glands produce thick mucus. At the end of proliferative phase, ovulation occurs[6].

2. Secretary Phase (Luteal Phase):

The secretary phase last for about 14 days. It starts after ovulation and extends upto the next menstrual cycle. The endometrium thickness upto 6 mm. The glands become bigger, tortuous, and filled with secretions. The stromal cells proliferate; spinal arteries become more colloid and dilated. This is caused due to the action of progesterone and estrogen on the uterine endometrium.

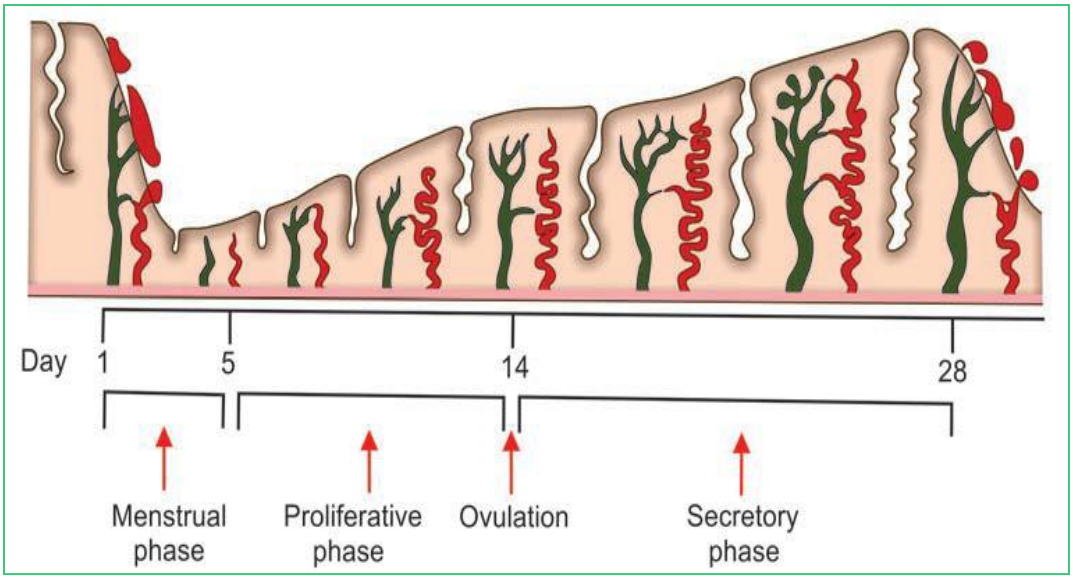


Figure 2: Phases of Menstrual cycle (Uterine changes)

(Source: Sembulingam K. Sembilingam P. Essentials of medical physiology, 6th ed. 2012)

3. Menstrual Phase: This phase lasts for about 3-5 days.

If the ovum is not fertilized, the corpus luteum regresses from the 24th day of the cycle. This causes a decrease in the estrogen and progesterone levels. It results in the shrinkage of the endometrium and coiling and spasm of the endometrium. The necrosed endometrium is shed along with blood and other secretions. The normal quantity of blood loss during menstruation is about 50-60 mL. The normal menstrual blood doesn not clot due to the release of fibrinolysin along with the necrotic endometrial material.

Ovarian changes during Menstruation:

During the proliferative phase, FSH from the anterior pituitary acts on the graafian follicle of the ovary to produce estrogen. By the end of proliferative phase, LH is produced in large amounts. LH surge results in ovulation.

During secretary phase, the ovary produces estrogen and progesterone. During the menstrual phase, involution of the corpus luteum causes sudden decrease in the levels of estrogen and progesterone[6].

3.6. Hormonal regulation of menstrual cycle:

The menstrual cycle is regulated by the cyclic changes in the levels of hormones secreted by the hypothalamus, anterior pituitary, ovaries and corpus luteum**(Figure 3)**. The periodic changes in the ovaries and reproductive tract do not occur, if any of these organs or removed or diseased. The hormones involved in the regulation of menstrual cycle are:

1) Gonadotropins releasing hormone-GnRH,

2) Gonadotropins-FSH and LH,

3) Estrogens and Progesterone.

1. Gonadotropin releasing hormone:

GnRH is secreted by the hypothalamus in a pulsatile manner. GnRH stimulates the release of LH and FSH by the anterior pituitary. A pulse generator in the medial-basal hypothalamus regulates the secretion of GnRH. The secretion of the gonadotropins depends on the pulsatile secretion of GnRH. Frequency of pulses is

increased by estrogen and decreased by progesterone. The pulse frequency is greater during late follicular phase, eventually evoking the LH surge coinciding with maximum sensitivity of pituitary gonadotropins to LH. In the luteal phase, pulse secretion decreases, owing to inhibitory effect of progesterone during luteal phase, but increases once again when the secretion of estrogen and progesterone decline at the end of cycle[5].

2. Gonadotropins - FSH and LH: FSH and LH are secreted by the anterior pituitary gland.

Actions of FSH:

- FSH causes the growth of primary follicles every month. It stimulates mitotic division of granulose cells increasing their numbers and layers.
- It helps the theca interna to form estrogen.
- It helps in the formation of new FSH and LH receptors on the granulosa cells.

Luteinizing Hormone (LH):

- Necessary for follicular growth and ovulation.
- Helps in the formation and maintenance of corpus luteum.
- Acts on the granulose cells and theca cells and converts them into progesterone secreting cells and it also changes granulosa and theca cells to lutein cells after ovulation[6].

2. Feedback regulation by estrogens:

Estrogen and progesterone regulate gonadotropin secretion by complex feedback mechanisms. In the follicular phase, estrogen modifies the pituitary gonadotropin secretion in response to GnRH. As a result, LH rises slowly, FSH level is modestly elevated in the early follicular phase. In the late follicular phase, GnRH pulse increases, estrogen secretion rises, exerts a positive feedback effect and triggers the LH surge that produces ovulation. During luteal phase, the levels of LH and FSH are low owing to negative feedback by the rise in estrogen and progesterone levels.

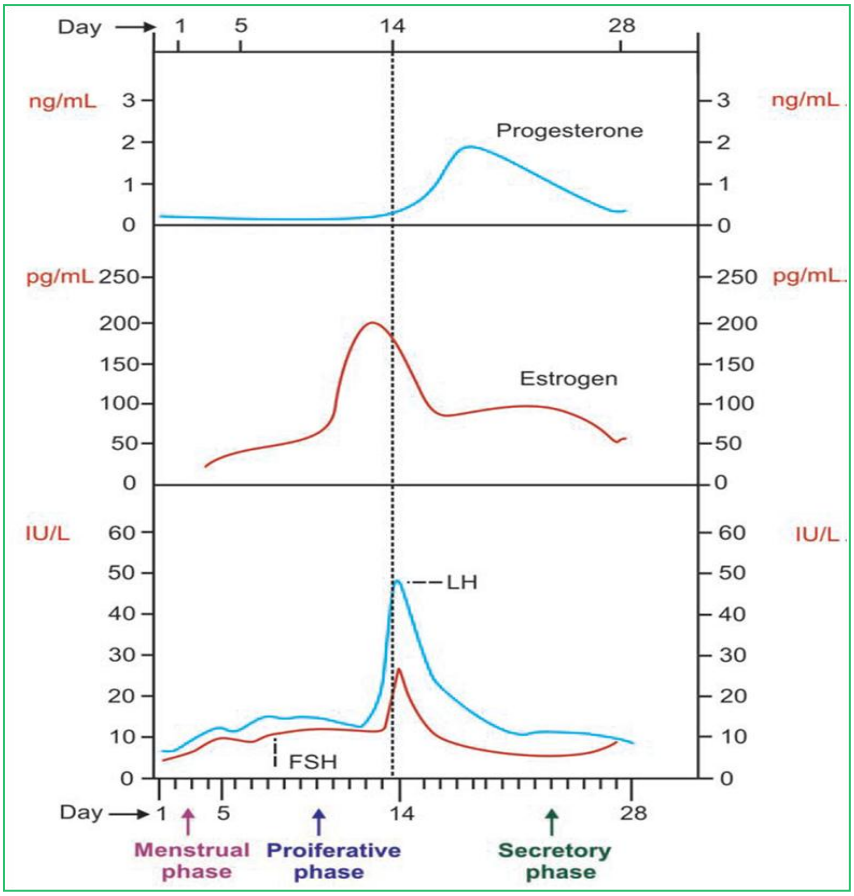


Figure 3: Hormonal levels during menstrual cycle

(Source: Sembulingam K. Semblingam P. Essentials of medical physiology, 6th ed, 2012)

The positive and negative feedback effects of estrogen are exerted on the medio basal hypothalamus, which is the pulse generator for GnRH secretion. Even though estrogen is the primary trigger for LH surge, progesterone also can increase the intensity of surge, quicken the onset of surge and reduce the duration of surge. Although progesterone facilitates the surge, its administration during other phases of a normal cycle, will inhibit gonadotropin secretion[5].

3.7. ENDOCRINE FUNCTIONS OF THE OVARIES:

The female sex hormones are of two types: 1) Estrogens and 2) Progestins.

The estrogens are: 1) 17 β-Estradiol, 2) Estrone 3) Eestriol

The progestins are: 1) Progesterone and 2) 17 α-Hydroxyprogesterone.

The principal ovarian estrogen is 17 β-Estradiol, secreted by the granulosa cells.

Estrone is secreted by the follicle or may be formed in peripheral tissues from estradiol or androgens. Estradiol is a week estrogen is formed mainly in the liver from estradiol or estrone. Estriol is also formed in the fetus and the placenta.

Progesterone is the principal progestin and is secreted by the corpus luteum in non-pregnant women. During pregnancy progesterone is secreted by placenta. Small amounts of 17 α-Hydroxy progesterone are secreted along with progesterone.

Biosynthesis:

Estrogens and progestins are steroids formed from cholesterol. Cholesterol is derived mainly from the blood and to a minor extent synthesized from acetyl Co-A.

Cholesterol is converted to androstenedione and testosterone. The enzyme, aromatase, present in the thecal cells and granulosa cells catalyze the conversion of androstenedione and testosterone to estradiol is regulated by LH in the thecal cells and by FSH in the granulosa cells.

Progesterone and 17α-hydroxyprogesterone are intermediates in the synthesis of estradiol. During the luteal phase, large amounts of these intermediary steroids are formed by corpus luteum and are not converted to estradiol. The ovaries secrete androgens to a minor extent, compared to the amounts secreted by testes[5].

Transport and metabolism:

Estrogen and progesterone are carried in the blood bound with albumin and with specific steroid-hormone binding globulins. In the liver, estrogens are conjugated with glucuronic acid and sulphate, and are excreted in the bile. Liver also converts the potent estrogens to estradiol. Progesterone is mainly degraded to pregnanediol, and liver is the major site for this conversion[5].

Biological actions of estrogens:

Mechanism of action: The mechanism of action of estrogen is similar to that of testosterone. Estrogens diffuse freely into the target cells and bind with nuclear receptors[5].

Estrogens exert their action on: 1) Reproduction and 2) Growth and metabolism.

1. Reproduction: At puberty estrogen is secreted in large amounts under the influence of pituitary gonadotropins. The estrogens are responsible for all the changes that transform a girl into an adult woman.

Changes in the female reproductive system are:

- Increase in the growth of ovaries, fallopian tubes, uterus and vagina.
- Enlargement of external genetalia to adult size.
- Stratification of vaginal epithelium.
- Initiation and maintenance of menstrual cycle.
- Proliferation of mucosa of fallopian tubes and increase in ciliated epithelial cells.
- Growth and maturation of ovarian follicles.
- Increased motility of uterine tubes.
- Secretion of copious watery cervical mucus.
- Stimulation of sebaceous gland secretion.
- Proliferation of duct system and deposition of fat in the breasts.
- Development of secondary sexual characteristics.

In pregnant women, estrogens bring about the bodily changes, the embedding of blastocyst in the endometrium and development of placenta[5].

2. Growth and metabolism:

a) Bone: Estrogens stimulate osteoblastic activity, activate growth rate of bones and cause closure of epiphyses. The effect on bone growth is more marked in women, and women normally stop growing earlier than men. After menopause, decrease in estrogen level leads to decreased osteoblastic activity, decreased formation of new bone and in severe deficiency can result in osteoporosis.

b) Skin: Estrogens are necessary for development of soft texture of skin, and also increase vascularity of the skin.

c) Protein metabolism: Estrogens are anabolic and produce a slight positive nitrogen balance. The effect is confined to sex organs, bone and few other tissues, unlike the general effects of testosterone

d) Lipid metabolism- Estrogens cause deposition of fat in the subcutaneous tissues, especially in the breast, thighs and gluteal region. Estrogens decrease serum cholesterol and increase serum HDL concentration.

e) Estrogens produce retention of water and sodium by increasing their reabsorption in the renal tubules. The effect is only slight, but may be of importance in pregnancy.

f) Liver- Estrogens stimulate liver to produce many of the hormone binding proteins, and clotting factors[5].

Biological actions of progesterone:

Mechanism of action of progesterone is similar to that of estrogens.

Progesterone exhibits on-1) Reproductive system 2) Metabolism and 3) Body temperature.

1. Reproductive system:

a) Uterus: Progesterone prepares the endometrium for the implantation of the fertilized ovum. In the luteal phase, progesterone activates estrogen sensitized endometrium and changes it into a highly secretory epithelium. It stimulates the secretion of scanty viscous cervical mucous. During pregnancy, it decreases the sensitivity of the myometrium to oxytocin, by decreasing their excitability and spontaneous electrical activity.

b) Breast: Progesterone facilitates development of breasts. It stimulates the growth of lobules and alveoli. The alveolar cells proliferate, enlarge and begin their secretory activity. However, milk secretion occurs only after exposure to prolactin.

3. Body temperature: Progesterone raises the 'set-point' for body temperature. The body temperature increases by 0.5ºC at the time of ovulation, and is the basis for rhythm method of contraception[5].

3.8. MENOPAUSE:

Menopause is defined as the time of cessation of ovarian function resulting in permanent amenorrhoea. It takes 12 months of amenorrhoea to confirm that menopause has set in, and therefore it is a retrospective diagnosis.

Age at Menopause:

Menopause sets in when the follicular number falls below 1000. Menopause normally occurs between the ages of 45 and 50 years, the average being 47 years. "It is not uncommon, however, to see a woman menstruate well beyond the age of 50". This delayed menopause may be related to good nutrition and better health. Late menopause is also common in women suffering from uterine fibroids and those at high risk of endometrial cancer. Menopause setting before the age of 40 is known as premature menopause.

Menopausal age is not related to menarche, race, and socio-economic status, number of pregnancies and lactation, or taking of oral contraceptives. It is however directly associated with smoking and genetic disposition. Smoking induces premature menopause[4].

The clinical diagnosis of menopause is confirmed following stoppage of menstruation (amenorrhoea) for twelve consecutive months without any other pathology. As such, a woman is declared to have attained menopause only retrospectively. Serum follicle-stimulating hormone (FSH) level is found elevated around the period of menopause (45-55 years)[3].

3.9. Premenopause:

According to WHO, **"**Pre menopause defined as the period ambiguously refers to one or two years immediately before the menopause or refer to whole reproductive period prior to menopause [7]".

3.10. Post menopause:

According to WHO, "Post menopause defined as the dating from the final menstrual period regardless of whether menopause of was induced or spontaneous[7]".

3.11 Endocrinology of menopausal transition and menopause:

"Few years prior to menopause, along with depletion of the ovarian follicles, the follicles become resistant to pituitary gonadotropins. As a result, effective folliculogenesis is impaired with diminished estradiol production". There is a significant fall in serum level of estradiol from 50-300 pg/mL before menopause to 10-20 pg/mL after menopause. This decreases the negative feedback effect on hypothalamopituitary axis resulting in increase in FSH. The increase in FSH is also due to diminished inhibin. Inhibin, a peptide secreted by the granulose cells of ovarian follicle. The increase of luteinizing hormone (LH) occurs subsequently.

Disturbed folliculogenesis during this period may result in anovulation, oligoovulation, premature corpus luteum or corpus luteal insufficiency. The sustained level of estrogens may even cause endometrial hyperplasia and clinical manifestation of menstrual abnormalities prior to menopause.

The mean cycle length is significantly shorter. This is due to shortening of follicular phase of the cycle. Luteal phase length remains constant. In late menopausal transition, there is accelerated rate of follicular depletion. Ultimately, no more follicles are available and even some exist, they are resistant to gonadotropins. Estradiol production drops down to the optimal level of 20 pg/mL which leads to no endometrial growth and there is absence of menstruation[3].

The secretions of both FSH and LH are increased due to absent negative feedback effect of estradiol and inhibin or due to enhanced responsiveness of pituitary to gonadotropin-releasing hormone (GnRH). Rise in FSH is about 10-20 fold where as that of LH is about 3-fold. GnRH pulse secretion is increased both in frequency and amplitude.

During menopause, there is fall in the level of prolactin and inhibin lead to increase in the level of FSH from the pituitary. Ultimately, due to physiologic aging, GnRH and both FSH, LH decline along with decline of estrogens[3].

The following risk factors for menopause related diseases are as follows[4]:

- Early menopause, surgical menopause or radiation.
- Chemotherapy especially alkylating agents.
- Smoking, Caffeine, alcohol.
- Family history of menopausal diseases (genetic).
- Drugs related such as GnRH, heparin, corticosteroids and clomiphene(anti estrogen) when given over a prolonged period(over 6 months) can lead to estrogen deficiency.
- Diabetes.

Menopausal symptoms: Pattern of Menstruation prior to menopause:

- Abrupt cessation of menstruation (rare).
- Gradual decrease in both amount and duration. It may be spotting or delayed and ultimately lead to cessation.
- Irregular with or without excessive bleeding. One should exclude genital malignancy prior to declare it as the usual premenopausal pattern[3].

Symptoms of menopausal transition:

During menopausal transition, the changes associated with menstrual cycle are short cycle with irregular bleeding **(Table 6)**. The vasomotor symptoms associated with menopausal transition includes hot flashes, night sweats and sleep disturbances. The Psychological symptoms include mood swings, irritability, poor memory and depression. Sexual function decreases due to vaginal dryness and dyspareunia. There is also dysuria, incontinence, back aches and joint pains[3].

Table 6: Symptoms of Menopausal Transition

SYMPTOMS OF MENOPAUSAL TRANSITION	
A. Menstrual changes	Short cycles Irregular bleeding
B. Vasomotor symptoms	Hot flashes Night sweats Sleep disturbances
C. Psychological	Irritability Mood swings Poor memory Depression
D. Sexual dysfunction	Vaginal dryness Dyspareunia
E. Urinary	Incontinence Urgency Dysuria
F. Others	Back aches Joint aches

Diagnosis of Menopause:

- Cessation of menstruation for consecutive 12 months during climacteric.
- Average age of menopause:50 years.
- Appearance of menopausal symptoms 'hot flash' and 'night sweats'.
- Serum estradiol:<20pg/mL.
- Serum FSH and LH ; > 40mIU/mL (three values at weeks interval required)[3].

3.12. THYROID:

Thyroid disorder is the most common endocrine disorder affecting majority of the population particularly women. "Diseases of thyroid gland are among the most abundant endocrine disorder worldwide second only to diabetes, including in india[8]".

General Physiology:

Thyroid gland is butter fly shaped endocrine gland secretes hormones which regulate the growth and development through metabolism. Thyroid gland has two distinct features as it is the largest endocrine gland in the body weighing about 15-25 gm in adults. The second distinct feature is, it was the first endocrine gland to be recognized on the basis of symptoms associated with excess or deficient symptoms **(Figure 4)**.It consists of two lobes joined by an isthmus and is located on either side of trachea just below the larynx**.**

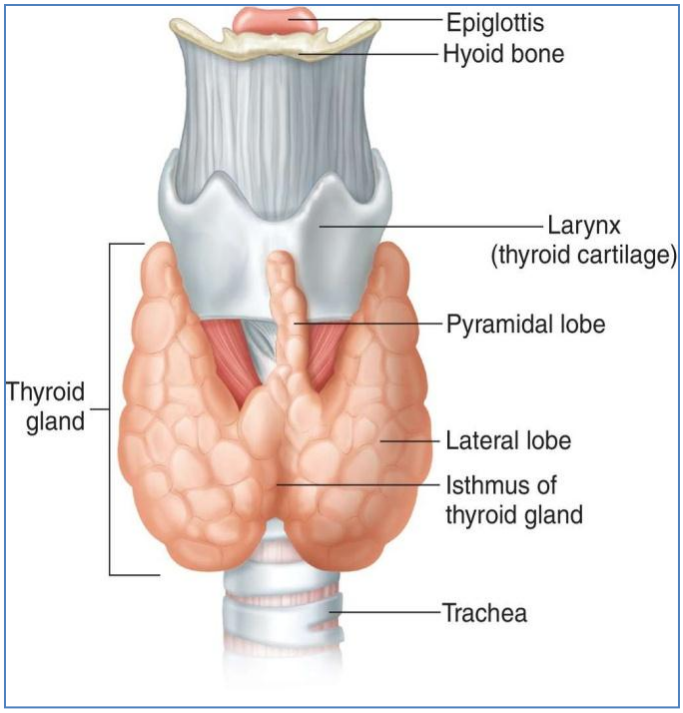


Figure 4:THYRODGLAND

(Source: Sembulingam K, Sembulingam P. Essentials of Medical Physiology, 6th ed, 2012.)

3.12.1. THYROID HORMONES:

Thyroid gland secretes following hormones **(Figure 5).**

1) Thyroxine (T_4): It is also called as Tetraiodothyronine.

- It constitutes about 90% of total secretion of thyroid hormones.

2) Tri-iodothyronine (T_3):

- It constitutes about 9-10% of Total secretion of thyroid hormones.

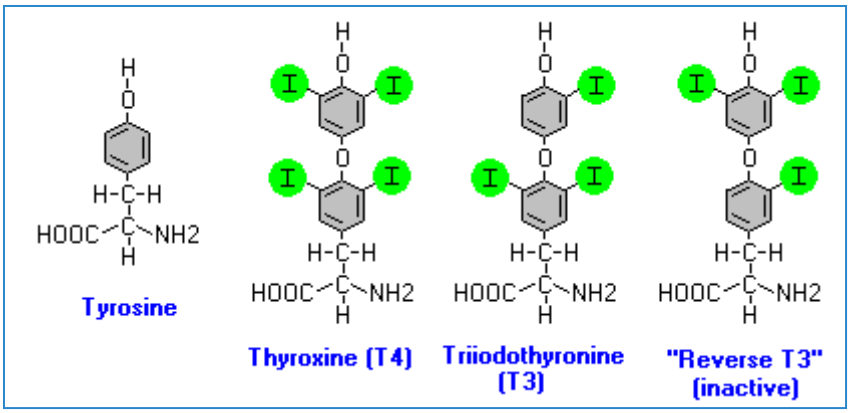


Figure 5: Structure of Thyroid Hormones

(Source:http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/thyroid/chem.html)

3) Reverse T_3 (rT_3): It is not biologically active.

- It constitutes about 1% of total secretion of thyroid hormones.

4) Calcitonin: It is a hormone secreted by parafollicular cells of thyroid gland takes part in calcium homeostasis.

Tyrosine molecules are iodinated to form monoiodotyrosine and di-iodotyrosine which are biologically inactive[1].

3.12.2. Applied aspects: Thyroid disorders can be categorized based on hypo or hypersecretion of thyroid hormones.

Hyperthyroidism: Increased secretion of thyroid hormones (T_3,T_4) refers to hyperthyroidism. The high concentration of thyroid hormones caused by Thyroid stimulating antibodies suppresses the TSH production.

Hypothyroidism: It is caused by low levels or decreased secretion of thyroid hormones. TSH levels elevated in hypothyroidism[1].

Normal values of TSH, T_3 and T_4.

Laboratory assessment of thyroid function tests helps us to diagnose the disorders of thyroid[9]. Every laboratory has its own standard reference values but the most commonly used reference values are shown in the below **Table no 7.**

Table 7 : Normal values of thyroid hormones

Test	Reference range	Hyperthyroidism	Hypothyroidism
TSH	0.4-4.2 mU/L	↓	↑
T_3	0.92-2.78 nmol/L	↑	↓
T_4	58-140 nmol/L	↑	↓

3.12.3.THYROID AND AGING:

Aging is one of the physiological processes associated with many morphological and physiological changes in the systems of the body. The endocrine organs including thyroid undergo essential functional changes with aging.

A well known numerous morphological and physiological changes of thyroid associated with the process of aging[10-12].Thyroid gland increases in its size as age advances although it is not consistent all the times[13]. Some studies revealed that there is increased rate of atrophy of gland and fibrosis, this may be due to underlying autoimmune disease of thyroid in some of the population. Among them, thyroid nodules are most common with aging[14].

Hypothyroidism and aging:

It is very important to monitor and diagnoses the diseases of the thyroid especially in aged people. Thyroid diseases are the most prevalent and frequently affecting aging women[15].

The prevalence of hypothyroidism also varies with so many factors like age, gender, iodine content in the diet, or an underlying prevalence of antithyroid antibodies and it may be overt hypothyroidism(elevated TSH, low T_3) or subclinical hypothyroidism(elevated TSH, normal T_4 levels). The prevalence rate of overt hypothyroidism ranges between 2-10% in the people aged above 60 years and subclinical hypothyroidism is upto 20% in the people aged above 80 years[15,16-18]. These are usually common in women and it may reflect increased rates of autoimmune thyroid disease in the women[19].

Hyperthyrodism and aging:

Age is considered as the most important determining factor which is associated with hyperthyroidism and the mechanism behind it not completely understood. One of the hyperthyroid cases in old age is may be due to multinodular goiter. Though TSH production is fully suppressed, adenomas of the gland produce and secrete excessive thyroid hormones[20]. Hyperthyroidism may be overt hyperthyroidism (low TSH, elevated T_4) or subclinical hyperthyroidism (low TSH, normal T_4). The National Health and Nutrition Examination Survey III (NHANES III) stated that there is increased rate of hyperthyroidism as age advances[18]. Women usually tend to have increased rates of hyperthyroidism than men and which reflects autoimmune disease of thyroid. It becomes less apparent with aging. In some cases toxic nodular goiter and use of medication also become more prevalent. The prevalence rate of hyperthyroidism may vary according to the type of population studied[16].

So, "The process of aging affects both the prevalence and clinical presentation of hypo and hyperthyroidism[21, 22]". As age advances, changes occur in all the system of the body which may be due to the amount of the secretion of the hormones. It may be due to sensitivity of the target organs. Some times change in the metabolic rate as there is increased peripheral degradation of thyroid hormones which might be associated with process of aging[23].

3.12.4. THYROID AND MENSTRUATION:

There is an important relationship exists between thyroid hormones and reproductive hormones. Menstruation is considered as one of the important period of ovarian cycle. It includes a series of many changes in the ovary, uterus and vagina, breast etc[24].

On an average, 28 days of menstrual cycle, some of the women may easily get through their menstrual period within the time with very few or no physiological symptoms, every month. But, most of the women may suffers from menorrhagia or missed periods before or during menstruation. It is associated with the symptoms of physical or emotional disturbances which ultimately lead to mood swings in most of the women life. Thyroid hormones plays prominent role in reproductive function by directly exerting their effect on the ovaries or interacting indirectly with the sex hormone binding proteins. So the dysfunction of thyroid may lead to menstrual irregularities and infertility.

Menstrual Disorders Associated with Thyroid:

In women, reproductive disorders mostly menstrual abnormalities are linked either with hypothyroidism or hyperthyroidism. Thyrotropin–releasing hormone (TRH) is the main hormone which mediates the relationship between menstrual cycle and thyroid hormone levels. It produces direct effect on the ovary and thyroid dysfunction can leads to the alteration of the levels of sex hormone binding globulin, prolactin and gonadotropin-releasing hormone (GnRH). Alteration of these hormones ultimately leads to menstrual abnormalities. So, there is a possibility of raised levels of TRH which may increase prolactin levels and it may contribute to the absence of menstruation associated with Hypothyroidism[25].

Thyroid autoimmunity is one of the important causes of thyroid abnormalities in their reproductive period of women life[26,27]. Studies showed that there is 26% estimated prevalence of pre and post menopausal women suffering from thyroid dysfunction[26]. The Hypothalamo pituitary ovarian axis (HPO) regulates the menstrual cycle. Any interruption in the regulation of this axis may result in menstrual abnormalities[28]. The length of menstrual cycle may vary because of the disruption in the HPO axis[29].

Role of Hypothyroidism and Hyperthyroidism in Menstruation:

Hypothyroidism may be associated with disruption of the functioning of Hypothalamo pituitary ovarian (HPO) axis. So it may lead to infertility, menstrual irregularity, anovulation and sometimes there is a chance of miscarriage of pregnancy[30,31]. Decreased levels of thyroxin associated with ovulatory dysfunction are the main clinical feature of hypothyroidism. "This condition leads to menstrual dysfunction 3 times more than usual prevalence". Most common type of menstrual dysfunction is infrequent menstruation also called as oligomenorrhea[32].

There is insufficiency of corpus luteum found to be interrupting the release of LH and hyperprolactinaemia due to increased levels of TRH release may contribute the negative influences on normal menstrual cycle[33, 34].

In hypothyroidism, despite the fact that increased levels of TSH may decrease the sex hormone binding proteins (SHBP), increasing free testosterone and estradiol level, add up to the serum levels of these hormones are generally low[35]. Also decrease in the metabolic clearance of androstenedione and estrone may also leads to increase in the aromatization of peripheral estrogens[36,37].

These mechanisms along with hemostatic decreasing factors, may also lead to the symptoms such as absence of menstruation, irregular menstruation, infertility in hypothyroidism[38].

Hyperthyroidism is also associated with abnormal pattern of menstruation like hypothyroidism. There is increased production of sex hormone binding protein (SHBP) in hyperthyroid women which ultimately leads to altered metabolism of estrogen and there is increased conversion of androgens to estrogens may occur [39,40]. A part from this, the patients also have altered GnRH-induced LH secretion, which is shown by the increased levels of serum LH and the pulsatile pattern of LH seem to be disturbed, leading to differential changes in the pattern of menstruation[41].

Studies showed that there are 65% of menstrual irregularities in hyperthyroid women compared to controls (17%) [42]. In other studies, it has been observed that, 46 out of 214 women with hyperthyroidism (22%) had menstrual cycle irregularities in which 24 women had hypomenorrhea, 15 women had polymerrhoea,5 oligomenorrhoea, 2 menorrhagia and no one is with amenorheoa[38].

3.12.5. THYROID AND MENOPAUSE:

There is a relationship exists between thyroid hormones and menopause. Thyroid disorders affect women more than men and the prevalence increases with age.

Changes associated with thyroid physiology and function with aging[43]:

- Reduction in the uptake iodine.
- Reduction in the synthesis of free T_3 and T_4 and catabolism of free thyroxine (T_4) and increase of reverse T_3.
- There is no change in TSH with occasionally a tendency to the higher limits.

Hyper and hypothyroid states may lead to many alterations in the steroid metabolism. Mainly during menopausal transition, hyperthyroidism has been associated with decreased free testosterone levels, with decreased estradiol concentrations and with increased sex hormone binding proteins (SHBP). Here FSH concentration may also increases and opposite effects could be observed in hypothyroidism [36, 44-46]. Though thyroid does not influence the menopause directly, in the presence of hypothyroidism or hyperthyroidism, complications of menopausal symptoms may be aggravated like coronary atherosclerosis, osteoporosis[47].

Hypothyroidism and menopause are the two important conditions and both were affected by the female hormone estrogen. Studies have shown that there are decreased levels of estrogen during the menopause and it may also affect the normal functioning of thyroid. Apart from this, thyroid function is also disturbed when there are decreased levels of progesterone and the ratio of estrogen/progesterone levels altered, and this condition is known as estrogen dominance[48].

3.13. DIABETES:

Diabetes mellitus is a clinical condition characterized by increased blood glucose level (hyperglycaemia) due to insufficient or inefficient (incompetent) insulin[49].

3.13.1. Insulin:

Insulin is a polypeptide hormone produced by the β-cells of islets of Langerhans of pancreas. It has profound influence on the metabolism of carbohydrate, fat and protein. Insulin is considered as anabolic hormone, as it promotes the synthesis of glycogen, triacylglycerols and proteins. This hormone has been implicated in the development of diabetes mellitus.

Insulin plays a key role in the regulation of carbohydrate, lipid and protein metabolisms. Insulin exerts anabolic and anticatabolic influences on the body metabolism.

Glucose is the most important stimulus for insulin release. The effect is more predominant when glucose is administered orally (either direct or through a carbohydrate-rich meal). A rise in blood glucose level is a signal for insulin secretion[49].

3.13.2. BLOOD GLUCOSE HOMEOSTASIS:

Regulation of Blood Glucose level:

Glucose is carbohydrate currency of the body. An adult human body contains about 18g free glucose. This amount is just sufficient to meet the basal energy requirements of the body for one hour. The liver has about 100 g stored glycogen. Besides this, it is capable of producing about 125-150 mg glucose/minute or 180-220 g/24 hrs[49].

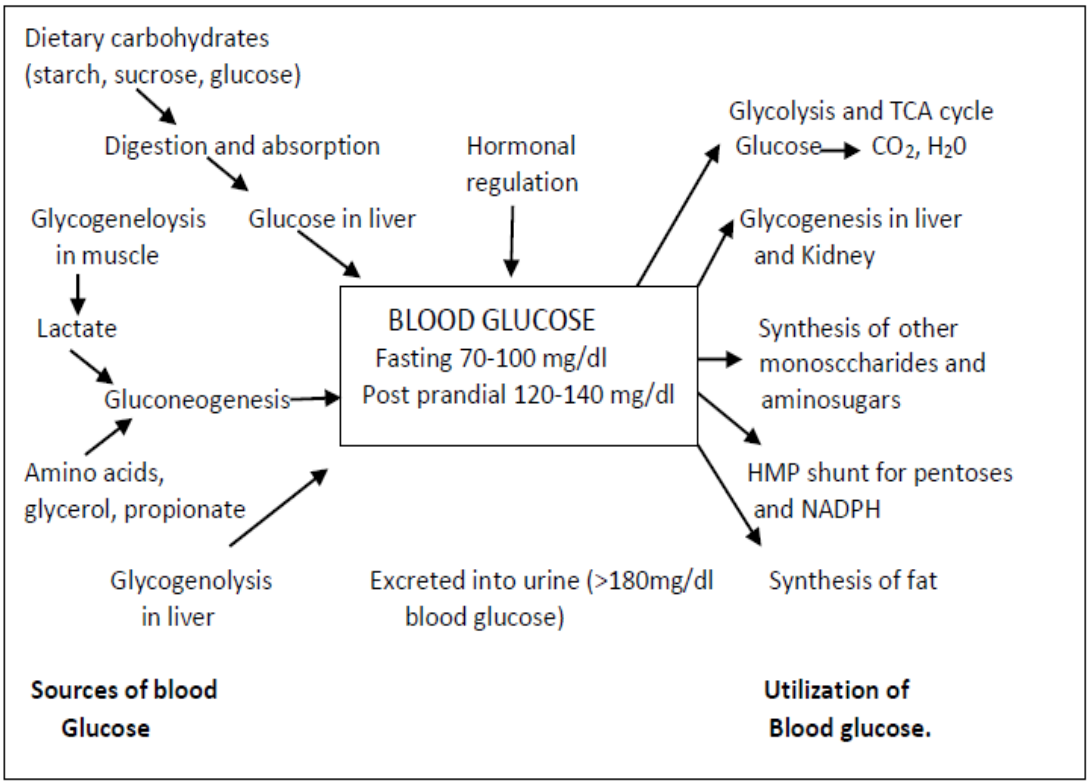


Figure 6: Overview of Blood glucose homeostasis
(Source: Satyanarayana U, Chakrapani U. Biochemistry. 3rd ed, 2006.)

A healthy individual is capable of maintaining the blood glucose concentration within a narrow range. The fasting blood glucose level in a post absorptive state is 70-100 mg/dl (plasma glucose 80-120 mg/dl)**(Figure 6)**. Following the ingestion of a carbohydrate meal, blood glucose may rise to 120-140 mg/dl[49].

Role of hormones in blood glucose homeostasis:

Hormones play a significant role in the regulation of blood glucose concentration. Primarily, insulin lowers blood glucose level (hypoglycemic) while the rest of the hormones oppose the actions of insulin (hyperglycaemia)[49].

Insulin: Insulin is produced by β-cells of the islets of Langerhans in response to hyperglycaemia (elevated blood glucose level). Insulin is basically a hypoglycaemic hormone that lowers in blood glucose level through various means. It is an anti-diabetogenic hormone.

Glucagon: Hypoglycaemia (low blood glucose level) stimulates glucagon production. Glucagon is basically involved in elevating blood glucose concentration. It enhances gluconeogenesis and glycogenolysis.

Epinephrine: It acts both on muscle and liver to bring about glycogenolysis by increasing phosphorylase activity. The end product is glucose in liver and lactate in muscle. The net outcome is that epinephrine increases blood glucose level.

Thyroxine: It elevates blood glucose level by stimulating hepatic glycogenolysis and gluconeogenesis.

Glucocorticoids: Glucocorticoids stimulate protein metabolism and increase gluconeogenesis. The glucose utilization by extrahepatic tissues is inhibited by glucocorticoids. The overall effect of glucocorticoids is to elevate blood glucose concentration.

Growth hormone and adreno corticotropic hormone (ACTH. The uptake of glucose by certain tissues (muscle, adipose tissue etc.) is decreased by growth hormone. ACTH decreases glucose utilization. The net effect of both these hormones is hyperglycemic[49].

Diagnostic criteria for diabetes:

World health organization (WHO) recommended the diagnostic criteria for diabetes. The following **Table 8** summarizes the diagnostic criteria and reference range for diabetes.

Table 8: WHO diagnostic criteria for diabetes[50, 51]

Fasting plasma glucose	---- ≥7.01mmol/l (126mg/dl)
2-h plasma glucose	---- ≥ 7.8 m mol/1(140mg/dl) ---- ≥11.01mmol/l (200 mg/dl)
HbA1c	---- ≥ 6.5%

3.13.3. DIABETES AND AGING:

Aging is considered as one of the important determinant risk factors of the metabolic disorders like diabetes, obesity etc. studies showed that prevalence of diabetes increases with age and it attains maximum peak at an older age[52-54]. Diabetes may appear at any age but it prevalence dramatically increases with age in older populations. It is estimated that persons aged 65yr olds have 16.5% and the persons aged 85 yr olds have 26% of diabetes reported by the National commission on diabetes[55]. The possible mechanisms why the incidence of diabetes increases with age is not clear. It's been stated that one of the reason might be impairment of glucose tolerance which includes poor nutrition, physical inactivity, decreased lean body mass, reduced insulin secretion and insulin resistance[56].

Pathogenesis of diabetes in aging.

It is characterized mainly by two factors[57]**.**

1) Peripheral insulin resistance.
2) Impaired insulin secretion from β cell.

Aging and Insulin secretion:

Studies showed that defects in the secretion of insulin due to age related factors have been demonstrated in rodents as well as humans. The major stimuli for the release of insulin from the pancreatic β cell. There is reduced secretion of insulin with increase in aging which stimulates glucose and at the same time aminoacid arginine[58].

In old age, there is an increased need for insulin due to superimposition of secretary defects of insulin which ultimately leads to intolerance of glucose, impaired glucose homeostasis and finally diabetes. Factors decreasing secretion of insulin which includes reduced mitochondrial functional activity and increased oxidative stress associated with aging[59].

Studies well documented that aging is associated with decreased activity of insulin and it is assumed to be in elderly people that may contributes to high prevalence of glucose intolerance and diabetes[60-64]. But some other studies showing contradictory results like decrease in the action of insulin in elderly people are due to increase in the abdominal fat rather than aging[65]. However the mechanism is not clear. So glucose homeostasis is a complex phenomenon which is regulated by the insulin

actions and may show defect in the secretions of insulin from β cells affects with aging.

3.13.4. DIABETES AND MENSTRUATION:

Menstrual cycle is common physiological phenomenon and it is affected by diabetes in many cases. Menstrual cycle irregularities are more commonly associated with systemic diseases and particularly diabetes in women frequently associated with irregular menstrual cycles[66].

Onset of diabetes is one of the major important determinant factors which initiate menstrual irregularities and at least 50% of these patients can be affected[67].

Possible mechanisms have been elucidated to know the link between menstrual irregularities and diabetes. It is assumed that obesity takes a major role with enhanced peripheral adipose tissue conversion of androgens to estrogens[68].

Women suffering from diabetes may also experience a rise in their blood glucose concentration a week prior to their menstrual cycle, just after the ovulation. Once the initiation of menstruation starts, her blood glucose levels will tend to fall. This blood glucose fluctuations might be due to rise in estrogen and progesterone levels which may interfere with the metabolism of insulin sesntivity[69].

Women suffering from diabetes mellitus may prone to early menopause and short span of reproductive life etc. It might be due to deregulation of hypothalamo-hyppituitary axis that may lead to delayed menarche, menstrual abnormlities [70,71].

3.13.5. DIABETES AND MENOPAUSE:

Menopause is an important transition period which represents the end of reproductive period. Disturbances in the metabolism of carbohydrates and lipids which may lead to the features of metabolic syndrome which includes diabetes, hypertension, osteoporosis etc. Like other disorders of metabolism, diabetes also effects women in their midlife and mainly affects their menopausal life and the transition of menopause[72].

Women with type 1 diabetes may have a chance of early menopause transition.There might be some autoimmune mechanisms which may be associated with menopausal age, because type 1 diabetic females may lead to ovarian dysfunction which is caused by the glycosylation of proteins and it ultimately leads to decrease in the production of endogenous estrogens[73].

Women with type II diabetes (insulin resistant diabetes mellitus) is the most common form of diabetes. Women in the menopausal age more likely tend to diagnose with type II diabetes, due to reduced levels of estrogen and progesterone hormones and human growth hormone which may contribute to decreased metabolism, which may produce obesity and all these complications together may prone for type II diabetes[74]**.** So menopause is the important factor which influences diabetes.

REFERENCES:

1. Khurana I. Textbook of Medical Physiology. 5th ed. Gurgaon(Haryana):Reed Elsevier India(P) Ltd; 2012.
2. Sembulingam K, Sembulingam P. Essentials of Medical Physiology. 6th ed. New Delhi: JP medical(P) Ltd; 2012.
3. Konar H. DC Dutta's textbook of gynecology. 7th ed. New Delhi: JP Medical(P) Ltd;2016.
4. Padubidri VG, Daftary SN. Hawkins & Bourne Shaw's Textbook of Gynaecology.16th ed. Gurgaon(Haryana): RELX India(P) Ltd; 2016.
5. Suresh R. Essentials of Human Physiology. 1st ed. Kolkata: Books and allied(P) Ltd; 2012.
6. Venkatesh D, Sudhakar H.H. Basics of medical physiology. 2nd ed. New Delhi: Wolters Kluwer(P) Ltd; 2010.
7. Research on the menopause in the 1990s: report of a WHO scientific group. World Health Organ Tech Rep Ser 1996; 866:1-107.
8. Nimmy NJ, Aneesh PM, Narmadha MP, Udupi RH, Binu KM. A survey on the prevalence of thyroid disorder induced by demography and food habits in South Indian population. IJOPP 2012;5(2).
9. Babu MS, Shivaswamy RP. Laboratory evaluation of thyroid function. International Journal of Health & Allied Sciences 2016;5(2):69.
10. Lewinski A, Sewerynek E, Karbownik M. Aging processes and the thyroid gland. Aging and Age Related Diseases: The Basics. New York: Nova Science Publishers Inc 2006:131-72.
11. Faggiano A, Del MP, Marciello F, Marotta V, Ramundo V, Colao A. Thyroid diseases in elderly. Minerva endocrinologica 2011;36(3):211-31.
12. Papaleontiou M, Haymart MR. Approach to and treatment of thyroid disorders in the elderly. Medical Clinics 2012 ;96(2):297-310.
13. Hassani S, Hershman JM. Thyroid diseases. Principles of Geriartric Medicine and Gerentology, 6th ed. 1999;4:973-89.
14. Mazzaferri EL. Management of a solitary thyroid nodule. N Engl J Med 1993 ;328(8):553-9.

15. Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol 1977;7(6):481-93.
16. Samuels MH. Subclinical thyroid disease in the elderly. Thyroid 1998; 8(9):803-13.
17. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. Arch Intern *Med* 2000;160(4):526-34.
18. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002;87(2):489-99.
19. Vanderpump MP, Tunbrldge WM, French J, Appleton D, Bates D, Clark F, Evans JG, Hasan DM, Rodgers H, Tunbridge F, Young ET. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol 1995;43(1):55-68.
20. Diez JJ. Hyperthyroidism in patients older than 55 years: an analysis of the etiology and management. Gerontology 2003;49(5):316-23.
21. Cooper DS. Thyroid disease in the oldest old: the exception to the rule. Jama 2004 ;292(21):2651-4.
22. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, Franklyn JA, Hershman JM, Burman KD, Denke MA, Gorman C. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. Jama 2004 ;291(2):228-38.
23. Mariotti S, Franceschi C, Cossarizza A, Pinchera A. The aging thyroid. Endocr Rev 1995;16(6):686-715.
24. Mohan S, Page LM, Higham, JM. Diagnosis of abnormal uterine bleeding. Best Pract Res Clin Obstet Gynaecol 2007**;** 18:73-74.
25. Stern L. Thyroid dysfunction and the menstrual cycle. Clinical Advisor Forum. 2009.
26. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition

Examination Survey (NHANES III). J Clin Endocrinol Metab 2002;87(2):489-99.

27. Vanderpump MP, Tunbrldge WM, French J, Appleton D, Bates D, Clark F, Evans JG, Hasan DM, Rodgers H, Tunbridge F, Young ET. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey.Clin Endocrinol 1995; 43(1):55-68.
28. Popat VB, Prodanov T, Calis KA, Nelson LM. The menstrual cycle: a biological marker of general health in adolescents. Ann N Y Acad Sci 2008; 1135:43-51.
29. Braunstein GD. The Endocrine Society Clinical Practice Guideline and The North American Menopause Society position statement on androgen therapy in women: another one of Yogi's forks. J Clin Endocrinol & Metab 2007 ;92 (11):4091-3.
30. Poppe K, Glinoer D. Thyroid autoimmunity and hypothyroidism before and during pregnancy. Hum Reprod update 2003;9(2):149-61.
31. Gerhard I, Becker T, Eggert-Kruse W, Klinga K, Runnebaum B. Thyroid and ovarian function in infertile women. Hum Reprod 1991;6(3):338-45.
32. Krassas GE, Pontikides N, Kaltsas TH, Papadopoulou P, Paunkovic J, Paunkovic N, H Duntas L. Disturbances of menstruation in hypothyroidism. Clin Endocrinol 1999;50(5):655-9.
33. Poppe K, Glinoer D, Tournaye H, Schiettecatte J, Devroey P, Van Steirteghem A, Haentjens P, Velkeniers B. Impact of ovarian hyperstimulation on thyroid function in women with and without thyroid autoimmunity. J Clin Endocrinol & Metab 2004;89(8):3808-12.
34. Negro R, Formoso G, Mangieri T, Pezzarossa A, Dazzi D, Hassan H. Levothyroxine treatment in euthyroid pregnant women with autoimmune thyroid disease: effects on obstetrical complications. J Clin Endocrinol & Metab 2006 ;91(7):2587-91.
35. Gordon GG, Southren AL. Thyroid-hormone effects on steroid-hormone metabolism. Bull N Y Acad Med 1977;53(3):241-59.
36. Longcope C, Abend S, Braverman LE, Emerson CH. Androstenedione and estrone dynamics in hypothyroid women. J Clin Endocrinol & Metab 1990 ;70(4):903-7.

37. Gallagher TF, Fukushima DK, Noguchi SH, Fishman JA, Bradlow HL, Cassouto JO, Zumoff BA, Hellman LE. Recent studies in steroid hormone metabolism in man. Recent Prog Horm Res 1966; 22:283-303.
38. Krassas GE, Pontikides N, Kaltsas TH, Papadopoulou P, Batrinos M. Menstrual disturbances in thyrotoxicosis. Clin Endocrinol 1994;40(5):641-4.
39. Southren AL, Olivo J, Gordon GG, Vittek J, Brener J, Rafii F. The conversion of androgens to estrogens in hyperthyroidism. J Clin Endocrinol & Metab.1974 ;38(2):207-214.
40. Krassas GE, Pontikides N. Gonadal effect of radiation from ^{131}I in male patients with thyroid carcinoma. Arch Androl 2005; 51(3):171-5.
41. Akande EO. The effect of estrogen on plasma levels of luteinizing hormone in euthyroid and thyrotoxic postmenopausal women. BJOG: An International Journal of Obstetrics & Gynaecology 1974;81(10):795-803.
42. Joshi JV, Bhandarkar SD, Chadha M, Balaiah D, Shah R. Menstrual irregularities and lactation failure may precede thyroid dysfunction or goitre. J Postgrad Med. 1993 ; 39(3):137.
43. Mariotti S, Franceschi C, Cossarizza A, Pinchera A. The aging thyroid. Endocr Rev 1995; 16(6):686-715.
44. Koutras DA. Disturbances of menstruation in thyroid disease. Ann N Y Acad Sci 1997 ;816(1):280-4.
45. Brenta G, Schnitman M, Gurfinkiel M, Damilano S, Pierini A, Sinay I, Pisarev MA. Variations of sex hormone-binding globulin in thyroid dysfunction. Thyroid 1999 ;9(3):273-7.
46. Ford HC, Cooke RR, Kelghtley EA, Feek CM. Serum levels of free and bound testosterone in hyperthyroidism. Clin Endocrinol 1992;36(2):187-92.
47. Del Ghianda S, Tonacchera M, Vitti P. Thyroid and menopause. Climacteric. 2014;17(3):225-34.
48. Cutolo M, Capellino S, Sulli A, Serioli B, Secchi ME, Villaggio B, Straub RH. Estrogens and autoimmune diseases. Ann N Y Acad Sci 2006;1089(1):538-47.
49. Satyanarayana U, Chakrapani U. Biochemistry. 3rd ed. Kolkata:Books and allied (P) Ltd;2006.
50. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. World Health Org;2006.

51. World Health Organization. Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. World Health Org; 2011.
52. Gunasekaran U, Gannon M. Type 2 diabetes and the aging pancreatic beta cell. Aging (Albany NY) 2011;3(6):565.
53. Iozzo P, Beck-Nielsen H, Laakso M, Smith U, Yki-Järvinen H, Ferrannini E, European Group for the Study of Insulin Resistance. Independent influence of age on basal insulin secretion in nondiabetic humans. J Clin Endocrinol & Metab 1999 ;84(3):863-8.
54. Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelgau MM, Saydah SH, Williams DE, Geiss LS, Gregg EW. Prevalence of diabetes and impaired fasting glucose in adults in the US population: National Health And Nutrition Examination Survey 1999–2002. Diabetes care 2006;29(6):1263-8.
55. Bennett PH. Report of work group on epidemiology. National Commission of Diabetes 1976; 3(part 2):65-135.
56. Davidson MB. The effect of aging on carbohydrate metabolism: a review of the English literature and a practical approach to the diagnosis of diabetes mellitus in the elderly. Metab Clin Exp 1979 ; 28(6):688-705.
57. Kadowaki T. Insights into insulin resistance and type 2 diabetes from knockout mouse models. J Clin Invest 2000;106(4):459-65.
58. Chen M, Bergman RN, Pacini G, Porte Jr D. Pathogenesis of age-related glucose intolerance in man: insulin resistance and decreased β-cell function. J Clin Endocrinol & Metab 1985;60(1):13-20.
59. Cooksey RC, Jouihan HA, Ajioka RS, Hazel MW, Jones DL, Kushner JP, McClain DA. Oxidative stress, β-cell apoptosis, and decreased insulin secretory capacity in mouse models of hemochromatosis. Endocrinology 2004 ;145(11):5305-12.
60. Chen M, Bergman RN, Porte Jr D. Insulin resistance and β-cell dysfunction in aging: the importance of dietary carbohydrate. J Clin Endocrinol & Metab 1988 ;67(5):951-7.
61. DeFronzo RA. Glucose intolerance and aging. Diabetes care 1981;4(4):493-501.

62. Rowe JW, Minaker KL, Pallotta JA, Flier JS. Characterization of the insulin resistance of aging. J Clin Invest 1983 ;71(6):1581-7.
63. Harris MI, Hadden WC, Knowler WC, Bennett PH. Prevalence of diabetes and impaired glucose tolerance and plasma glucose levels in US population aged 20–74 yr. Diabetes 1987;36(4):523-34.
64. Yki-Järvinen H. Role of insulin resistance in the pathogenesis of NIDDM. Diabetologia 1995;38(12):1378-88.
65. Bryhni B, Jenssen TG, Olafsen K, Eikrem JH. Age or waist as determinant of insulin action? Metab Clin Exp 2003;52(7):850-7.
66. Edelman SV. Type II diabetes mellitus. Adv Intern Med 1998; 43:449-500.
67. Djursing H, Nyholm HC, Hagen C, Carstensen L, Pedersen LM. Clinical and hormonal characteristics in women with anovulation and insulin-treated diabetes mellitus. Am J Obstet Gynecol 1982 ;143(8):876-82.
68. Longcope C, Baker R, Johnston CC. Androgen and estrogen metabolism: relationship to obesity. Metab Clin Exp 1986;35(3):235-7.
69. Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Müller-Wieland D, Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. Science 2000 ; 289(5487):2122-5.
70. Dorman JS, Steenkiste AR, Foley TP, Strotmeyer ES, Burke JP, Kuller LH, Kwoh CK. Menopause in type 1 diabetic women: is it premature? Diabetes 2001 ;50(8):1857-62.
71. Griffin ML, South SA, Yankov VI, Booth RA, Asplin CM, Veldhuis JD, Evans WS. Insulin-dependent diabetes mellitus and menstrual dysfunction. Ann Med 1994 ;26(5):331-40.
72. Narayan KV, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. Jama 2003;290(14):1884-90.
73. Kritz-Silverstein D, Barrett-Connor E, Wingard DL. Hysterectomy, oophorectomy, and heart disease risk factors in older women. Am J Public Health 1997; 87(4):676-80.
74. Lizcano F, Guzmán G. Estrogen deficiency and the origin of obesity during menopause. Biomed Res In 2014; 2014: 757461.

CHAPTER 4

MATERIALS AND METHODS

4.0. MATERIALS AND METHODS.

4.1. Study design: Human cross sectional study.

4.2. Sample size: 200 subjects.

4.2.1. Sample size calculation:

With 95% of confidence interval, expected prevalence of Diabetes Mellitus having thyroid disorders among women as 30% and desired precision as ± 13%. The minimum sample size is 48 per Group, rounded to 50 per group.

n=48 ~100 per group(rounded to 50 per group)

So now total sample size in each group= 4x 50=200

The formula used here to calculate the sample size is following.

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where n= sample size.

Z= 1.96 at 95% confidence level.

P= Expected prevalence (30%).

d= Precision.

The entire sample (200) is divided into 4 groups.

Table 9: classification of groups

Group I(n=50)	Pre menopause	Non-Diabetic	25-45 Yrs
Group II(n=50)	Pre menopause	Diabetic	25-45 Yrs
Group III(n=50)	Post menopause	Non-Diabetic	46-65 Yrs
Group IV(n=50)	Post menopause	Diabetic	46-65 Yrs

4.3. INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria:

- Pre menopausal women (with regular menstruation every month without any history of menstrual disorders).
- Post menopausal women (women not menstruating for a period of 12 consecutive months without any abnormality).
- Non- smokers.
- Non- alcoholics.

Exclusion criteria:

- Evidence of Hypertension.
- Hysterectomy.
- Pregnant women.
- On medications (diabetic, thyroid and hormonal replacement therapy).

4.4. ETHICS

Informed consent:

Informed consent form was obtained from the participants.

Institutional approval:

Source of data:

The source of study from the subjects, who were attending the outpatient clinic and OBG departments, Shri B. M. Patil Medical College, Hospital and Research center, Vijayapur.

An institutional ethical clearance was obtained from the institutional ethical committee of Shri B.M. Patil Medical College, Hospital and Research center, BLDE (Deemed to be university) on April 10th, 2015.

Declaration of Helsinki

We followed declaration of Helsinki during the entire study.

4.5. STUDY PROTOCOL:

All the study participants were interviewed using a stuctured questionare format that is specific to our study. Participants were asked to provide their relevant biodata like history of diabetes, history of menopause and the details about smoking, alcoholic etc were also collected.

Further we have collected the information of physical anthropometric, physiological parameters.

Later we collected the fasting blood samples and analyzed for further tests which includes Hematological profile, Blood glucose profile, Thyroid hormonal assay and Reproductive hormonal assays.

4.6. Physical Anthropometry parameters:

a. **Height (cm):** Height was measured with the subject in standing position without foot wear nearest to 0.1 cms.
b. **Weight (kg):** weight was measured using a standard weighing machine, with a minimum of clothing nearest to 0.1 kgs.
c. **Body surface Area (square meters):** Body surface area was calculated in each subject by using Duboi's Nomogram.
d. **Body Mass Index (kilogram/meter^2):** Body Mass Index was calculated from weight and height by dividing weight in kilogram by height in meter square and the formula was expressed as Kg/m^2.
e. **Waist Hip Ratio (cm):** It was calculated by measuring waist at narrowest point under lowest rib and hips at the widest portion of buttocks using a tape and the ratio was calculated by dividing waist circumference by hip circumference and expressed in cm.

4.7. Physiological Parameters:

a. **Systolic and Diastolic Blood Pressure (mmHg):** Blood pressure was measured by using Diamond mercury sphygmomanometer in sitting posture in each subject and it was expressed in mmhg.
b. **Pulse Pressure (PP) (mmHg):** It is the pressure difference between Systolic and Diastolic Blood Pressure and is expressed in mmHg.
c. **Mean arterial Pressure (mmHg):** It is the pressure existing in the arteries. It was calculated by the formula= DBP+1/3rd of PP and is expressed in mmHg.
d. **Pulse rate (bpm):** It was calculated by pulsating radial artery and was expressed as beats per minute (bpm).

Sample collection:

Blood samples were collected from the subjects at the morning hours after overnight fasting in the test tubes containing EDTA in which sodium fluoride act as an anticoagulant for the estimation of completed blood picture count (CBC) and Glycosylated haemoglobin (HbA1c) where as plain test tubes were used for the remaining investigations.

4.8. Haematological Parameters:

In haematological parameters we have estimated the complete blood picture count(CBC).

For complete blood picture count (CBP), about 1 ml of blood was collected in commercial tubes containing about 40 µl potassium EDTA as anticoagulant and the blood cell count was analyzed within 24 hrs by automated cell counter by (SYSMEX XN-1000).

4.9. Blood Glucose profile:

4.9.1. Estimation of Fasting blood sugar (FBS): Fasting blood sugar level was analyzed by using GOD-PAP method. (Trinder P., 1969)[1]. (Agappe Dianostics).

Principle:

Glucose is oxidized to gluconic acid and hydrogen peroxidase in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of colour formed is directly proportional to the amount of glucose present in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\textbf{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2.$$

$$2H_2O_2 + 4\text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\textbf{Peroxidase}} \text{Red quinoneimine dye} + 4H_2O.$$

Table 10 : Reagents for Fasting blood glucose

Reagents	**concentration**
Phosphate buffer(pH 7.40)	100 mmol/l
Phenol	10 mmol/l
Glucose Oxidase	>1000 U/L
peroxidase	>600 U/L
4- Aminoantipyrine	270mmol/l

Procedure

Working reagent	1000µl	1000µl	1000µl
Glucose	-	10µl	
Sample	-	-	10µl

The standard and sample mixed with reagent respectively and incubated at 37 ºC for ten minutes. The change in the absorbance was measured at 505/630 nm against reagent blank.

The program was set in the instrument as follows

Mode of Reaction	End Point
Slope of reaction	Increasing
Wavelength	505/630
Temperature	37ºC
Standard concentration	100.0 mg/dl
Blank	Reagent Blank
Linearity	500.mg/dl
Incubation time	10 min
Sample volume	10 µl
Reagent Volume	1000 µl
Cuvette	1 cm light path

The calculation:

$$\text{Glucose conc(mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

4.9.2. Estimation of Glycosylated Haemoglobin (HbA1c) (Processed on Roche C311 Autonalyzer)

"Tina Quant" method (Turbidimetric Inhibition Immunoassay).(Chang J. et al 1998)[2].

Principle and Method:

This method uses Tetradecyltrimethylammoniumbromide(TTAB) as the detergent in the hemolyzing reagent to eliminate interference from leucocytes(TTAB does not lyse leucocytes). Sample pre treatment to remove labile HbA1c is not necessary.

All haemoglobin which are glycated at the β chain N-terminus and which have antibody-recognizable regions identical to that HbA1c are determined by this assay.

The concentration of A**1**c (HbA1c) is measured as a percentage of total haemoglobin in the whole blood of human (% HbA1c). HbA1c and haemoglobin levels in the sample are determined from the obtained hemolysate by two independent reactions.

During first stage of reaction in the sample reacts with the anti-HbA1c specific antibody (Reagent A1) to form soluble antigen-antibody complexes. Then the polyhapten is added. (Reagent A2). The polyhapten reacts with the specific antibody excess from the first reaction, producing insoluble immune complexes which can be measured turbidimetrically at 340 nm.

Reagents:

Reagent A1: Monospecific antibodies anti-HbA1c in pH 6.2 buffer.

Reagent A2: Polyhapten-HbA1c in pH 6.2 buff

The programme was set in the instrument as follow

Reaction type	End point
Wave length	340 nm
Temperature	37ºC
Sample volume	10µl
Reagent A1 volume	250 µl
Reagent A2 volume	50 µl
Reagent A1 incubation	300 seconds
Reagent A2 incubation	300 seconds

Calculation:

% HbA1c = 91.5 x HbA1c (g/dL)/Hb(gm/dl) + 2.15

4.10. Estimation of Thyroid Hormonal Assay (TSH, T_3, T_4): ELFA (Enzyme Linked Fluoroscent Assay)[3].

4.10.1. Estimation of Thyroid Stimulating Hormone (TSH):

Principle:

Thyroid Hormone Profile was analyzed by One-Step Enzyme immune sand wich method (TSH) with final fluorescent detection (ELFA) by mini-VIDAS.

- Solid Phase Receptacle (SPR) functions as solid phase as well as the pipetting device for the assay. Now the reagents are ready to use and pre dispensed in the sealed reagents.
- As the entire assay steps are performed automatically by the instrument, the reaction medium is cycled in and out of the SPR several times.
- Transferring the samples into the well containing anti TSH antibody labeled with alkaline phosphatase(conjugate) and now this mixture is cycled in and

out of the SPR. So that the antigen binds to antibody coated on the SPR and to the conjugate forming a "sandwich".

- Unbound components are eliminated during washing.
- During the final detection step, the substrate (4-Methyl-umbelliferyl Phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm.
- The intensity of the fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to calibration curve which was stored in the memory, and then printed out.

REAGENTS:

The SPR (Solid Phase Receptacle):

- The interior of the SPR is coated during production with anti-TSH immunoglobulins(mouse).
- Each SPR is identified by the TSH code. Only required number of SPRs will be removed from the pouch and carefully should be resealed after opening.

The strip:

- The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date.
- The foil of the first well is perforated to facilitate the introduction of the sample.
- The last well of each strip is a cuvette in which the fluorometric reading is performed.
- The wells in the center section of the strip contain the various reagents required for the assay.

Table 11: Reagents and Description of the TSH strip

Wells	Reagents
1	Sample well.
2-3-4-5	Empty wells.
6	Conjugate: alkaline phosphastase–labeled monoclonal anti-TSH immunoglobulins(mouse)+1g/l sodium azide (400µl).
7-8	Wash buffer: sodium phosphate (0.01 mol/l) pH 7.4+1g/l sodium azide(600µl).
9	Wash buffer: diethanalomine(1.1mol/l or 11.5%, pH 9.8) + 1g/l sodium azide(600µl).
10	Cuvette with substrate: 4-methyl-ombellyferyl phosphate (0.6mmol) + diethanolamine (DEA) (0.62mol/l or 6.6%, pH 9.2) +1g/l sodium azide(300µl).

Procedure:

1. Only required reagents removed from the refrigerator and allowed them to come to room temperature for at least 30 minutes.
2. One "TSH" strip and one "TSH" SPR used for each sample, control or calibrator to be tested. Make sure that the storage pouch has been carefully resealed after the required solid phase receptacles have been removed.
3. The test is identified by the "TSH" code on the instrument. The calibrator must be identified by "S1", and tested in duplicate. If the control needs to be tested, it should be identified by "C1".
4. If necessary, clarify samples by centrifugation.
5. Mixed the calibrator, control and samples using a vortex type mixer (for serum or plasma separated from the pellet).
6. For this test, the calibrator, control, and sample test portion is 200µl.

7. Inserted the "TSH" solid phase receptacles (SPRs) and "TSH" strips into the instrument. Check to make sure the colour labels with the assay code on the SPRs and the reagent strips should be matched.
8. All the assay steps performed automatically by the instrument as initiated and directed in the user's manual.
9. Reclosed the vials and return them to the required temperature after pipetting.
10. The assay will be completed within approximately 40 minutes. After the assay is completed, removed the SPRs and strips from the instrument.
11. Disposed the used SPRs and reagent strips in an appropriated recipient.

Results and Interpretation:

Once the assay is completed, results were analyzed by the computer automatically. Fluorescence is measured twice in the reagent strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluoroscence Value) is calculated by substracting the background reading from the final result. This calculation appears on the result sheet. The results are automatically calculated by the instrument using calibration curves which are stored by the instrument.

4.10.2. Estimation of Tri-iodothyronine (T_3):

Principle:

Triiodothyronine (T_3) was analyzed by enzyme immuno assay competition method with final fluorescent detection (ELFA) by mini-VIDAS.

- Solid Phase Receptacle (SPR) functions as solid phase as well as the pipetting device for the assay. Now the reagents are ready to use and pre dispensed in the sealed reagents.
- As the entire assay steps are performed automatically by the instrument, the reaction medium is cycled in and out of the SPR several times.
- Transferring the samples into the well containing T3 antigen labeled with alkaline phosphatase(conjugate) .

- Competition occurs between the antigen present in the sample and the labeled antigen for the specific anti-T_3 antibodies (sheep) coated on the interior of the SPR.
- Unbound components are eliminated during washing.
- During the final detection step, the substrate (4-Methyl-umbelliferyl Phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm.
- The intensity of the fluorescence is inversely proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to calibration curve which was stored in the memory, and then printed out.

REAGENTS:

The SPR (Solid Phase Receptacle):

- The interior of the SPR is coated during production with anti-T_3 monoclonal antibodies (sheep).
- Each SPR is identified by the T_3 code. Only required number of SPRs will be removed from the pouch and carefully should be resealed after opening.

The strip:

- The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date.
- The foil of the first well is perforated to facilitate the introduction of the sample.
- The last well of each strip is a cuvette in which the fluorometric reading is performed.
- The wells in the center section of the strip contain the various reagents required for the assay.

Table 12: Reagents and description of the T_3 strip

Wells	Reagents
1	Sample well
2-3-4-5	Empty wells
6	Conjugate: alkaline phosphastase labeled T_3 derivative +ANS(0.95 mmol/l)+ sodium salicylate(11.9 mmol/l) +1g/l sodium azide(400µl)
7-8-9	Wash buffer: Tris-Tween-Nacl(1 mmol/l) pH 7.4 +1g/l of sodiumazide(600µl).
10	Cuvette with substrate: 4-methyl-ombellyferyl phosphate(0.6mmol)+ diethanolamine(DEA)(0.62mol/l or 6.6%, pH 9.2)+1g/l sodium azide(300µl)

Procedure:

1. Only required reagents removed from the refrigerator and allowed them to come to room temperature for at least 30 minutes.
2. One "T_3" strip and one "T_3" SPR used for each sample, control or calibrator to be tested. Make sure that the storage pouch has been carefully resealed after the required solid phase receptacles have been removed.
3. The test is identified by the "T_3" code on the instrument. The calibrator must be identified by "S1", and tested in duplicate. If the control needs to be tested, it should be identified by "C1".
4. If necessary, clarify samples by centrifugation.
5. Mixed the calibrator, control and samples using a vortex type mixer(for serum or plasma separated from the pellet)
6. For this test, the calibrator, control, and sample test portion is 100µl.
7. Inserted the "T_3" solid phase receptacles (SPRs) and "T_3" strips into the instrument. Check to make sure the colour labels with the assay code on the SPRs and the reagent strips should be matched.
8. All the assay steps performed automatically by the instrument as initiated and directed in the user's manual.

9. Reclosed the vials and returned them 2-8ºC after pipetting.
10. The assay will be completed within approximately 40 minutes. After the assay is completed, removed the SPRs and strips from the instrument.
11. Disposed the used SPRs and reagent strips in an appropriated recipient.

Results and Interpretation:

Once the assay is completed, results were analyzed by the computer automatically. Fluorescence is measured twice in the reagent strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluoroscence Value) is calculated by substracting the background reading from the final result. This calculation appears on the result sheet. The results are automatically calculated by the instrument using calibration curves which are stored by the instrument.

4.10.3. Estimation of Thyroxine or Tetraidothyronine (T_4):

Principle:

Triiodothyronine (T_4) was analyzed by enzyme immuno assay competition method with final fluorescent detection (ELFA) by mini-VIDAS.

- Solid Phase Receptacle (SPR) functions as solid phase as well as the pipetting device for the assay. Now the reagents are ready to use and pre dispensed in the sealed reagents.
- As the entire assay steps are performed automatically by the instrument, the reaction medium is cycled in and out of the SPR several times.
- Transferring the samples into the well containing T_4 antigen labeled with alkaline phosphatase(conjugate) .
- Competition occurs between the antigen present in the sample and the labeled antigen for the specific anti-T_4 antibodies(sheep) coated on the interior of the SPR.
- Unbound components are eliminated during washing.

- During the final detection step, the substrate(4-Methyl-umbelliferyl Phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm.
- The intensity of the fluorescence is inversely proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to calibration curve which was stored in the memory, and then printed out.

REAGENTS:

The SPR (Solid Phase Receptacle):

- The interior of the SPR is coated during production with anti-T_4 monoclonal antibodies(mouse).
- Each SPR is identified by the T_4 code. Only required number of SPRs will be removed from the pouch and carefully should be resealed after opening.

The strip:

- The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date.
- The foil of the first well is perforated to facilitate the introduction of the sample.
- The last well of each strip is a cuvette in which the fluorometric reading is performed.
- The wells in the center section of the strip contain the various reagents required for the assay.

Table 13: Reagents and description of T_4 strip

Wells	Reagents
1	Sample well
2-3-4-5	Empty wells
6	Conjugate: alkaline phophatase-labeled T4 derivatives+ANS(0.8 mmol/l)+sodium salicylate(9.3mmol/l) + 1g/l sodium azide(400µl).
7	Wash buffer: Tris, Nacl(0.05 mol/l) pH 7.4+1g/l of sodium azide(600 µl)
8	Wash buffer: Tris-Tween, Nacl(0.05 mmol/l)pH 7.4 +1g/l sodium azide(600µl).
9	Wash buffer: diethanolamine(1.1mol/l or 11.5%) pH 9.8+1g/l sodium azide(600µl)
10	Cuvette with substrate: 4-methyl-ombellyferyl phosphate(0.6mmol)+ diethanolamine(0.62mol/l or 6.6%, pH 9.2)+1g/l sodium azide(300µl)

Procedure:

1. Only required reagents removed from the refrigerator and allowed them to come to room temperature for at least 30 minutes.
2. One "T_4" strip and one "T_4" SPR used for each sample, control or calibrator to be tested. Make sure that the storage pouch has been carefully resealed after the required solid phase receptacles have been removed.
3. The test is identified by the "T_4" code on the instrument. The calibrator must be identified by "S1", and tested in duplicate. If the control needs to be tested, it should be identified by "C1".
4. If necessary, clarify samples by centrifugation.
5. Mixed the calibrator, control and samples using a vortex type mixer(for serum or plasma separated from the pellet).
6. For this test, the calibrator, control, and sample test portion is 200µl.
7. Inserted the "T_4" solid phase receptacles (SPRs) and "T_4" strips into the instrument. Check to make sure the colour labels with the assay code on the SPRs and the reagent strips should be matched.
8. All the assay steps performed automatically by the instrument as initiated and directed in the user's manual.
9. Restopper the vials and returned them 2-8ºC after pipetting.

10. The assay will be completed within approximately 40 minutes. After the assay is completed, removed the SPRs and strips from the instrument.
11. Disposed the used SPRs and reagent strips in an appropriated recipient.

Results and Interpretation:

Once the assay is completed, results were analyzed by the computer automatically. Fluorescence is measured twice in the reagent strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluoroscence Value) is calculated by substracting the background reading from the final result. This calculation appears on the result sheet. The results are automatically calculated by the instrument using calibration curves which are stored by the instrument.

4.11. Estimation of Reproductive Hormonal Assays:

4.11.1. Estimation of Luteinizing Hormone (LH): ELFA (Enzyme Linked Fluoroscent Assay)[3].

Principle:

Luteinizing Hormone (LH) was analyzed by One-Step Enzyme immune sandwich method (TSH) with final fluorescent detection (ELFA) by mini-VIDAS.

- Solid Phase Receptacle (SPR) functions as solid phase as well as the pipetting device for the assay. Now the Reagents are ready to use and pre dispensed in the sealed reagents.
- As the entire assay steps are performed automatically by the instrument, the reaction medium is cycled in and out of the SPR several times.
- Transferring the samples into the well containing anti LH antibody labeled with alkaline phosphatase(conjugate) and now this mixture is cycled in and out of the SPR. So that the antigen binds to antibody coated on the SPR and to the conjugate forming a "sandwich".
- Unbound components are eliminated during washing.

- During the final detection step, the substrate(4-Methyl-umbelliferyl Phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm.
- The intensity of the fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to calibration curve which was stored in the memory, and then printed out.

REAGENTS:

The SPR (Solid Phase Receptacle):

- The interior of the SPR is coated during production with monoclonal anti-LH immunoglobulins.
- Each SPR is identified by the LH code. Only required number of SPRs will be removed from the pouch and carefully should be resealed after opening.

The strip:

- The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date.
- The foil of the first well is perforated to facilitate the introduction of the sample.
- The last well of each strip is a cuvette in which the fluorometric reading is performed.
- The wells in the center section of the strip contain the various reagents required for the assay.

Table 14: Reagents and Description of the LH reagent strip

Wells	Reagents
1	Sample well
2-3-4-5	Empty wells
6	Conjugate: alkaline phosphastase–labeled monoclonal anti-LH immunoglobulins (mouse)+1g/l sodium azide (400µl)
7-8	Wash buffer: sodium phosphate (0.01 mol/l/) pH 7.4+1g/l sodium azide(600µl).
9	Wash buffer: diethanalomine(1.1mol/l or 11.5%, pH 9.8) + 1g/l sodium azide(600µl)
10	Cuvette with substrate: 4-methyl-ombellyferyl phosphate (0.6mmol) + diethanolamine (DEA) (0.62mol/l or 6.6%, pH 9.2) +1g/l sodium azide(300µl)

Procedure:

1. Only required reagents removed from the refrigerator and allowed them to come to room temperature for at least 30 minutes.
2. One "LH" strip and one "LH" SPR used for each sample, control or calibrator to be tested. Make sure that the storage pouch has been carefully resealed after the required solid phase receptacles have been removed.
3. The test is identified by the "LH" code on the instrument. The calibrator must be identified by "S1", and tested in duplicate. If the control needs to be tested, it should be identified by "C1".
4. If necessary, clarify samples by centrifugation.
5. Mixed the calibrator, control and samples using a vortex type mixer(for serum or plasma separated from the pellet)
6. For this test, the calibrator, control, and sample test portion is 200µl.

7. Inserted the "LH" solid phase receptacles (SPRs) and "LH" strips into the instrument. Check to make sure the colour labels with the assay code on the SPRs and the reagent strips should be matched.
8. All the assay steps performed automatically by the instrument as initiated and directed in the user's manual.
9. Reclosed the vials and return them to the required temperature after pipetting.
10. The assay will be completed within approximately 40 minutes. After the assay is completed, removed the SPRs and strips from the instrument.
11. Disposed the used SPRs and reagent strips in an appropriated recipient.

Results and Interpretation:

Once the assay is completed, results were analyzed by the computer automatically. Fluorescence is measured twice in the reagent strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluoroscence Value) is calculated by substracting the background reading from the final result. This calculation appears on the result sheet. The results are automatically calculated by the instrument using calibration curves which are stored by the instrument.

4.11.2. Estimation of Follicle Stimulating Hormone (FSH): ELFA (Enzyme Linked Fluoroscent Assay)[3].

Principle:

Follicle stimulating Hormone (FSH) was analyzed by One-Step nzyme immune sandwich method with final fluorescent detection (ELFA) by mini-VIDAS.

- Solid Phase Receptacle (SPR) functions as solid phase as well as the pipetting device for the assay. Now the Reagents are ready to use and pre dispensed in the sealed reagents.
- As the entire assay steps are performed automatically by the instrument, the reaction medium is cycled in and out of the SPR several times.

- Transferring the samples into the well containing anti- FSH antibody labeled with alkaline phosphatase(conjugate) and now this mixture is cycled in and out of the SPR. So that the antigen binds to antibody coated on the SPR and to the conjugate forming a "sandwich".
- Unbound components are eliminated during washing.
- During the final detection step, the substrate (4-Methyl-umbelliferyl Phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm.
- The intensity of the fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to calibration curve which was stored in the memory, and then printed out.

REAGENTS:

The SPR (Solid Phase Receptacle):

- The interior of the SPR is coated during production with mouse monoclonal anti-FSH immunoglobulins.
- Each SPR is identified by the FSH code. Only required number of SPRs will be removed from the pouch and carefully should be resealed after opening.

The strip:

- The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date.
- The foil of the first well is perforated to facilitate the introduction of the sample.
- The last well of each strip is a cuvette in which the fluorometric reading is performed.
- The wells in the center section of the strip contain the various reagents required for the assay.

Table 15: Reagents and Description of the FSH strip

Wells	Reagents
1	Sample well
2-3-4-5	Empty wells
6	Conjugate: alkaline phosphastase–labeled monoclonal anti- FSH immunoglobulins(mouse)+1g/l sodium azide(400µl)
7-8	Wash buffer: sodium phosphate (0.01 mol/l/) pH 7.4+1g/l sodium azide(600µl).
9	Wash buffer: diethanalomine(1.1mol/l or 11.5%, pH 9.8) + 1g/l sodium azide(600µl)
10	Cuvette with substrate: 4-methyl-ombellyferyl phosphate (0.6mmol) + diethanolamine (DEA) (0.62mol/l or 6.6%, pH 9.2) +1g/l sodium azide(300µl)

Procedure:

1. Only required reagents removed from the refrigerator and allowed them to come to room temperature for at least 30 minutes.
2. One "FSH" strip and one "FSH" SPR used for each sample, control or calibrator to be tested. Make sure that the storage pouch has been carefully resealed after the required solid phase receptacles have been removed.
3. The test is identified by the "FSH" code on the instrument. The calibrator must be identified by "S1", and tested in duplicate. If the control needs to be tested, it should be identified by "C1".
4. If necessary, clarify samples by centrifugation.
5. Mixed the calibrator, control and samples using a vortex type mixer (for serum or plasma separated from the pellet).
6. For this test, the calibrator, control, and sample test portion is 200µl.

7. Inserted the "FSH" solid phase receptacles (SPRs) and "FSH" strips into the instrument. Check to make sure the colour labels with the assay code on the SPRs and the reagent strips should be matched.
8. All the assay steps performed automatically by the instrument as initiated and directed in the user's manual.
9. Reclosed the vials and return them to the required temperature after pipetting.
10. The assay will be completed within approximately 40 minutes. After the assay is completed, removed the SPRs and strips from the instrument.
11. Disposed the used SPRs and reagent strips in an appropriated recipient.

Results and Interpretation:

The assay is completed, results were analyzed by the computer automatically. Fluorescence is measured twice in the reagent strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluoroscence Value) is calculated by substracting the background reading from the final result. This calculation appears on the result sheet. The results are automatically calculated by the instrument using calibration curves which are stored by the instrument.

4.11.3. Estimation of Estradiol (E_2) .

Method: Chemiluminescence Immunoassay (CLIA)[4].

Principle:

In the presence of complimentary antigen and antibody, the paratope of the antibody binds to the epitope of the antigen to form an antigen-antibody or an immune complex. Estimating the levels of such immune complex by use of labelled antibodies form the basis of CLIA. It involves use of stationary solid particles coated either with the antigen or antibody of interest. Post incubation, which ensures intact immune complexes are formed, substrate is added. This result in generation of light, the intensity of which is directly proportional to the amount of labeled complexes present and which indirectly aids in quantification of the analyte of interest. The intensity of light is measured in terms of Relative Light Units (RLU).

REAGENTS:

Table 16: Reagents for Estradiol (E_2)

Reagents	Conc
Goat Anti-Rabbit IgG-coated microtiter wells	96 wells
Estradiol Reference Standards: 0,10,30,100,300 and 1000 pg/ml. Liquid, ready to use.	0.50 ml/each
Rabbit Anti-Estradiol Reagent	7.0 ml
Estradiol-HRP Conjugate Reagent	12 ml
50 x Wash Buffer Concentrate	15 ml
Chemiluminiscence Reagent A	6.0 ml
Chemiluminiscence Reagent B	6.0 ml

Assay Procedure:

Preparation of Reagents:

- All reagents should be allowed to reach room temperature (18-25.c) before use, and mixed by gently inverting or swirling prior to use. Avoid foaming.
- To prepare substrate solution, make a 1:1 mixing of reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
- Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735ml of distilled water to prepare 750 ml of washing buffer (1X). Mix well before use.

Procedure:

- Secured the desired number of coated wells in the holder.
- Dispense 25µl of standards, specimens and controls into the appropriate wells
- Dispense 50µl of rabbit anti-Estradiol(E_2) reagent to each well
- Dispense 100µl of Estradiol-HRP Conjugate Reagent into each well.
- Throughly mix for 30 seconds. It is very important to mix them completely.
- Incubate at room temperature (18-25°C) for 90 minutes.
- Rinse and flick the microwells 5 times with washing buffer (1X).
- Strike the wells sharply onto absorbent paper to remove residual water droplets.
- Dispense 100µl chemilumiscence microwell reader 5 minutes later(Between 5 and 20 min. after dispensing the substrates the substrates).
- Read well with a chemiluminescence microwell reader 5 minutes later. (Between 5 and 20 min after dispensing the substrates).

Calculation of results:

- Calculated the average read relative light units (RLU) for each set of reference standards, control and samples. In the absence of software, construct a standard curve by plotting the mean RLU obtained for each reference standard against E_2 Pg/ml on linear graph paper,with RLU on the vertical(y) axis and concentration on the horizontal(x) axis. Using the mean absorbance value for each sample, determine the corresponding concentrate of E_2 in pg/ml from the standard curve.

Example of standard curve:

- Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

E_2(pg/ml)	Relative Lights Units(RLU) (10^5)
0	15.2
10	12.5
30	9.6
100	6.7
300	3.6
1000	1.8

4.11.4. Estimation of Progesterone (P_4):

Method: Chemiluminescence Immunoassay (CLIA)[5].

Principle:

In the presence of complimentary antigen and antibody, the paratope of the antibody binds to the epitope of the antigen to form an antigen-antibody or an immune complex. Estimating the levels of such immune complex by use of labelled antibodies form the basis of CLIA. It involves use of stationary solid particles coated either with the antigen or antibody of interest. Post incubation, which ensures intact immune complexes are formed, substrate is added. This result in generation of light, the intensity of which is directly proportional to the amount of labeled complexes present and which indirectly aids in quantification of the analyte of interest. The intensity of light is measured in terms of Relative Light Units (RLU).

Table 17: Reagents for Progesterone:

Reagents	Conc
Goat Anti-Rabbit IgG-coated microtiter wells	96 wells
Reference Standards: 0, 0.5, 3.0, 10, 25 and 50ng/ml, 0.5 ml each, ready for use.	1 set
Rabbit Anti-Progesterone Reagent	7.0 ml
Progesterone-HRP Conjugate concentrate	1.3 ml
Progesterone-HRP Conjugate Diluent	13.0ml
50 x Wash Buffer Concentrate	15 ml
Chemiluminiscence Reagent A	6.0 ml
Chemiluminiscence Reagent B	6.0 ml

Assay procedure:

Preparation of Reagents:

- To prepare substrate solution, make a 1:1 mixing of reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
- Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735ml of distilled water to prepare 750 ml of washing buffer (1X). Mix well before use.
- To prepare working progesterone-HRP conjugate Reagent, add 0.1ml of progesterone-HRP conjugate concentrate(11x) to 1.0 ml of progesterone-HRP Conjugate Diluent(1:10 dilution) and mix well. The amount of conjugate diluted depends on your assay size. Discard the excess after use.

Procedure:

- Secure the desired number of coated wells in the holder.
- Dispense 25µl of standards, specimens and controls into the appropriate wells
- Dispense 50µl of working progesterone-HRP Conjugate reagent into each well
- Gently Mix it for 5 seconds
- Dispense 50 µl of rabbit anti-progesterone Reagent to each well.
- Throughly mix for 30 seconds. It is very important to mix them completely.
- Incubate at room temperature (18-25°C) for 90 minutes.
- Rinse and flick the microwells 5 times with washing solution
- Dispense 100µl chemilumiscence microwell substrate reagent into each well and mix for 5 seconds.
- Read well with a chemiluminescence microwell reader 5 minutes later(Between 5 and 20 min after dispense the substrates).

Calculation of results

- Calculated the average read relative light units (RLU) for each set of reference standards, control and samples. In the absence of software, construct a standard curve by plotting the mean RLU obtained for each reference standard against P4 conc in ng/ml on linear graph paper,with RLU on the vertical(y) axis and concentration on the horizontal(x) axis. Using the mean absorbance value for each sample, determine the corresponding concentrate of P4 in ng/ml from the standard curve.

Example of standard curve:

- Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

P_4(ng/ml)	Relative Lights Units(RLU) (10^3)
0	97.4
0.5	59.7
3	40.8
10	22.7
25	11.3
50	4.4

4.12. STATISTICAL ANAYLYSIS:

- The obtained data was analyzed and the values are expressed in terms of Mean $\pm$ standard deviation.
- To determine the significance of inter group differences, One Way Anova followed by 'Post Hoc t tests' were done using SPSS software version 16.
- Regression coefficient analysis is done to know the mean change in the dependant variable for one unit of change in the independent variable.
- Level of significance established at $p \leq 0.05$.

REFERENCES:

1. Trinder P. Determination of blood glucose using 4-Aminophenazone as oxygen carrier acceptor. J ClinPathol. 1969; 22:246.

2. Chang J, Hoke C, Ettinger B, et al. Evaluation and Interference Study of HemoglobinA1c Measured by Turbidimetric Inhibition Immunoassay Am J Clin Pathol 1998; 109(3):274-8.

3. Yolken RH, Stopa PJ. Enzyme-linked fluorescence assay: Ultrasensitive solid-phase assay for detection of human rotavirus. J clin microbiol. 1979; 10(3):317-21.

4. http://www.abnova.com/protocol_pdf/KA3641.pdf.

5. http://www.abnova.com/protocol_pdf/KA2803.pdf.

CHAPTER 5

RESULTS

5.1. Physical Anthropometric parameters:

Table 18 shows the Results of Analysis of variance (ANOVA) of anthropometric parameters in all the four groups. Here we found there is significant increase in weight, Body surface area (BSA), Body mass index and Waist to hip ratio in post menopausal diabetic group (Group IV) along with increase in the age compared to all the other groups.

Table 18: Results of Analysis of Variance(ANOVA) of Physiological parameters.

Parameter N=50	**Pre Menopause Non-Diabetic** **Mean±SD Group I [a]**	**Pre Menopause Diabetic** **Mean±SD Group II [b]**	**Post Menopause Non-Diabetic** **Mean±SD Group III [c]**	**Post Menopause Diabetic** **Mean±SD Group IV [d]**	**ANOVA**	
					F-value	**P-Value**
Age(Yrs)	34.6 ±6.3	35.3±5.4	54.90±5.9[a,b]	56.0 ± 5.8[a,b]	194.841	0.000*
Height(cm)	153.6±5.7	152.6±4.2	150.9 ± 6.4	152.1± 6.0	1.996	0.116
Weight(kg)	58.4±7.57	55.9±7.6	55.3±7.2	60.62±7.51[b,c]	5.514	0.001*
BSA(m^2)	1.55±0.09	1.51±0.10	1.49± 0.11[a]	1.56±0.11[c]	4.364	0.005*
BMI(kg/m^2)	24.9±3.80	23.9±3.1	24.3± 2.8	26.13±2.21[b,c]	4.986	0.002*
Waist to Hip Ratio(cm)	0.90±0.10	0.95±0.25	0.94±0.15	1.06±0.23[a,b,c]	5.712	0.001*
Data expressed as Mean±SD. Means of different variables are compared by ANOVA test followed by Post-Hoc test. Superscripts a,b,c, suggests significant difference between groups.						

*p≤0.05, **p≤0.01, *p≤0.001

5.2. Physiological parameters.

Table 19 shows the Results of Analysis of variance(ANOVA)physiological parameters in all the four groups. Here we found Systolic Blood Pressure (SBP) showed significant increase in Group IV compared to all other groups. Diastolic Blood Pressure (DBP) significantly increased in Group III compared to Group II whereas it showed significant decrease in Group II compared to Group I (controls).Pulse Pressure (PP) (mmHg) showed significant increase in Group IV

compared to all other groups and significantly decreased in Group III compared to Group II. Mean Arterial Pressure (MAP) and Pulse rate did not show any significant change among all the groups.

Table 19 : Results of Analysis of variance(ANOVA) of Physiological parameters

Parameter N=50	**Pre Menopause Non-Diabetic Mean±SD Group I [a]**	**Pre Menopause Diabetic Mean±SD Group II [b]**	**Post Menopause Non-Diabetic Mean±SD Group III [c]**	**Post Menopause Diabetic Mean±SD Group IV [d]**	**ANOVA**	
					F-value	**P-Value**
SBP(mmHg)	117.0±9.4	123.3±7.7[a]	122.8±8.5[a]	125.6±8.20[a]	9.464	0.000*
DBP(mmHg)	76.6±5.0	72.8±6.4[a]	77.0±5.3[b]	75.1±5.0	6.072	0.001*
PP(mmHg)	40.3+8.08	50.4+8.74[a]	45.8±8.12[a,b]	51.32± 9.97[a]	16.589	0.000*
MAP(mmHg)	90.06+5.62	89.5+ 5.44	92.1± 5.29	92.1±5.49	3.191	0.065
PR(bpm)	71.9±6.0	71.4±4.2	73.2±4.2	72.5±4.7	0.600	0.616
Data expressed as Mean±SD. Means of different variables are compared by ANOVA test followed by Post-Hoc test. Superscripts a,b,c, suggests significant difference between groups.						

*p≤0.05, **p≤0.01, *p≤0.001

5.3. Haematological parameters:

Table 20 shows the Results of Analysis of variance(ANOVA) of haematological parameters in all the four groups. Here we did not find any statistically significant change in haematological parameters among the groups except white blood cells(WBC), which is significantly increased in Post menopausal diabetics (Group IV) compared to other groups.

Table 20: Results of Analysis of variance (ANOVA) of Haematological paramaters.

Parameter N=50	Pre Menopause Non-Diabetic Mean±SD Group I [a]	Pre Menopause Diabetic Mean±SD Group II [b]	Post Menopause Non-Diabetic Mean±SD Group III [c]	Post Menopause Diabetic Mean±SD Group IV [d]	ANOVA	
					F-value	P-Value
RBC (millions/ mm^3)	4.74±0.49	4.7±0.57	4.68±0.38	4.62±0.44	0.924	0.430
WBC (thousands/ mm^3)	7320.9± 1666.7	7347.4± 2297.3	8565.6± 2035.6[a,b]	8636.2± 2279.1[a,b]	6.16	0.001*
Hb(gm%)	12.6±1.57	12.7±1.37	12.32±1.29	12.63±1.29	0.923	0.430
PCV(%)	38.7±3.85	37.26±4.46	37.51±4.41	38.01±3.99	1.290	0.279
MCV(fl)	82.2±8.07	82.16±6.37	83.72±6.72	83.99±6.42	0.976	0.405
MCH(pg)	26.8±3.37	26.82±2.54	26.33±2.65	27.07±3.12	0.559	0.643
MCHC (g/DL)	32.5±1.41	32.23±1.51	31.740±2.05	32.05±1.91	2.059	0.107
Platelet count (lacks/mm^3)	3.11±0.80	3.53±0.86	3.31±0.86	3.16±0.81	2.512	0.060
Data expressed as Mean±SD. Means of different variables are compared by ANOVA test followed by Post-Hoc test. Superscripts a,b,c, suggests significant difference between groups.						

*p≤0.05, **p≤0.01, *p≤0.001

5.4. Blood Glucose Profile: FBS and HbA1c.

Table 21 shows the Results of Analysis of variance (ANOVA) of Blood glucose profile. Here Fasting blood sugar significantly increased in Group II and IV compared to controls (Group I). FBS significantly decreased in Group III and IV compared to Group II. HbA1c significantly increased in Group II and IV compared to controls (Group I). HbA1c significantly increased in Group IV compared to Group III and significantly decreased in Group III compared to Group II.

Table 21: Results of Analysis of variance (ANOVA) of Blood glucose profile

Parameter N=50	Pre Menopause Non-Diabetic Mean±SD Group I[a]	Pre Menopause Diabetic Mean±SD Group II [b]	Post Menopause Non-Diabetic Mean±SD Group III[c]	Post Menopause Diabetic Mean±SD Group IV [d]	ANOVA	
					F-value	P-Value
FBS(mg/dl)	83.0±10.7	200.8±86.8[a]	89.3 ± 13.1[b]	149.4± 51.7 [a,b,c]	58.738	0.000*
HbA1c(%)	5.3±0.7	7.9±1.5[a]	5.5 ± 0.5[b]	7.9±1.9 [a, c]	54.991	0.000*
Data expressed as Mean±SD. Means of different variables are compared by ANOVA test followed by Post-Hoc test. Superscripts a,b,c, suggests significant difference between groups.						

*p≤0.05, **p≤0.01, *p≤0.001

Percentage Difference of Blood Glucose Profile-(Fasting Blood Sugar (FBS) and Glycosylated Haemoglobin (HbA1c):

The percentage difference among all the groups is expressed as E_1, E_2 and E_3 considering Pre Menopause Non-Diabetics as controls:

E_1= Pre Menopause Non Diabetic - Pre Menopause Diabetic (G1-G2).

E_2= Pre Menopause Non Diabetic - Post Menopause Non-Diabetic (G1-G3).

E_3= Pre Menopause Non Diabetic - Post Menopause Diabetic (G1-G4).

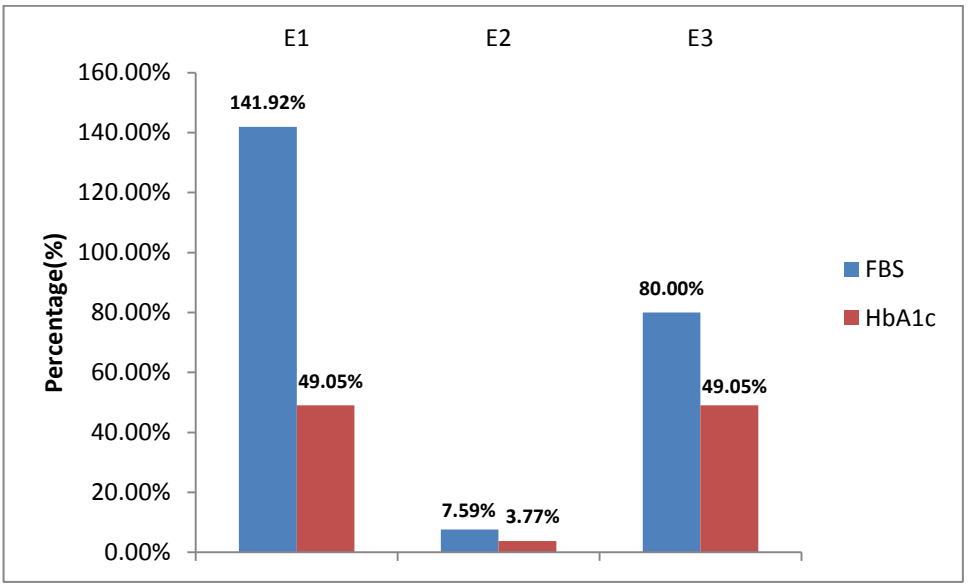


Figure 7: Percentage Difference of FBS and HbA1c

Figure 7 shows that Fasting Blood Sugar(FBS) levels is 141.92% increased in Pre menopausal diabetics(E_1) compared to other groups(E_2,E_3) where as Glycosylated haemoglobin(HbA1c) remains same(49.05%) in both E_1, E_3(Pre and Post menopausal diabetics) and is decreased 3.77% in E2(Post menopausal non-diabetics) group.

5.5.Thyroid Hormonal Assay (TSH, T_3, T_4):

Table 22 shows the Results of Analysis of variance(ANOVA) of thyroid hormonal assay.

- TSH did not show any significant change among all the groups.
- T_3 and T_4 showed significant increase in Post menopausal diabetics (Group IV) compared to controls (Group I).

Table 22: Results of Analysis of variance(ANOVA) of Thyroid Hormonal Assay.

Parameter N=50	Pre Menopause Non-Diabetic Mean±SD Group I [a]	Pre Menopause Diabetic Mean±SD Group II [b]	Post Menopause Non-Diabetic Mean±SD Group III [c]	Post Menopause Diabetic Mean±SD Group IV [d]	ANOVA	
					F-value	P-Value
TSH(mμIU/m)	3.0±1.49	2.55±1.49	2.8 ± 1.49	2.5±1.58	1.327	0.26
T_3(nmol/L)	1.4+0.5	1.6+0.4	1.7± 0.3	1.8±0.6 [a]	3.576	0.01*
T_4(nmol/ml)	90.01+11.3	93.3+15.98	94.5± 19.9	98.7±18.15[a]	2.367	0.07
Data expressed as Mean±SD. Means of different variables are compared by ANOVA test followed by Post-Hoc test. Superscripts a,b,c, suggests significant difference between groups.						

*p≤0.05, **p≤0.01, *p≤0.001

Percentage difference of Thyroid hormonal profile among the groups.

The percentage difference among all the groups is expressed as E_1, E_2 and E_3 considering Pre Menopause Non-Diabetics as controls:

E_1= Pre Menopause Non Diabetic - Pre Menopause Diabetic (G1-G2).

E_2= Pre Menopause Non Diabetic - Post Menopause Non-Diabetic (G1-G3).

E_3= Pre Menopause Non Diabetic - Post Menopause Diabetic (G1-G4).

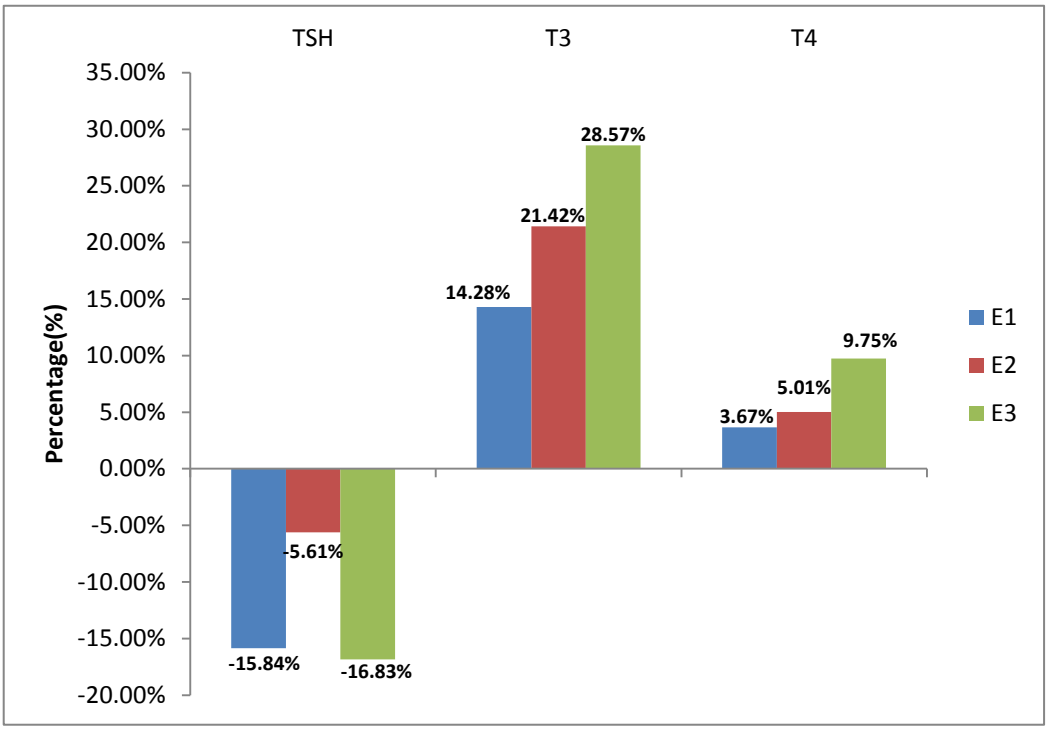


Figure 8: Percentage difference of Thyroid Hormonal Assay (TSH, T_3, T_4):

- **Figure 8** shows that, there is 28.57% increase in T_3 and 9.75% increase in T_4 levels in post menopausal diabetics compared to other groups
- TSH levels shows -16.83% decrease in post menopausal diabetics compared to other groups.

5.6. Reproductive Hormonal Assay:

Table 23 shows the Results of Analysis of variance(ANOVA) of Reproductive Hormonal assay**(**Luteinizing hormone and Follicle stimulating Hormone).

- This table indicates that there is significant increase in LH and FSH in Group III and IV compared to Group I and II.
- There is significant decrease in Group IV compared to Group III.

Table 23: Results of Analysis of variance(ANOVA) of LH & FSH

Parameter N=50	Pre Menopause Non-Diabetic Mean±SD Group I[a]	Pre Menopause Diabetic Mean±SD Group II[b]	Post Menopause Non-Diabetic Mean±SD Group III[c]	Post Menopause Diabetic Mean±SD Group IV[d]	ANOVA	
					F-value	P-Value
LH(mIU/ml)	9.7±6.8	5.8± 3.6	26.2±12.2[a,b]	20.2±10.5[a,b,c]	50.846	0.000*
FSH(IU/L)	9.1±6.0	8.5± 5.3	67.4±29.9[a,b]	55.4±29.0[a,b,c]	103.02	0.000*
Data expressed as Mean±SD. Means of different variables are compared by ANOVA test followed by Post-Hoc test. Superscripts a,b,c, suggests significant difference between groups.						

*p≤0.05, **p≤0.01, *p≤0.001

Percentage difference of LH and FSH among the groups.

The percentage difference among all the groups is expressed as E_1, E_2 and E_3 considering Pre Menopause Non-Diabetics as controls:

E_1= Pre Menopause Non Diabetic - Pre Menopause Diabetic (G1-G2)

E_2= Pre Menopause Non Diabetic - Post Menopause Non-Diabetic (G1-G3)

E_3= Pre Menopause Non Diabetic - Post Menopause Diabetic (G1-G4)

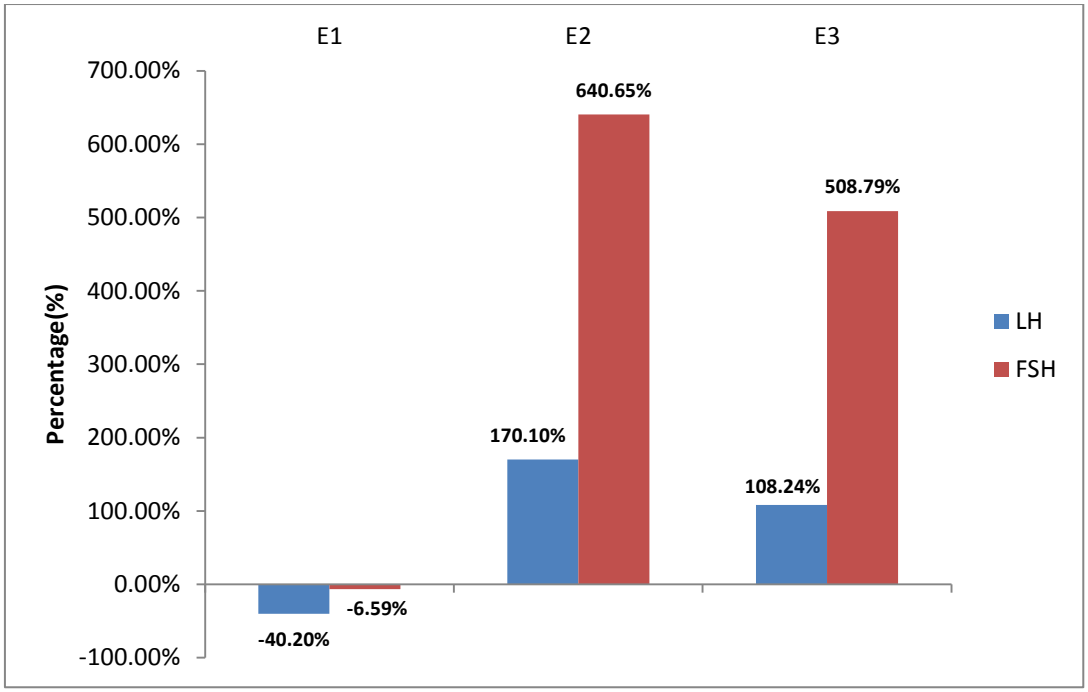


Figure 9: Percentage difference of LH and FSH

Figure 9 shows that, there is 108.24% of LH and 508.79% of FSH levels are decreased in post menopause diabetics compared to post menopausal non-diabetics.

Estradiol(E_2) & Progesterone(P_4):

Table 24 shows the Results of Analysis of variance(ANOVA) of Reproductive Hormonal assay(Estradiol and Progesterone).

- Estradiol and Progesterone showed significant decrease in Group III and IV compared to Group I and II.
- Progesterone showed significant decrease in Group II compared to Group I.

Table 24: Results of Analysis of variance(ANOVA) of E_2 & P_4

Parameter N=50	Pre Menopause Non-Diabetic Mean±SD Group I[a]	Pre Menopause Diabetic Mean±SD Group II[b]	Post Menopause Non-Diabetic Mean±SD Group III[c]	Post Menopause Diabetic Mean±SD Group IV[d]	ANOVA	
					F-value	P-Value
Estradiol (pg/ml)	107.6±85.4	87.0±62.2	22.2±12.3[a,b]	17.9±10.3[a,b]	35.952	0.000*
Progesterone (ng/ml)	3.1±1.9	2.2±0.9[a]	0.8±0.2[a,b]	0.2±0.1[a,b]	27.372	0.000*
Data expressed as Mean±SD. Means of different variables are compared by ANOVA test followed by Post-Hoc test. Superscripts a,b,c, suggests significant difference between groups.						

Percentage difference of Estradiol (E_2) and Progesterone(P_4) among the groups.

The percentage difference among all the groups is expressed as E_1, E_2 and E_3 considering Pre Menopause Non-Diabetics as controls:

E_1= Pre Menopause Non Diabetic - Pre Menopause Diabetic.

E_2= Pre Menopause Non Diabetic - Post Menopause Non-Diabetic.

E_3= Pre Menopause Non Diabetic - Post Menopause Diabetic.

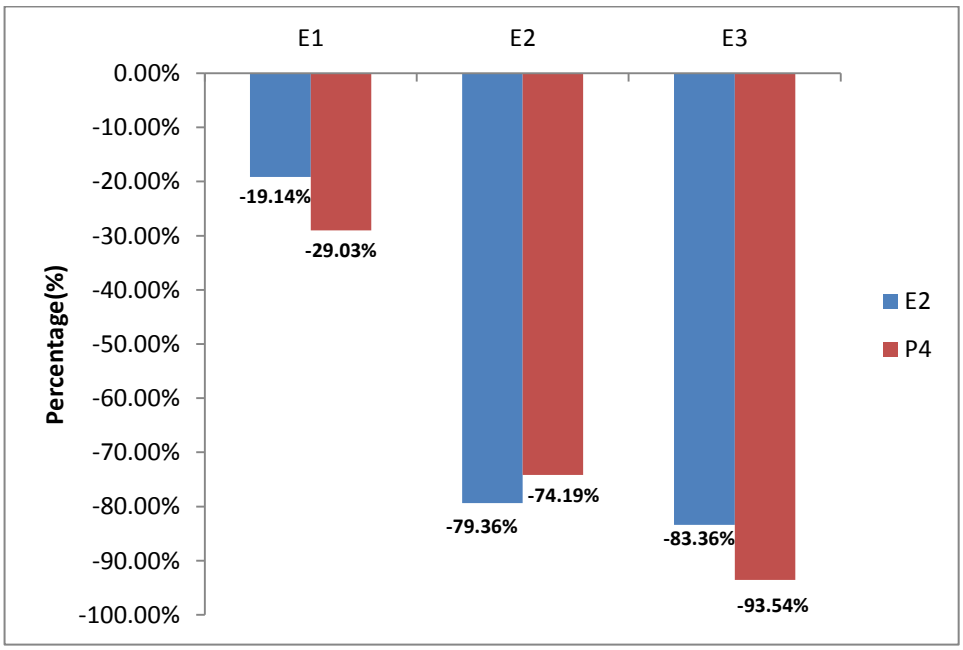


Figure 10: Percentage difference of Estradiol (E_2) and Progesterone (P_4)

Figure 10 shows that, there is -83.36% of Estradiol and -93.54% of Progesterone values were decreased in Post menopausal diabetics compared to other groups.

5.7. Regression coefficient analysis:

Regression co-efficient analysis was done between Thyroid profile (TSH, T_3, T_4) as dependable variables and remaining other variables as independent variables by holding other variables Constant.

Regression coefficient analysis: TSH(dependent variable)

Table 25 shows Analysis of correlation between TSH and Hormonal profile and glucose homeostasis in pre and post menopausal non-diabetic and diabetic women. Here we found 1mIU/ml in LH results in 0.058 muIU/ml increase (p=0.04) in TSH, as well as 1IU/L of increase in FSH results in 0.028 muIU/ml decrease (p=0.01) in TSH in post menopausal non-diabetic women.

Table 25 :**Analysis of correlation between TSH and Hormonal profile**

GROUPS	Parameter	B	Beta	t	sig
Pre Menopause Non-Diabetic	**FBS**	-0.023	-0.168	-1.088	0.283
	HbA1c	-0.116	-0.071	-0.44	0.662
	Estradiol	-0.004	-0.218	-1.443	0.156
	Progesterone	-0.018	-0.034	-0.21	0.834
	LH	-0.019	-0.102	-0.599	0.552
	FSH	0.041	0.198	1.088	0.283
	MAP	0.039	0.145	0.993	0.326
Pre Menopause Diabetic	**FBS**	0.000	-0.032	-0.198	0.844
	HbA1c	-0.213	-0.223	-1.343	0.186
	Estradiol	0.002	0.064	0.353	0.726
	Progesterone	-0.093	-0.124	-0.801	0.427
	LH	-0.047	-0.146	-0.702	0.487
	FSH	0.031	0.141	0.617	0.54
	MAP	0.029	0.106	0.67	0.507
Post Menopause Non-Diabetic	**FBS**	-0.01	-0.087	-0.542	0.591
	HbA1c	0.254	0.097	0.619	0.531
	Estradiol	-0.031	-0.259	-1.776	0.083
	Progesterone	-0.379	-0.182	-1.334	0.189
	LH	0.058	0.483	2.037	0.048*
	FSH	-0.028	-0.579	-2.43	0.019*
	MAP	0.067	0.241	1.735	0.09
Post Menopause Diabetic	**FBS**	0.002	0.052	0.324	0.747
	HbA1c	0.125	0.151	0.945	0.35
	Estradiol	-0.008	-0.062	-0.389	0.699
	Progesterone	0.326	0.036	0.238	0.813
	LH	0.043	0.282	1.486	0.145
	FSH	-0.011	-0.181	-0.985	0.33
	MAP	-0.056	-0.193	-1.247	0.219

$p \leq 0.05$, **$p \leq 0.01$, *$p \leq 0.001$

Regression coefficient analysis: T_3(dependent variable)

Table 26 shows Analysis of correlation between T_3 and Hormonal profile and glucose homeostasis in pre and post menopausal non-diabetic and diabetic women. Here we could not find any significant changes between dependant (T_3) and independent variables.

Table 26: Results of Analysis of correlation between T_3 and other hormonal profile.

GROUPS	Parameter	B	Beta	t	sig
Pre Menopause Non-Diabetic	**FBS**	-0.004	-0.078	-0.511	0.612
	HbA1c	0.064	0.107	0.671	0.506
	Estradiol	-0.001	-0.172	-1.152	0.256
	Progesterone	0.045	0.24	1.505	0.14
	LH	0.016	0.226	1.343	0.186
	FSH	-0.017	-0.23	-1.279	0.208
	MAP	0.006	0.065	0.445	0.659
Pre Menopause Diabetic	**FBS**	0.001	0.145	0.947	0.349
	HbA1c	-0.079	-0.302	-1.903	0.064
	Estradiol	-0.001	-0.211	-1.222	0.228
	Progesterone	0.05	0.244	1.66	0.104
	LH	0.002	0.026	0.132	0.896
	FSH	0.012	0.193	0.887	0.38
	MAP	0.009	0.117	0.776	0.442
Post Menopause Non-Diabetic	**FBS**	0.005	0.165	0.963	0.341
	HbA1c	-0.09	-0.131	-0.786	0.436
	Estradiol	0.001	0.041	0.262	0.794
	Progesterone	0.011	0.019	0.134	0.894
	LH	0.015	0.458	1.814	0.077
	FSH	-0.007	-0.526	-2.076	0.066
	MAP	-0.011	-0.153	-1.013	0.317
Post Menopause Diabetic	**FBS**	-0.002	-0.164	-1.059	0.296
	HbA1c	0.055	0.161	1.034	0.307
	Estradiol	-0.002	-0.036	-0.231	0.819
	Progesterone	0.823	0.209	1.425	0.161
	LH	0.02	0.31	1.681	0.1
	FSH	-0.005	-0.22	-1.23	0.225
	MAP	-0.024	-0.189	-1.254	0.217

Regression coefficient analysis: T_4 (dependent variable **)**

Table 27 shows Analysis of correlation between T_4 and Hormonal profile and glucose homeostasis.Here we found 1gm% of increase in Glycosylated haemoglobin(HbA1c) results in 4.169 nmol/l increase(p=0.04) in T_4 in Pre menopause Non diabetic women compared to all other groups.

Table 27: Analysis of correlation between T_4 and Hormonal profile.

GROUPS	Parameter	B	Beta	t	sig
Pre Menopause Non-Diabetic	**FBS**	0.247	0.232	1.515	0.137
	HbA1c	4.169	0.337	2.098	0.042*
	Estradiol	-0.007	-0.054	-0.359	0.721
	Progesterone	-0.177	-0.045	-0.282	0.779
	LH	-0.196	-0.135	-0.8	0.428
	FSH	0.115	0.073	0.405	0.687
	MAP	-0.107	-0.053	-0.362	0.719
Pre Menopause Diabetic	**FBS**	0.022	0.12	0.81	0.422
	HbA1c	-1.634	-0.16	-1.047	0.301
	Estradiol	0.051	0.2	1.2	0.237
	Progesterone	1.465	0.182	1.285	0.206
	LH	-1.237	-0.362	-1.895	0.065
	FSH	0.355	0.152	0.723	0.474
	MAP	0.283	0.096	0.663	0.511
Post Menopause Non-Diabetic	**FBS**	0.237	0.156	0.916	0.365
	HbA1c	-1.941	-0.055	-0.331	0.742
	Estradiol	0.357	0.221	1.435	0.159
	Progesterone	5.14	0.183	1.268	0.212
	LH	0.566	0.348	1.386	0.173
	FSH	-0.153	-0.23	-0.912	0.367
	MAP	-1.048	-0.279	-1.905	0.064
Post Menopause Diabetic	**FBS**	-0.017	-0.05	-0.307	0.761
	HbA1c	0.305	0.034	0.206	0.838
	Estradiol	-0.331	-0.224	-1.379	0.175
	Progesterone	1.484	0.014	0.093	0.926
	LH	0.135	0.078	0.405	0.687
	FSH	-0.089	-0.143	-0.763	0.45
	MAP	-0.129	-0.039	-0.242	0.81

*$p \leq 0.05$, **$p \leq 0.01$, *$p \leq 0.001$

Percentage difference between premenopausal diabetic and non-diabetic women:

Table 28 : Percentage difference between Premenopausal diabetic and non-diabetic women.

- Among Anthropometric Parameters, Age (2.02%), Wst.Hip ratio (5.55%) increased where as Height (-0.65%), Weight (-4.28%), BSA (-2.58%), BMI (-4.01%) values were decreased.
- Among Physiological Parameters, SBP (5.38%), P.P(25.06%) values were increased where as DBP(-4.96%), MAP(-0.62%), Pulse rate(-0.69%) showed decreased values.
- Among Haematological parameters, RBC (-0.84%), PCV (-3.72%), MCV (-0.04%), MCHC (-0.83%) values were decreased where as WBC (0.36%), Hb (1.19%), Platelets (13.50%), values were increased.
- In Blood glucose profile, FBS showed 141.92% and HbA1c showed 49.05% increase in their values.
- In Thyroid Hormonal Assay, TSH is decreased to -15.84% where as T_3 is increased to 14.28% and T_4 are increased to 3.67%.
- In Reproductive hormonal Assay, E_2 is decreased to -19.14%, P_4 is decreased to -20.03%.
- In Reproductive hormonal assay, LH is decreased to -40.20%, FSH is decreased to -6.59%.

Anthropometric Parameters							
Age	**Height**	**weight**	**BSA**	**BMI**	**Waist Hip ratio**		
2.02%	-0.65%	-4.28%	-2.58%	-4.01%	5.55%		
Physiological Parameters							
SBP	**DBP**	**PP**	**MAP**	**P.R**			
5.38%	-4.96%	25.06%	-0.62%	-0.69%			
Haematological Parameters							
RBC	**WBC**	**Hb**	**PCV**	**MCV**	**MCH**	**MCHC**	**Platelets**
-0.84%	0.36%	1.19%	-3.72%	-0.04%	0.07%	-0.83%	13.50%
Blood Glucose Profile							
		FBS	**HbA_1C**				
		141.92%	49.05%				
Thyroid Hormonal Assay							
		TSH	**T_3**	**T_4**			
		-15.84%	14.28%	3.67%			
Reproductive Hormonal Assay							
		E_2	**P_4**		**LH**	**FSH**	
		-19.14%	-29.03%		-40.20	-6.59%	

Table 29: Percentage difference between Post menopausal Diabetic and Non-Diabetic women

- Among Anthropometric Parameters, Age (2.00%), Height (0.79%), Weight (9.62%), BSA (4.69%), BMI (7.53%), Waist Hip ratio (12.76%) increased in their values.
- Among Physiological Parameters, SBP (2.28%), PP (12.05%) values were increased where as DBP (-2.46%), P.R (-0.95%) values were decreased.
- Among Haematological Parameters, RBC(-1.28%), Platelets(-4.53%) values were decreased where as WBC(0.82%), Hb(2.51%), PCV(1.33%), MCV(0.32%), MCH(2.81%), MCHC(0.97%) Values were increased.
- In Blood glucose profile, FBS is increased to 67.30%, HbA1c is increased to 43.63%.
- In Thyroid Hormonal Assay TSH is decreased to -10.71% where as T_3 is increased to 5.88% and T_4 is increased to 4.44%.
- In Reproductive Hormonal Assay, E_2 is decreased to –19.36%, P_4 is decreased to -75.00%.
- In Reproductive Hormonal Assay,LH is decreased to – 22.90%, FSH is decreased to -17.80%.

Anthropometric Parameters							
Age	**Height**	**weight**	**BSA**	**BMI**	**Waist Hip ratio**		
2.00%	0.79%	9.62%	4.69%	7.53%	12.76%		
Physiological Parameters							
SBP	**DBP**	**PP**	**MAP**	**P.R**			
2.28%	-2.46%	12.05%	0.00%	-0.95%			
Haematological Parameters							
RBC	**WBC**	**Hb**	**PCV**	**MCV**	**MCH**	**MCHC**	**Platelets**
-1.28%	0.82%	2.51%	1.33%	0.32%	2.81%	0.97%	-4.53%
Blood Glucose Profile							
		FBS	**HbA_1C**				
		67.30%	43.63%				
Thyroid Hormonal Assay							
		TSH	**T_3**	**T_4**			
		-10.71%	5.88%	4.44%			
Reproductive Hormonal Assay							
		E_2	**P_4**		**LH**	**FSH**	
		-19.36%	-75.00%		-22.90%	-17.80%	

Table 30: Current research findings with other research findings.

Author	Research findings
Suresh DR et al., 2014	Found Dysfunction of thyroid in diabetes and there was no association between glycaemic control and thyroid function status.
Riyaz M., 2015	Found higher prevalence of hypothyroidism among Type 2 Diabetic patient and females are more vulnerable to thyroid disorder as compared to males.
Venkateshwarlu N et al., 2013.	Found that diabetic1s are at increased risk for hypothyroidism and their Fasting glucose levels do not predict the risk.
Mukherjee S et al,2015	Found Primary Hypothyroidism is highly prevalent in patients with T2DM
Alam MJ et al., 2013	Found high incident of abnormal thyroid hormones in diabetic subjects. Found good comparison between hypothyroidism and diabetic subjects.
Gupta PK., 2013.	Found high frequency of hyperlipidemia and hypothyroidism in type-2 diabetes mellitus subjects.
Singh T et al., 2014	Found high prevalence of thyroid disorders in diabetes. Thyroid disorders are more common in females and the most common thyroid disorder is hypothyroidism. The association of thyroid disorders is more frequent in diabetics who have deranged metabolic control.
Gosavi RV et al., 2017	Found Thyroid dysfunction is more common among type 1 diabetics, especially females. So the estimation of anti-TPO antibodies is necessary in detecting thyroid dysfunction in type 1 diabetics.
Umpierrez GE et al., 2013.	Found the association between autoimmune thyroid dysfunction and type 1 diabetes.
Present study	The current findings of our research are thyroid disorders deteriorates the glucose metabolism in post menopausal women suffering from Diabetes Mellitus. So monitoring of thyroid profile in post menopausal diabetic women would be a better therapy in future.

CHAPTER 6

DISCUSSION

6.1. Physical Anthropometric parameters:

In the present study, Weight, Body surface Area (BSA), Body Mass Index (BMI) and Waist to Hip Ratio were found to be significantly more in Post menopausal diabetic women compared to the other groups which may be due to obesity. Obesity is considered as one of the commonest causes of diabetes in post menopausal women. "Wing RR and Svendesen OL showed that women tend to gain weight and undergo an alteration to their fat distribution in mid-life"[1, 2].

At menopause, decreased production of ovarian hormones which leads to many functional and endocrinological disturbances, including increase in the body weight and decrease in the basal body metabolism. These may contribute to weight gain[3].

In women, decreased production of estrogen at menopause may be due to alteration in their body fat distribution and these changes are due to change in the metabolism of adipose tissue. Estrogen is considered as one of the influencing factor for the activity of adipose tissue lipoprotein lipase (LPL) and lipolysis[4]. Decreased levels of estrogen favours for central body obesity by exerting negative effect on the metabolism of fat [5,6].

Reduction in the receptors of insulin on the insulin responsive cells may also leads to the common diabetogenic factor obesity[7]. There is an increased prevalence of diabetes which is associated with the increased rate of excess weight and obesity. It has been assessed that over 90% of type 2 diabetic subjects owed to excess weight. Diabetes often coexists with obesity[8,9].

Both obesity and physical inactivity are responsible for the development of insulin resistance. The insulin resistance together with dysfunction of β-cell and apoptosis are the two important fundamental mechanisms for the development of diabetes in menopausal women [10,11].

The adipose tissue produces and releases some hormones along with the other biologically active molecules know as 'adipokines' which plays an important role in the regulation of various metabolic activities[12].

So among these adipokines, 'adiponectin' may directly or indirectly affect the sensitivity of insulin through the modulation of insulin signaling and the glucose molecules and the metabolism of lipid. Decline in the circulating levels of adiponectin by the genetic and environmental factors is associated with the development of diabetes mellitus[13,14]

There is a strong link between increase in the body fat and production of adipokine especially, leptin[15]. Leptin is an adipose derived hormone and has been involved in the regulation of adipose mass. So it plays an important role in the alteration of both insulin sensitivity and secretion of insulin[16-21].

Studies also revealed that the deficiency of leptin or the leptin receptor, not only contributes to the obesity, but also becomes both insulin resistant and glucose intolerant and they are at high risk of developing diabetes mellitus[22,23]. This suggests that leptin may act directly and affect the function of pancreatic β- cells[24]

The other factors which are involved in the development of obesity in post menopausal diabetic women are discovery of macrophage infiltration in the abdominal adipose tissue. Unbalanced production of adipokines. Apart from this, there is increased cellular uptake of non-esterified fatty acids (NEFA) aggravated in an obese state without any subsequent β oxidation. This may leads to the accumulation of lipid metabolites and causes defects in the pathway of insulin signaling[25,26].

. There are several mechanisms proposed to explain about the weight gain associated with diabetes in post menopausal women. There is a decrease in the sensitivity of insulin by the accumulation of fat mass in general. Excess abdominal adipose tissue closely associated with glucose intolerance and actions of insulin[27].

Within the adipocyte an increase in the expression of products such as tumour necrosis factor α(TNF-α) along with leptin may contribute to the deterioration of sensitivity of insulin. Recently a novel protein has been discovered known as resistin which plays an important role in providing the molecular link between diabetes and obesity[28].

"The metabolic effects of obesity on circulating estrogen hormone is a progressive decrease of sex hormone binding globulin (SHBG) with increase in the body mass index (BMI) in both pre and post menopausal women[29].

The other secondary causes of obesity which may leads to the diabetes in women may be due to principal glucocorticoid and cortisol significantly contributes to the regulation of carbohydrates, proteins, lipids etc. These factors enhance the blood glucose production by opposing the insulin secretions and its actions. So that there is increase in the peripheral break down of proteins which enhances the activation of lipoprotein lipase in the adipocytes and this in turn increases the accumulation of fat[30].

So the post menopausal diabetic women have increased tendency in gaining weight. Menopausal transition itself makes them to gain body weight is still unclear. But, it is known that the physiological withdrawal of estrogen at menopausal stage itself brings the changes in distribution of fat[31].

Mechanism between Menopause, Obesity and diabetes:

In menopausal women, the level of sex hormone binding globulin (SHBG) is decreased which leads to the depletion of estrogen and increases the androgen levels. The decrease of the sex steroids (estrogen and progesterone) in post menopausal women leads to increased activity of lipoprotein lipase in the abdominal subcutaneous adipose tissue. These changes contributes to obesity by triggering the changes in the body metabolism **(Figure 11)**. This in turn make the adipose tissue to release the amount of fatty acids,glycerol, hormones, proinflammatory cytokines and other factors. All these factors may contribute to the development of insulin resistance which is accompanied by the dysfunction of pancreatic islet betacells. It is difficult to control the blood glucose level. These all features together contribute to the development of diabetes in post menopausal women[32-34].

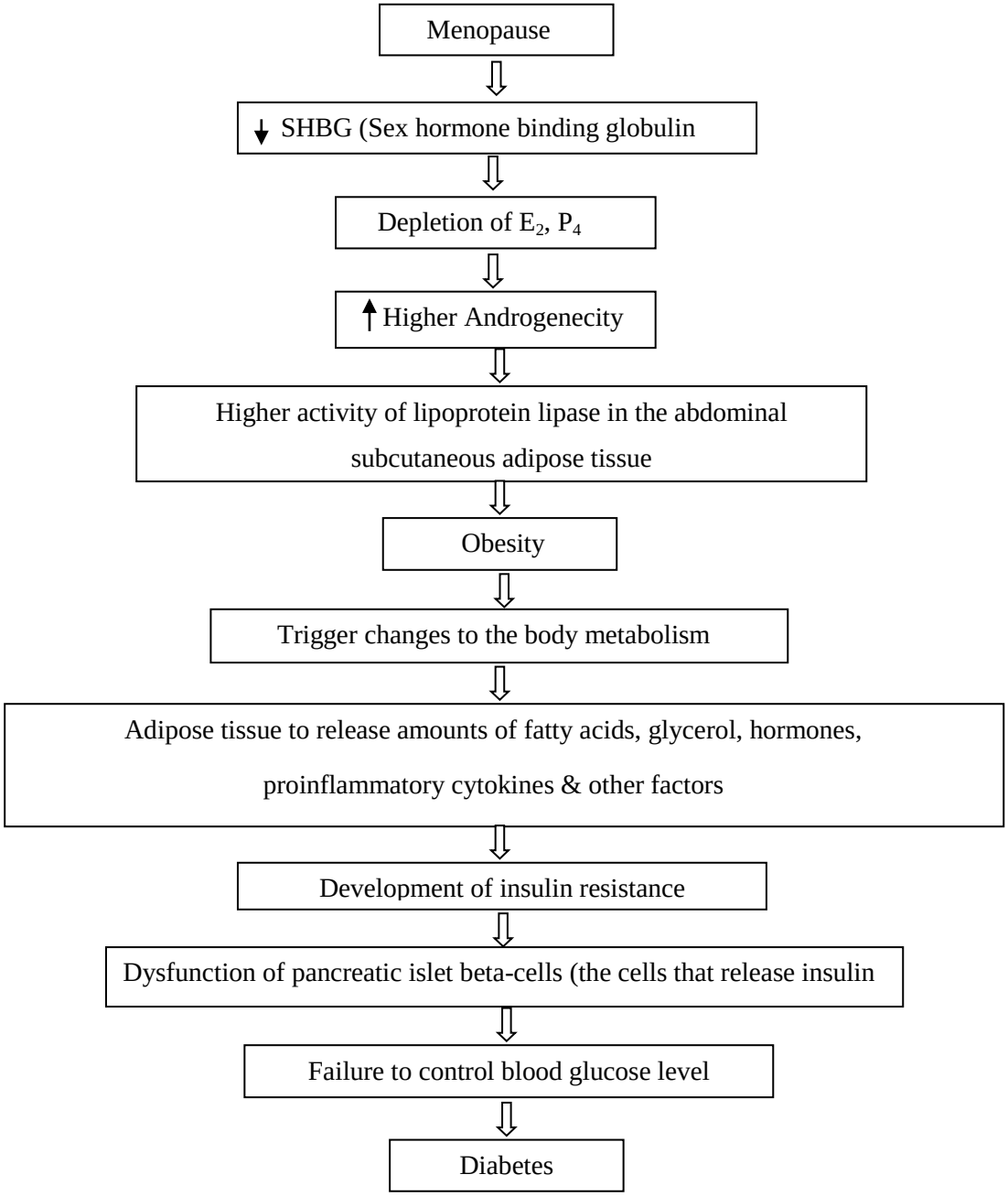


Figure 11: Mechanism between Menopause, Obesity, Diabetes

6.2. Physiological Parameters:

In the present study, SBP increased and DBP decreased where as pulse pressure increased in post menopausal diabetics compared to the other groups and there is no change in mean arterial pressure and pulse rate.

Increase in the SBP in post menopausal diabetics might be due to reduced levels of estrogen. Our finding is in agreement with seely et.al, that there is increase in the blood pressure by decreasing the effect of Estradiol [35].

The reason for decrease in the DBP might be due to age related decline, which is assumed to be from early recoil of pressure wave because of increase in the stiffness of artery and deficient state of compliance of proper larger artery. So there is a shifting of reflection wave from diastole to systole and it results in increasing the systolic and decreasing the diastolic blood pressures. These observations were supported by Ronnback M et al [36].

The change in the arterial or arteriolar stiffness in menopausal women is associated with increase in the blood pressure. The increase in the diastolic blood pressure up to the age of 50 is due to the increase in peripheral vascular resistance (PVR). So the stiffness of artery and peripheral vascular resistance may contributes towards the increase in systolic blood pressure (SBP), though, there is increase in the diastolic pressure along with peripheral vascular resistance but it decreases with the large artery stiffness(LAS). The acceleration of large artery stiffness that may contributes to the steeper increase in systolic blood pressure as the age advances[37].

It has been suggested that, sex steroids protect the women from hypertension in fertile phase and the withdrawal of gonadal steroids may play a role in modifications of B.P control. Infact, at menopause, the role played by with drawl of steroids is still controversial. These steroids may also regulate the functions of blood vessels[38].

"The endothelium-mediated dilating effect of estrogens disappears in women with the alterations in endothelium and it appears to decrease with time since menopause, being lost several years after menopause[39,40] ".The activity of the endothelial cells via genomic and non-genomic signaling is influenced by estrogens[41-44].

Glycaemia or insulin resistance affects arterial stiffening through several mechanisms. “Chronic hyperglycaemia may increase sympathetic tone, promotes reabsorption of sodium, activates renin-angiotension aldosterone system and increases systemic and vascular inflammation[45,46].

The complex mechanisms where hyperglycaemia damages the blood vessel endothelial cells includes, activation of protein kinase C, increased expression of adhesion molecules, adherence and uptake of leukocytes, production of proliferative substances and proliferation of the cells of endothelium, synthesis of collagen IV and fibronectin etc[47].

As some studies showed that, diabetes is associated with an increase in the arterial stiffness or a decrease in the vascular compliance[48-51]. Although it is probable that diabetes increases pulse pressure by increasing stiffness of the artery, there has been no exploration of the direct relation between pulse pressure and progression to diabetes. In one study, it has been postulated that the association between pulse pressure and diabetes leads to increase in the stiffness of the arteries[52].

There are usually high to normal glucose levels and mild hyperglycaemia is constantly observed in the insulin resistant subjects as well as in the subjects of hypertensives[53].Some other findings also suggest that the increased blood pressure may contribute to increased risk of type 2 diabetes[54-57].

The other mechanism where blood pressure is high in post menopausal women associated with aging is the activation of renin angiotensin system (RAS). The plasma renin activity increases in the post menopausal women which suggest the activation of renin angiotensin system (RAS). The genetic component of RAS as renin gene polymorphisms that contributes to post menopausal hypertension in women aged 40 to 70[58-60].

Still it is not clear about the mechanisms where increase in the blood pressure shows negative effect on diabetes risk. The association of BP and endothelial dysfunction could be one of the possible explanations which may be related to insulin resistance and development of diabetes[61].

Mechanism between Menopause, Diabetes and Blood pressure:

There are diverse mechanisms for elevating blood pressure in post menopausal diabetic women. The decrease in the sex steroids, 'estrogen' and 'progesterone' leads to obesity by decreasing the levels of leptin. This increases the sympathetic activity of kidney. It increases renal vascular resistance and it ultimately it increases the blood pressure. Possibly, the decrease in the estrogen and progesterone levels and increasing the androgen levels leads to increase in the angiotensin II . These factors leads to the dysfunction of endothelium. It increases renal vascular resistance(RVS) and thus increase in the blood pressure. At the same time, the other mechanism which is responsible for the elevation of blood pressure is, decline in the estrogen levels which may have direct effect on insulin sensitive cells **(Figure 12)**. Therefore it develops hyperglycaemia which also induces renal sodium retention and extra vascular shift of fluid and sodium. These all factors contributes to the development of insulin resistance thus by increasing renal vascular resistance which may finally elevates the blood pressure in post menopausal diabetics[62-64].

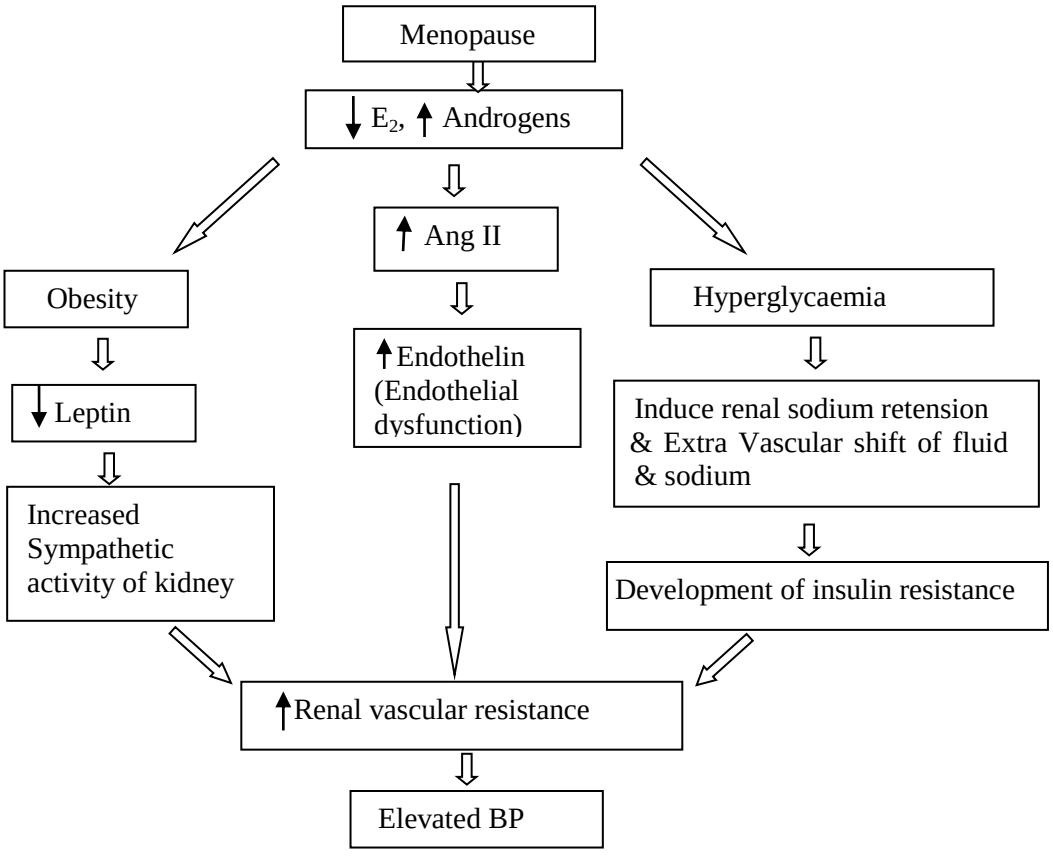


Figure 12: Mechanism between Menopause, Diabetes and Blood Pressure

6.3.Haematological profile:

In the present study, there is no much difference observed in haematological profile.WBC count significantly increased in post menopausal diabetic women compared to the other groups and one of the causes may be insulin resistance.

Chronic low grade inflammation has been considered as an important component in the pathogenesis of development of insulin resistance and type 2 diabetes mellitus[65].

If there is defect in the action of insulin on the insulin sensitive tissues like adipose tissues, muscle and liver may contributes to a chronic, low grade state of inflammation. So the process which may be linked to chronic state of inflammation may lead to reduced action of insulin and insulin resistance[66].

"Inflammation on its own can impair insulin signaling and promote β cell death[67,68]." There is an evidence from some of the epidemiological studies suggests that there is an "association between the white blood cell count (WBC), a non-specific marker of inflammation and risk of diabetes[69].

Many studies demonstrated that higher white blood cell count is linked to metabolic syndrome but the possible explanation is not clear. The pathophysiological mechanism that may link increase in the white blood cell count to insulin resistance was not completely understood. Some authors have concluded that both the white blood cell count (WBC) and insulin resistance may depend on the activation of the immune system[70].

Our finding is in agreement with other researchers that, increase in the WBC count in post menopausal diabetic women might be associated with the deterioration of glucose metabolism[71-74].

6.4. Blood glucose profile:

In the present study, glucose levels are more in pre menopause diabetics compared to other groups. The exact reason is not known but it might be assumed that early menarcheal age might be one of the reasons for increased risk of developing diabetes which is in agreement with several studies[75-77].

"Baek et al. studied in Korean women and defined early age at menarche as less than 13 years and average age at menarche as 13-16 years. Early menarche was significantly associated with pre diabetes, diabetes, and hyperglycemia" [78].

Similar results were observed in a study of women in the USA by Chen et al. defining early menarche as ≤12 years and average age at menarche as 12–14 years of age[79]. Decreased menarcheal age associated with diabetes suggests that there are several factors aside from reproductive aged hormonal factors which may also effects the glucose metabolism. However in our study, menarcheal age may not taken as important factor but we assumed that menarche at earlier age may also play a significant role in development of diabetes in further phases of women life.

We also predict the other reason for increased blood glucose levels in pre menopausal diabetics compared to post menopausal diabetics is, premenopausal women may be prone to more stress at the time of menstruation and has increased secretion of cortisol. Excess secretion of cortisol may counteract insulin secretion and it might have contributed to hyperglycaemia by causing hepatic gluconeogenesis which may ultimately leads to the development of insulin resistance[80]. The increased secretion of glucocorticoids associated with stress in premenopausal diabetic women may counteract and interfere with the glucose regulation and leading to hyperglycaemia.

6.5. Thyroid Hormonal Assay:

Thyroid disorder is a pathological state that negatively affects the diabetic control. It is found to be common in most forms of diabetes mellitus which is associated with type 2 diabetes as age advances[81].

There is an underlying relationship between diabetes and thyroid disorders. Hyper and hypothyroidism have been associated with insulin resistance. This has been reported to be the major cause of impaired glucose metabolism in diabetes[82].

In the present study, there are increased levels of T_3, T_4 and decreased levels of TSH in post menopausal diabetics. The possible reason may be due to negative feedback mechanism. Glucose metabolism may be affected by thyroid hormones by its different actions on intermediary metabolism

Thyroid hormones in excess play a major role in promoting hyperglycemia by increasing the intestinal glucose absorption, facilitating the clearance of insulin, increasing glycogenolysis and gluconeogenesis[83].

Thyroid hormones also take part in the regulation of expression of genes such as GLUT-4 and Phosphoglycerate kinase that are usually involved in the glucose transport and glycolysis respectively. Thus acting together with insulin and also facilitates the disposal of glucose and peripheral utilization[84, 85].

However thyroid hormones contributes to many changes in the expression of gene and many of these actions shows complexity[86]. " It might be due to promotion of glycation of malondialdehyde which is produced by peroxidation of lipid which may be brought by increased level of thyroid hormones" [87,88].

In women with excess thyroid hormone secretion, there is reduced insulin stimulated peripheral utilization of glucose[89]. "There is impaired uptake of glucose in the tissues of thyrotoxic women is because of lower glucose extraction from serum in proportion to increased blood flow" [90].

The actions of thyroid hormones on peripheral tissues further explain the overall effects of functions of thyroid on insulin action and uptake of glucose. The secretion of insulin is under direct control of thyroid hormones. The response of β-cell

to glucose and stimulation of catecholamines is increased in hyperthyroidism. This is also accompanied by increased mass of β-cell[91,92].

The disposal of glucose is intervened by combining effect of insulin and hyperglycaemia modulates the most important three basic phenomenons.

First phenomena includes the weakening of the endogenous hepatic glucose production.

Second phenomena is enhancement of glucose uptake.

Third phenomena is glucose upregulation by the peripheral tissues (skeletal muscles). The process of glycolysis and synthesis of glycogen modulates the uptake of glucose into the muscles[93].

Recent studies revealed that thyroid hormones increases the apoptosis of β cell and this could be one of the major cause for the worsening of glucose tolerance in hyperthyroid subjects[94,95].

The most important novel finding which includes the hepatic glucose production stimulation by thyroid hormones acts through sympathetic pathway from the hypothalamus. So the discovery of the transcription regulatory factors of metabolism and genes of mitochondria which were influenced by the levels of intracellular tri-iodo thyronine (T_3) may also leads to the development of insulin resistance[96,97].

The other explanation for the development of peripheral insulin resistance is increased secretion of adipokines includes interleukin 6 (IL6) and tumour necrosis factor α (TNF α) from the adipose tissue and these adipokines exerts pro inflammatory effects of insulin resistance and found to be elevated in hyperthyroid females[98].

Some studies showed that there is increased insulin degradation in the subjects with increased thyroid hormone levels. In long term run, severe thyrotoxicosis may also lead to irreversible pancreatic damage [99-102].

"The term harmonious quartet is used to address the core pathology of insulin resistance[103]". The most important factors which play a key role in pathogenesis of

type 2 diabetes are the deregulation of the disposal of glucose and metabolism in adipocytes, muscles and liver. This may contributes to the impairment in the secretion of insulin by the pancreatic β cells[104].

"It is worth considering that insulin resistance has been a proven condition in hyperthyroidism as well as hypothyroidism", suggesting that there is a possible link between insulin resistance, diabetes and thyroid dysfunction" in post menopausal women"[105].

In regression coefficient analysis, by taking thyroid hormones (T_3,T_4,TSH) as dependent variables and other variables as independent, we found HbA1c showed significance increase with T_4 in pre menopausal non-diabetic women.

It might be due to differential actions of thyroid hormones on liver, skeletal muscle, adipose tissue. It's been known from long time that thyroid hormones may act differentially on the main target organs of insulin action such as liver, skeletal muscle, adipose tissue. Thyroid hormones opposes and inhibits the action of insulin by stimulating hepatic gluconeogenesis and glycogenolysis[106,107].

In our study, LH, FSH showed significant increase with decreased TSH levels in post menopausal non-diabetics. The exact reason was not known and it may be due to decreased activity of TRH which in turn decreases TSH in menopausal women.

Clinical and experimental studies have suggested a close relationship between the hypothalamic-pituitary-thyroid axis and the hypothalamic-pituitary-ovarian axis[108].

Mechanism between Thyroid(Euthyroid) and glucose homeostasis:

"Thyroid hormones exert profound effects on glucose homeostasis". These effects includes modification of circulating levels of insulin, counter- regulatory hormones, intestinal absorption, hepatic production and peripheral uptake of glucose by tissues(fat and muscles)**(Figure 13)**. As mentioned earlier, "thyroid hormones opposes the action of insulin and stimulates hepatic gluconeogenesis and glycogenolysis by up regulating the expression of genes GLUT-4 and phosphoglycerate kinase thus acting synergistically with insulin" [109-112].

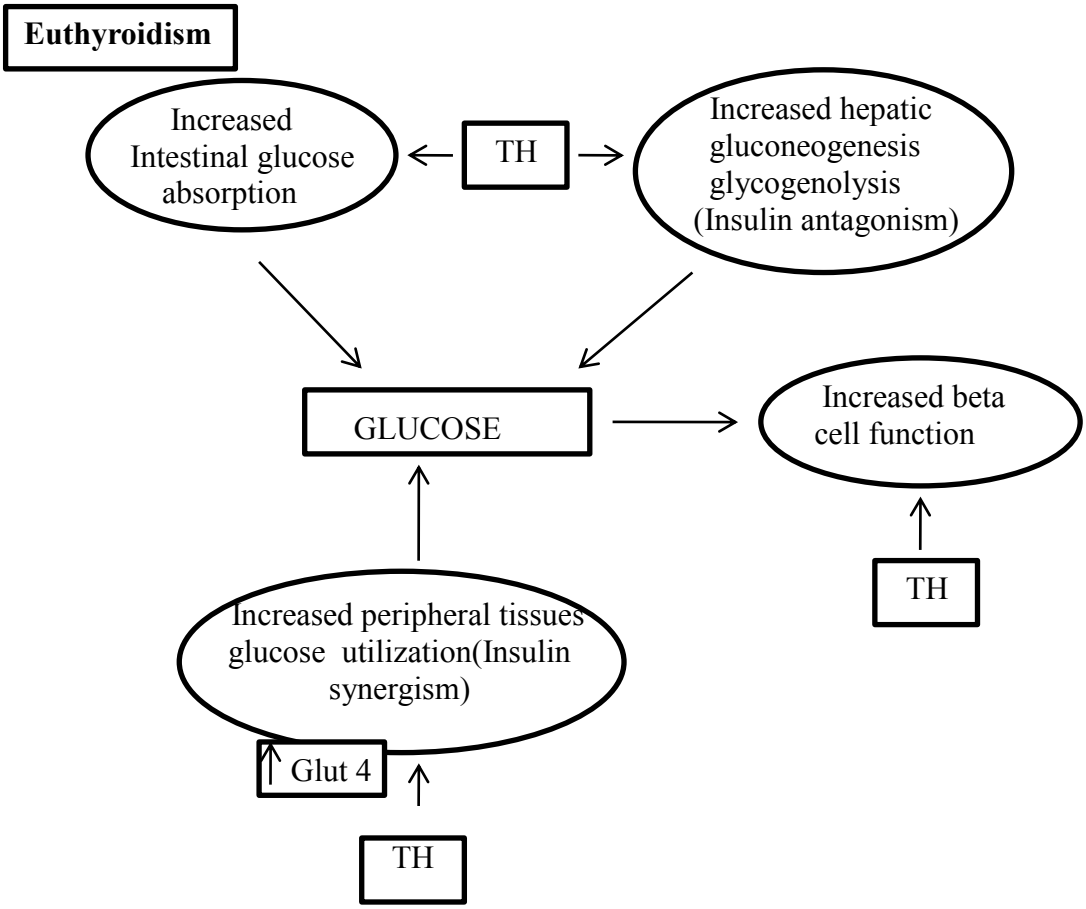


Figure 13 : Effects of thyroid hormone on glucose homeostasis (Euthyroidism)[113]

Mechanism between Hyperthyroidism (Thyrotoxicosis) and glucose homeostasis.

There is a great impact of thyroid hormones on glucose control. In post menopausal diabetic women, when there is dysfunction of thyroid, the homeostatic balance of glucose will be disrupted. Insulin resistance is associated mainly with increased hepatic gluconeogenesis**(Figure 14)** and it explains why glucose control deteriorates when diabetic patients develop hyperthyroidism[114].

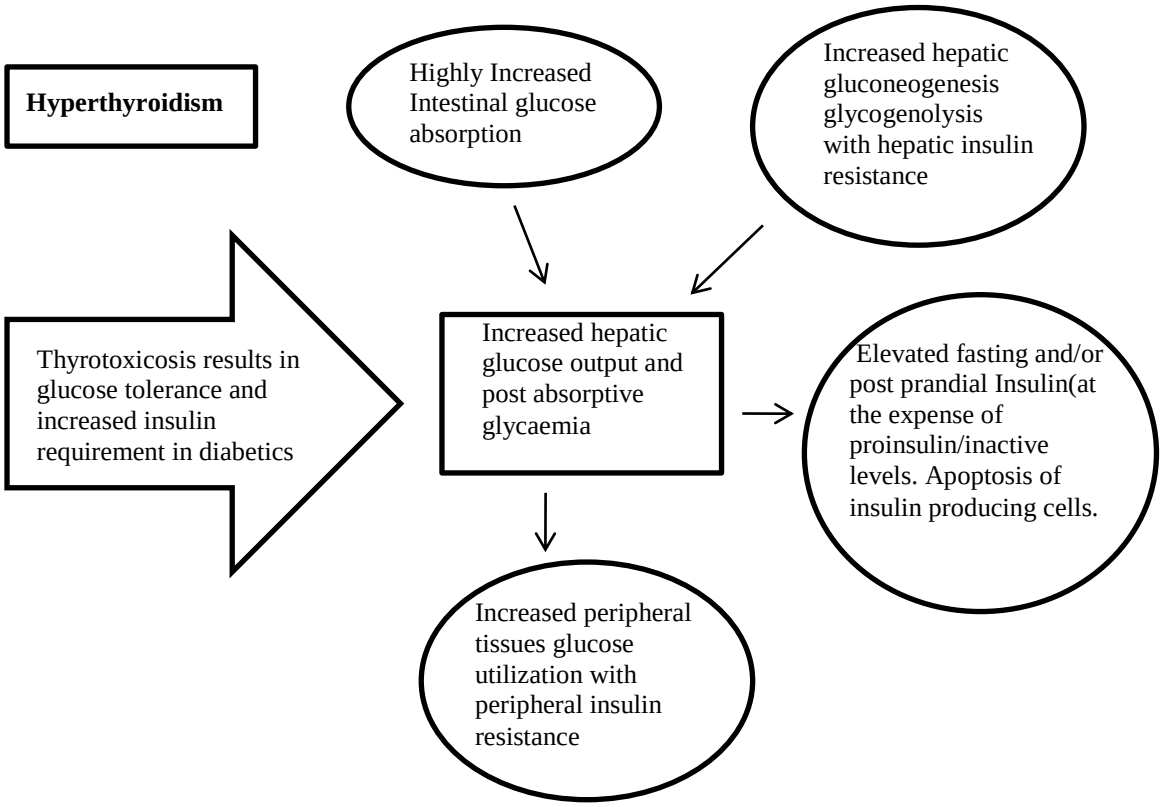


Figure 14 : Hyperthyroidism (Thyrotoxicosis) and glucose homeostasis[113].

Mechanism between Hypothyroidism and glucose homeostasis.

Glucose homeostasis is also affected in hypothyroidism .Hypothyroidism results in unimpaired or decreased hepatic output of glucose **(Figure 15)**. It is thereby compensating the insulin resistance present in peripheral tissues and it accounts for decreased requirement of insulin for glycaemic control in hypothyroid diabetic patients[115-117].

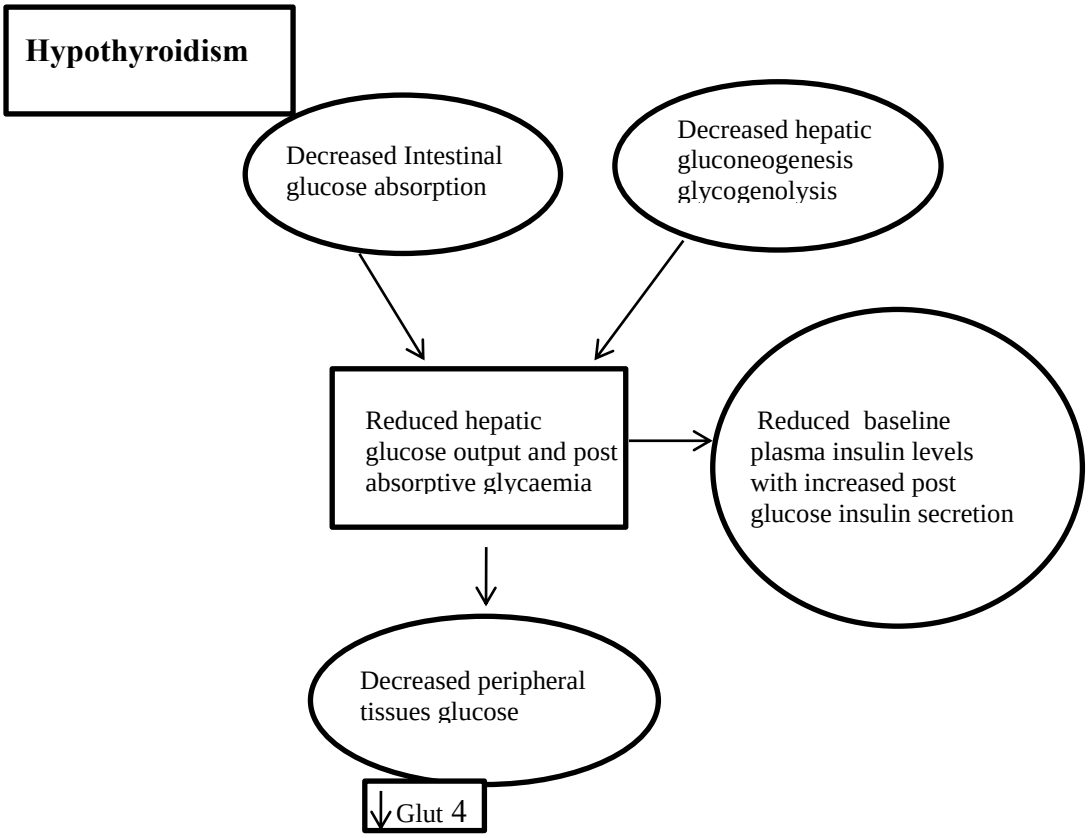


Figure 15 : Hypothyroidism and glucose homeostasis[113].

6.6 Reproducitve hormonal assay:

6.6.1. LH and FSH:

Here we found decreased levels of LH and FSH in Post menopausal diabetic group compared to non- diabetic group. Decreased levels of LH, FSH are associated with Higher Fasting blood sugar levels and HbA1c which might be associated with increasing prevalence of pre-diabetes and diabetes in post menopausal women.

Rise of LH and FSH levels in non-diabetics may be due to decrease in the estrogen which is mediated through Negative feedback mechanism.

"There is evidence that, the hyperglycaemia interferes with the basal levels of gonadotropins and with the levels following stimulation with either GnRH alone or with GnRH" [118].

Some of the studies hypothesized that FSH may be associated with diabetes through inflammatory markers such as C-reactive protein, TNF-α and IL-1β and it was postulated that low grade systemic inflammation was related to the development of diabetes and it is associated with the suppression of gonadotropins in diabetics[119-122].

FSH is also found to be associated with adiposity. It has been hypothesized that there is also a possible link between follicle stimulating hormone (FSH) and obesity in menopausal women."Obesity related patterns of relative change in FSH during the transition of menopause may also explain the observed change in FSH and incidence of diabetes but the mechanism is unclear"[123,124].

The low levels of FSH in diabetic women might be attributed to increased production of endogenous estrogens by mesenchymal tissue and all these factors may lead to the development of diabetes in post menopausal women[125-128]. "These associations might be explained by adiposity and insulin resistance to a limited extent. It is needed to find whether FSH is a protective biomarker in the metabolism of glucose, in the post menopausal women" [129].

6.6.2. Estradiol and Progesterone:

Estradiol and Progesterone levels decreased in post menopausal diabetic group compared to the post menopausal non-diabetic group." Our findings is in agreement with Godsland IF who showed that estrogen deficiency in post menopausal is associated with deterioration in glucose homeostasis and insulin resistance and estrogen replacement in humans is associated with increased insulin secretion[130],,.

In women, synthesis of sex hormone binding globulin (SHBG) is stimulated by estrogen but the presence of androgen, insulin and overweight may contributes to the decreased secretion of SHBG particularly in post menopausal women. There were so many studies postulated that the insulin is considered as the strong inhibitor of synthesis of SHBG. So the estrogen influences the secretion of SHBG which is a good indicator of insulin resistance in post menopausal women[131,132].

Several studies explained that low or decreased levels of sex hormone binding globulins (SHBG) may be linked to insulin resistance and incidence of diabetes in postmenopausal females[133-135].

The actions of insulin may be regulated by estradiol directly through actions on insulin sensitive tissues or indirectly by the different regulating factors like oxidative stress which may contributes to the development of insulin resistance[136].

It has not been clearly explained about the mechanism and action of estrogen on the glucose metabolism. Studies revealed that estrogen related mechanisms decreases in fasting glucose levels and it might be due to suppressed production of hepatic glucose [137,138].

While, other studies suggests that, estrogen increases the elimination of hepatic insulin along with no other compensatory increase in the main pancreatic response to insulin and it leads to relative decrease in the initial level of plasma insulin and it would results in the reduction of the elimination of glucose and that ultimately leads to rise in overall glucose concentration[139].

The effect of estradiol on post menopausal diabetics is a combination of different factors. There is "direct effect of estrogen on insulin signaling in insulin sensitive tissue. The effect of estrogen on pancreatic β cells regulating the release of

insulin, and its role in adipose tissue metabolism and energy expenditure. The effect on the hypothalamus in regulation of food intake, and its effects on energetics and metabolism[140]".

"It is well known that disruptors (chemicals which interfere with endocrine system) of endocrine system play a major part in the incidence of various metabolic diseases. The β cell of pancreas which plays an important role in blood glucose homeostasis is a target for estrogen. Among them the levels of estradiol may contribute to the insulin resistance and diabetes[141]".

"Fukui et al showed that the estrogen and progesterone could decrease the insulin sensitivity[142]. There is also a possible link between estrogen and obesity in post menopausal diabetics as adipose tissue is one of the important source of estrogen which is formed by aromatization of androgens after menopause"[143, 144]. So such changes in body fat distribution as well as there is an increased proinflamatory cytokines during the menopausal transition. These all changes have been associated with glucose homeostasis[145,146]. "The change in the level of estradiol during menopausal transition may slowly offset the effect of estradiol on diabetic risk"[123].

Women are more prone to thyroid disorders and this confirms the role of estrogen in the functioning of thyroid gland[147]. Thyroid hormone and estradiol modulate biological processes by binding to nuclear receptor proteins. "Thyroid hormone interactions with specific response elements in the regulatory regions of genes, modulate gene transcription. Both the estrogen receptor (ER) and thyroid hormone receptor (TR) are members of the nuclear receptor superfamily"[148].

There are certain evidences that estrogen may have direct actions in human thyroid cells by ER-dependent mechanisms , modulating proliferation, and function. Different patterns of distribution, expression, and ratios of ERα and ERß may have a role[149].

"Estrogen binding to ERα would promote cell proliferation and growth and ERß would promote apoptotic actions and other suppressive functions in thyroid tumors, as reviewed by Chen et al"[150].

6.6.3. Possible mechanism of the present study

We propose the possible mechanism of the influence of thyroid hormones on post menopausal diabetic women. **Figure 16** shows, the possible relationship exists between thyroid hormones and glucose homeostasis. At the same time there is also relationship exists between thyroid hormones and menopause. So there is differential regulation of blood glucose levels influenced by thyroid hormones in case of post menopausal women may be expected.

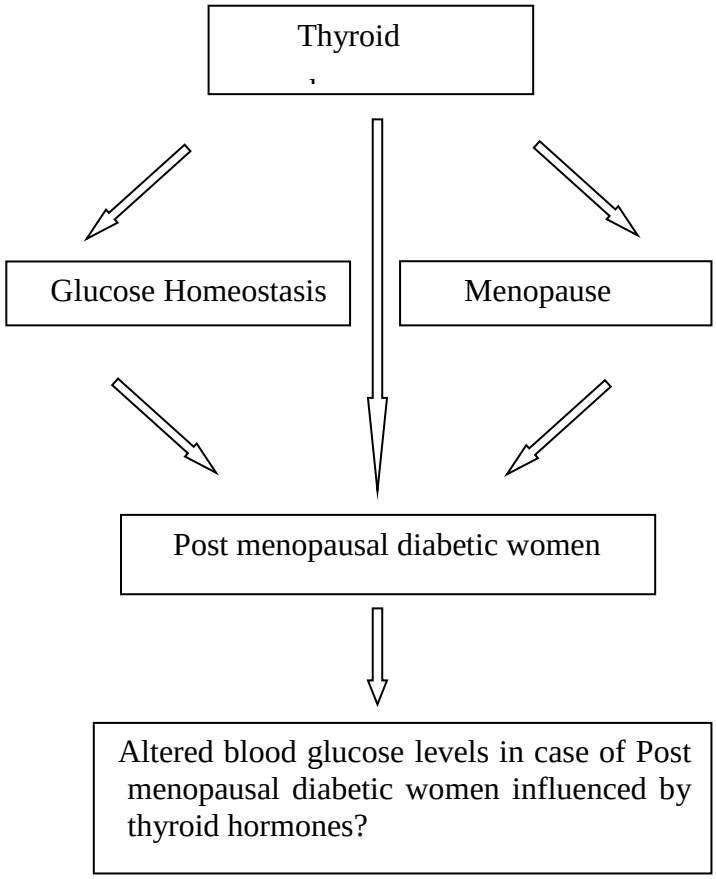


Figure 16: Possible mechanism of the effect of thyroid hormones on glucose homeostasis in post menopausal diabetic women.

REFERENCES:

1. Wing RR, Malthews KA, Kuller LH, Meilahn EN, Plantinga PL. Weight gain at the time of menopause. Arch Int Med 1991; 151(1):97-102.
2. Svendsen OL, Hassager C, Christiansen C. Age-and menopause-associated variations in body composition and fat distribution in healthy women as measured by dual-energy X-ray absorptiometry. Metabolism 1995; 44(3): 369-73.
3. The North American Menopause Society (NAMS): Menopause Guidebook.United States of America: 7th edition.2012.
4. Rebuffé-Scrive M, Eldh J, Hafström LO, Björntorp P. Metabolism of mammary, abdominal, and femoral adipocytes in women before and after menopause. Metab Clin Exp 1986; 35(9):792-7.
5. Douchi T, Kosha S, Uto H, Oki T, Nakae M, Yoshimitsu N, Nagata Y.Precedence of bone loss over changes in body composition and body fat distribution within a few years after menopause. Maturitas 2003; 46(2):133-8.
6. Sowers M, Zheng H, Tomey K, Karvonen-Gutierrez C, Jannausch M, Li X, Yosef M, Symons J. Changes in body composition in women over six years at midlife: ovarian and chronological aging. J Clin Endocrinol & Metab 2007 ; 92(3):895-901.
7. Satyanarayana U, Chakrapani U. Biochemistry. 3rd ed. Kolkata: Books and allied (P) Ltd;2006.
8. Lipscombe LL, Hux JE. Trends in diabetes prevalence, incidence, and mortality in Ontario, Canada 1995–2005: a population-based study. The Lancet. 2007;369(9563):750-6.
9. Hossain P, Kawar B, EI Nahas M. Obesity and diabetes in the developing world—a growing challenge. N Engl J Med 2007;356(3):213-5.
10. Gogelienė L, Strupas K. Comparison of changes in blood glucose, insulin resistance indices, and adipokine levels in diabetic and nondiabetic subjects with morbid obesity after laparoscopic adjustable gastric banding. Medicina (Kaunas) 2013;49(1):9-14.
11. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 2003;52(1):102-10.

12. Koerner A, Kratzsch J, Kiess W. Adipocytokines: leptin-the classsical, resistin-thecontroversial, adiponectin- the promissing, and more to come. Best Pract Res Clin Endocrinol Metab 2005; 19(4): 525-546.
13. Kadowaki T, Yamauchi T, Kubota N, et al. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116(7):1784-1792.
14. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, Nagai M, Matsuzawa Y, Funahashi T. Adiponectin as a biomarker of the metabolic syndrome. Circulation journal 2004;68(11):975-81.
15. Hattori A, Uemura K, Miura H, Ueda M, Tamaya N, Iwata F, Muraguchi M, Ohmoto Y, Iguchi A. Gender-related difference in relationship between insulin resistance and serum leptin level in Japanese type 2 diabetic and non-diabetic subjects. Endocr J 2000;47(5):615-21.
16. Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. Diabetes 1996; 45(11):1455-63.
17. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995 ; 269(5223):540-3.
18. Sivitz WI, Walsh SA, Morgan DA, Thomas MJ, Haynes WG. Effects of leptin on insulin sensitivity in normal rats. Endocrinology 1997;138(8):3395-401.
19. Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ. Acute stimulation of glucose metabolism in mice by leptin treatment. Nature 1997 ;389(6649):374.
20. Oral EA, Ruiz E, Andewelt A, Sebring N, Wagner AJ, Depaoli AM, Gorden P. Effect of leptin replacement on pituitary hormone regulation in patients with severe lipodystrophy. J Clin Endocrinol Metab 2002;87(7):3110-7.
21. Timothy J, Kiffer JF. The adipoinsular axis: effects of leptin on pancreatic β-cell. Am J Physiol 2000;278:E1-14.
22. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994 ;372(6505):425-432.
23. Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM. Abnormal splicing of the leptin receptor in diabetic mice. Nature 1996;379(6566):632-5.

24. Niswender KD, Magnuson MA. Obesity and the β cell: lessons from leptin. J Clin Invest 2007;117(10):2753-6.
25. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. Jama. 2003;289(1):76-9.
26. Gogelienė L, Strupas K. Comparison of changes in blood glucose, insulin resistance indices, and adipokine levels in diabetic and nondiabetic subjects with morbid obesity after laparoscopic adjustable gastric banding. Medicina (Kaunas) 2013;49(1):9-14.
27. Jung RT. Obesity and nutritional factors in the pathogenesis of non-insulin dependent diabetes mellitus. Textbook of diabetes. 2nd ed. London:Blackwell Science Ltd;1997.
28. Steppan CM, Bailey ST, Bhat S.The hormone resistin links obesity to diabetes. Nature 2001 ;409(6818):307-12.
29. Lukanova A, Lundin E, Zeleniuch-Jacquotte A. Body mass index, circulating levels of sex-steroid hormones, IGF-I and IGF-binding protein-3: a cross-sectional study in healthy women. Eur J Endocrinol 2004;150(2):161-71.
30. Block NE, Buse MG. Effects of hypercortisolemia and diabetes on skeletal muscle insulin receptor function in vitro and in vivo. Am J Physiol Endocrinol Metab 1989 ; 256(1):E39-48.
31. Dubnov-Raz G, Pines A, Berry EM. Diet and lifestyle in managing postmenopausal obesity. Climacteric 2007;10(sup2):38-41.
32. Svendsen OL, Hassager C, Christiansen C. Relationships and independence of body composition, sex hormones, fat distribution and other cardiovascular risk factors in overweight postmenopausal women. Int J Obes Relat Metab Disord 1993; 17(8):459-63.
33. Pasquali R, Vicennati V, Bertazzo D, Casimirri F, Pascal G, Tortelli O, Labate AM. Determinants of sex hormone—binding globulin blood concentrations in premenopausal and postmenopausal women with different estrogen status. Metabolism 1997;46(1):5-9.
34. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444(7121):840-6.

35. Seely EW, Walsh BW, Gerhard MD, Williams GH. Estradiol with or without progesterone and ambulatory blood pressure in postmenopausal women. Hypertension 1999; 33(5):1190-4.
36. Rönnback M, Fagerudd J, Forsblom C, Pettersson-Fernholm K, Reunanen A, Groop PH. Altered age-related blood pressure pattern in type 1 diabetes. Circulation 2004; 110(9):1076-82.
37. Nichols WW, O'Rourke MF . Ageing, high blood pressure and disease in humans. In: McDonald's blood flow in arteries, theoretical, experimental and clinical principals.4th ed. London:Edward Arnold; 1998:54–113.
38. Dubey RK, Oparil S, Imthurn B, Jackson EK. Sex hormones and hypertension. Cardiovasc Res 2002;53(3):688–708.
39. Oneda B, Cardoso Jr CG, Foriaz CL, et al. Effects of estrogen therapy and aerobic training on sympathetic activity and hemodynamics in healthy postmenopausal women: a double-blind randomized trial. Menopause 2014;21(4):369–375.
40. Novella S, Dantas AP, Segarra G, et al. Gathering of aging and estrogen withdrawal in vascular dysfunction of senescent accelerated mice. Exp Gerontol 2010;45(11):868–874.
41. Vegeto E, Belcredito S, Etteri S, Ghisletti S, Brusadelli A, Meda C, Krust A, Dupont S, Ciana P, hambon P, Maggi A. Estrogen receptor-α mediates the brain antiinflammatory activity of estradiol. Proc Natl Acad Sci.2003 ;100(16):9614-9.
42. Darblade B, Pendaries C, Krust A, Dupont S, Fouque MJ, Rami J, Chambon P, Bayard F, Arnal JF. Estradiol alters nitric oxide production in the mouse aorta through the α-, but not β-, estrogen receptor. *Circ Res* 2002;90(4):413-9.
43. Geary GG, McNeill AM, Ospina JA, Krause DN, Korach KS, Duckles SP. Selected contribution: cerebrovascular nos and cyclooxygenase are unaffected by estrogen in mice lacking estrogen receptor-α. J Appl Physiol 2001 ;91(5):2391-9.
44. Chakrabarti S, Morton JS, Davidge ST. Mechanisms of estrogen effects on the endothelium: an overview. Can J Cardiol 2014;30(7):705–712.

45. Brillante DG, O'Sullivan AJ, Howes LG. Arterial stiffness in insulin resistance: the role of nitric oxide and angiotensin II receptors. Vasc Health Risk Manag 2009;5:73–78.
46. Yki-Järvinen H., & Westerbacka J. Insulin resistance, arterial stiffness and wave reflection. Adv Cardiol 2007;44:252-60.
47. Lefebvre PJ, Scheen AJ. The postprandial state and risk of cardiovascular disease. Diabet Med 1998; 15 (suppl 4):S63–8.
48. Rydén Ahlgren A, Länne T, Wollmer P, Sonesson B et al. Increased arterial stiffness in women, but not in men, with IDDM. Diabetologia 1995;38(9):1082-9.
49. Salomaa V, Riley W, Kark JD, Nardo C, Folsom AR. Non–insulin-dependent diabetes mellitus and fasting glucose and insulin concentrations are associated with arterial stiffness indexes: the ARIC Study. Circulation 1995 ;91(5):1432-43.
50. Emoto M, Nishizawa Y, Kawagishi T et al. Stiffness index beta of the common carotid and femoral arteries are associated with insulin resistance in NIDDM. Diabetes Care 1998; 21(7): 1178–1182.
51. Vavuranakis M, Stefanadis C, Triandaphyllidi E, Toutouzas K, Toutouzas P. Coronary artery distensibility in diabetic patients with simultaneous measurements of luminal area and intracoronary pressure: evidence of impaired reactivity to nitroglycerin. J Am Coll Cardio 1999;34(4):1075-81.
52. Yasuno S, Ueshima K, Oba K et al. Is pulse pressure a predictor of new-onset diabetes in high-risk hypertensive patients? a subanalysis of the Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial. Diabetes care 2010;33(5):1122-7.
53. Cubeddu LX, Hoffmann IS. Insulin resistance and upper-normal glucose levels in hypertension: a review. J Hum Hypertens 2002;16(S1):S52-S55.
54. Conen D, Ridker PM, Mora S, Buring JE, Glynn RJ. Blood pressure and risk of developing type 2 diabetes mellitus: the Women's Health Study. Eur Heart J.2007;28(23):2937-43.
55. Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus.N Engl J Med 2000;342(13):905-12.

56. Stolk RP, Van Splunder IP, Schouten JS, Witteman JC, Hofman A, Grobbee DE. High blood pressure and the incidence of non-insulin dependent diabetes mellitus: findings in a 11.5 year follow-up study in The Netherlands. Eur J Epidemiol 1993 ;9(2):134-9.
57. Meisinger C, Döring A, Heier M. Blood pressure and risk of type 2 diabetes mellitus in men and women from the general population: the Monitoring Trends and Determinants on Cardiovascular Diseases/Cooperative Health Research in the Region of Augsburg Cohort Study. J Hypertens 2008;26(9):1809-15.
58. chunkert H, Danser AJ, Hense HW, Derkx FH, Ku S. Effects of estrogen replacement therapy on the renin-angiotensin system in postmenopausal women. Circulation 1997;95(1):39-45.
59. Fernández-Vega F, Abellán J, Vegazo O, De Vinuesa SG, Rodríguez JC, Maceira B, De Castro SS, Nicolás RR, Luño J. Angiotensin II type 1 receptor blockade to control blood pressure in postmenopausal women: influence of hormone replacement therapy. Kidney Int 2002;62:S36-41.
60. Mansego ML, Redon J, Marin R et al . Renin polymorphisms and haplotypes are associated with blood pressure levels and hypertension risk in postmenopausal women. J Hypertens 2008;26(2):230-7.
61. Julius S, Gudbrandsson T, Jamerson K, Shahab ST, Andersson O. The hemodynamic link between insulin resistance and hypertension. J Hypertens 1991; 9(11):983-6.
62. Rapelli A. Hypertension and obesity after the menopause. J Hypertens Suppl 2002 ; 20(2): S26-8.
63. Rahmouni K, Correia ML, Haynes WG, Mark AL. Obesity-associated hypertension:new insights into mechanisms.Hypertension 2005;45(1):9-14.
64. Yanes LL, Reckelhoff JF. Postmenopausal hypertension. Am J Hypertens 2011 ;24(7):740-9.
65. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 2011;11(2):98-107.
66. De Luca C, Olefsky JM. Inflammation and insulin resistance. FEBS Lett 2008 ;582(1):97-105.
67. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006 ;444(7121):860-7.

68. Donath MY, Ehses JA, Maedler K, Schumann DM, Ellingsgaard H, Eppler E, Reinecke M. Mechanisms of β-cell death in type 2 diabetes. Diabetes 2005 ;54(suppl 2):S108-13.
69. Twig G, Afek A, Shamiss A, Derazne E, Tzur D, Gordon B, Tirosh A. White blood cells count and incidence of type 2 diabetes in young men. Diabetes care 2013;36(2):276-82.
70. Leng S, Xue QL, Huang Y, Semba R, Chaves P, Bandeen-Roche K, Fried L, Walston J. Total and differential white blood cell counts and their associations with circulating interleukin-6 levels in community-dwelling older women. J Gerontol A Biol Sci Med Sci 2005;60(2):195-9.
71. Fritsche A, Häring H, Stumvoll M. White blood cell count as a predictor of glucose tolerance and insulin sensitivity. The role of inflammation in the pathogenesis of type 2 diabetes mellitus. Dtsch Med Wochenschr 2004;129(6):244-8.
72. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002 ;51(2):455-61.
73. Gokulakrishnan K, Deepa R, Sampathkumar R, Balasubramanyam M, Mohan V. Association of leukocyte count with varying degrees of glucose intolerance in Asian Indians: the Chennai Urban Rural Epidemiology Study (CURES-26). Metab Syndr Relat Disord 2009; 7(3):205-10.
74. Nakanishi N, Yoshida H, Matsuo Y, Suzuki K, Tatara K. White blood-cell count and the risk of impaired fasting glucose or Type II diabetes in middle-aged Japanese men. Diabetologia 2002;45(1):42-8.
75. Day FR, Elks CE, Murray A, et al. Puberty timing associated with diabetes, cardiovascular disease and also diverse health outcomes in men and women: the UK Biobank study. Sci Rep 2015; 18(5): 11208.
76. He C, Zhang C, Hunter DJ, et al. Age at menarche and risk of type 2 diabetes: results from 2 large prospective cohort studies. Am J Epidemiol 2010; 171(3):334–44.
77. Lakshman R, Forouhi N, Luben R, et al. Association between age at menarche and risk of diabetes in adults: results from the EPIC-Norfolk cohort study. Diabetologia 2008; 51(5):781–6.

78. Baek TH, Lim NK, Kim MJ, Lee J, Ryu S, Chang Y, Choi Y, Park HY. Age at menarche and its association with dysglycemia in Korean middle-aged women. Menopause (New York, NY) 2015; 22(5):542.
79. Chen L, Zhang C, Yeung E, Ye A, Mumford SL, Wactawski-Wende J, Schisterman EF. Age at menarche and metabolic markers for type 2 diabetes in premenopausal women: the BioCycle Study. J Clin Endocrinol & Metabo 2011; 96(6):E1007-12.
80. Brown DF, Brown DD. USMLE Step 1 Secrets: Questions You Will Be Asked on USMLE Step 1. Philadelphia: Hanley & Belfus; 2003: 63
81. Shah SN. Thyroid disease in diabetes mellitus. J Assoc Phys India 2007; 32(12): 1057-59.
82. Brenta G, Danzi S, Klein I. Potential therapeutic applications of thyroid hormone analogs. Nat Clin Pract Endocrinol Metab 2007;3(9):632-40.
83. Potenza M, Via MA, Yanagisawa RT. Excess thyroid hormone and carbohydrate metabolism. Endocr Pract 2009 ; 15(3): 254–62.
84. Clement K, Viguerie N, Diehn M, Alizadeh A, Barbe P, Thalamas C, Storey JD,Brown PO, Barsh GS, Langin D. Invivo regulation of human skeletal muscle gene expression by thyroid hormone. Genome Res 2002; 12(2):281-91.
85. Viguerie N, Millet L, Avizou S, Vidal H, Larrouy D, Langin D. Regulation of human adipocyte gene expression by thyroid hormone. J Clin Endocrinol Metab 2002; 87(2):630-34.
86. Sohal S, Wats A, ViJ C. Evaluation of glycosylated hemoglobin (HbA1c) levels in hypothyroid and hyperthyroid patients. Ann Appl Biosci 2016 ; 3(1):A101-107.
87. Mohan Kumar KM, Bobby Z, Selvaraj N, Kumar Das A, Chandra Koner B, Sen SK, et al. Possible link between glycated hemoglobin and lipid peroxidation in hyperthyroidism.ClinChimActa 2004;342:187-92.
88. Jain SK, Palmer M. The effect of oxygen radicals metabolites and Vitamin E on glycosylation of proteins. Free Radic Biol Med 1997; 22:593-6.
89. Shen DC, Davidson MB, Kuo SW, Sheu WH. Peripheral and hepatic insulin antagonism in hyperthyroidism. J Clin Endocrinol Metab 1988;66:565-9.

90. Dimitriadis G, Mitrou P, Lambadiari V et al. Insulin-stimulated rates of glucose uptake in muscle in hyperthyroidism: the importance of blood flow. J Clin Endocrinol Metab 2008;93:2413-15.
91. Stanická S, Vondra K, Pelikánová T, Vlček P, Hill M, Zamrazil V. Insulin sensitivity and counter-regulatory hormones in hypothyroidism and during thyroid hormone replacement therapy. Clin Chem Lab Med 2005 ;43(7):715-20.
92. Mitrou P, Raptis SA, Dimitriadis G. Insulin action in hyperthyroidism: a focus on muscle and adipose tissue. Endocr Rev 2010 ; 31(5):663-79.
93. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am 2004 ;88(4):787-835.
94. Klieverik LP, Janssen SF, van Riel A, Foppen E, Bisschop PH, Serlie MJ, Boelen A, Ackermans MT, Sauerwein HP, Fliers E, Kalsbeek A. Thyroid hormone modulates glucose production via a sympathetic pathway from the hypothalamic paraventricular nucleus to the liver. Proc Natl Acad Sci U S A 2009;106(14):5966-71.
95. Crunkhorn S, Patti ME. Links between thyroid hormone action, oxidative metabolism, and diabetes risk?. Thyroid 2008 ;18(2):227-37.
96. Ximenes HM, Lortz S, Jörns A, Lenzen S. Triiodothyronine (T3)- mediated toxicity and induction of apoptosis in insulin-producing INS-1 cells. Life Sci 2007; 80: 2045-2050.
97. Lenzen S, Bailey CJ. Thyroid hormones, gonadal and adrenocortical steroid and the function of the islets of langerhans. Endocr Rev 1984; 5: 411-434.
98. . Mitrou P, Boutati E, Lambadiari V, Tsegka A, Raptis AE, Tountas N, Economopoulos T, Raptis SA, Dimitriadis G. Insulin resistance in hyperthyroidism: the role of IL6 and TNFα. Eur J Endocrinol 2010 ; 162(1):121-6.
99. Dimitriadis G, Baker B, Marsh H, Mandarino L, Rizza R, Bergman R, Haymond M, Gerich J. Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans. Am J Physiol Endocrinol Metab 1985 ;248(5):E593-601.
100. Randin JP, Tappy L, Scazziga B, Jequier E, Felber JP. Insulin sensitivity and exogenous insulin clearance in Graves' disease: Measurement by the glucose

clamp technique and continuous indirect calorimetry. Diabetes 1986;35(2):178-81.

101. Lenzen S, Kücking H. Inhibition of insulin secretion by L-thyroxine and D-thyroxine treatment in rats under the influence of drugs affecting the adrenergic nervous system. Acta endocrinologica 1982 ;100(4):527-33.
102. Ximenes HM, Lortz S, Jörns A, Lenzen S. Triiodothyronine (T3)-mediated toxicity and induction of apoptosis in insulin-producing INS-1 cells. Life Sci 2007;80(22):2045-50.
103. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am 2004 ;88(4):787-835.
104. Kapadia KB, Bhatt PA, Shah JS. Association between altered thyroid state and insulin resistance. J Pharmacol Pharmacother 2012;3(2):156.
105. Wang C. The relationship between type 2 diabetes mellitus and related thyroid diseases. J Diabetes Res 2013;2013: 390534.
106. Raboudi N, Arem R, Jones RH, Chap Z, Pena J, Chou J, Field JB. Fasting and Postabsorptive hepatic glucose and insulin metabolism in hyperthyroidism. Am J Physiol 1989;256(1):159-66.
107. Weinstein SP, O'Boyel E, Fisher M, Haber RS. Regulation of GLUTA glucose transporter expression in liver by thyroid hormone; evidence for hormonal regulation of the hepatic glucose transport system. Endocrinology 1994; 135 (2):649-54.
108. Fupare S, Gadhiya BM, Jambhulkar RK, Tale A. Correlation of Thyroid Hormones with FSH, LH and Prolactin in Infertility in the Reproductive Age Group Women. IJCBR 2015;2(4):216-222 .
109. Raboudi NE, Arem RI, Jones RH, Chap Z, Pena JA, Chou JE, Field JB. Fasting and postabsorptive hepatic glucose and insulin metabolism in hyperthyroidism. Am J Physiol 1989 ;256(1):E159-66.
110. Weinstein SP, O'Boyle E, Fisher M, Haber RS. Regulation of GLUT2 glucose transporter expression in liver by thyroid hormone: evidence for hormonal regulation of the hepatic glucose transport system. Endocrinology 1994;135(2):649-54.
111. Viguerie N, Millet L, Avizou S et al. Regulation of human adipocyte gene expression by thyroid hormone. J Clin Endocrinol Metab 2002;87:630-4.

112. Clement K, Viguerie N, Diehn M et al. In vivo regulation of human skeletal muscle gene expression by thyroid hormone. Genome Res 2002; 12:281-91.
113. Brenta G. Diabetes and thyroid disorders. Br J Diabetes Vasc Dis 2010;10(4):172-7.
114. Potenza M, Via MA, Yanagisawa RT et al. Excess thyroid hormone and carbohydrate metabolism. Endocr Pract 2009;15:254-62.
115. Muller MJ, Paschen U, Seitz HJ. Thyroid hormone regulation of glucose homeostasis in the miniature pig. Endocrinology 1983;112:2025-31.
116. Okajima F, Ui M. Metabolism of glucose in hyper and hypothyroid rats in vivo. Glucose turnover values and futile cycle activities obtained with 14 C and 3H labelled glucose. Biochem J 1979;182:565-75.
117. McCulloch AJ, Nosadini R, Pernet A et al. Glucose turnover and indicesof recycling in thyrotoxicosis and primary thyroid failure. Clin Sci 1983;64:41-7.
118. Vierhapper H, Grubeck-Loebenstein BE, Bratusch-Marrain P, Panzer S, Waldhäusl W. The impact of euglycemia and hyperglycemia on stimulated pituitary hormone release in insulin-dependent diabetics. J Clin Endocrinol Metab 1981;52(6):1230-4.
119. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, Hoogeveen R, Folsom AR, Heiss G. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes 2003 ;52(7):1799-805.
120. Folsom AR, Golden SH, Boland LL, Szklo M. Association of endogenous hormones with C-reactive protein, fibrinogen, and white blood count in post-menopausal women. Eur J Epidemiol 2005;20(12):1015-22.
121. Watanobe H, Hayakawa Y. Hypothalamic interleukin-1beta and tumor necrosis factor-alpha, but not interleukin-6, mediate the endotoxin-induced suppression of the reproductive axis in rats. Endocrinology 2003;144(11):4868-75.
122. Dandona P, Dhindsa S. Update: hypogonadotropic hypogonadism in type 2 diabetes and obesity. J Clin Endocrinol Metab 2011;96(9):2643-5.
123. Randolph JF, Jr, Zheng H, Sowers MR, Crandall C, Crawford S, Gold EB et al. Change in follicle-stimulating hormone and estradiol across the menopausal transition: effect of age at the final menstrual period. J Clin Endocrinol Metab 2011;96(3):746-54

124. Freeman EW, Sammel MD, Lin H, Gracia CR. Obesity and reproductive hormone levels in the transition to menopause. Menopause 2010;17(4):718-26.
125. Freeman EW, Sammel MD, Lin H, Gracia CR. Obesity and reproductive hormone levels in the transition to menopause. Menopause 2010;17(4):718-26.
126. Kim C, Randolph JF, Golden SH, Labrie F, Kong S, Nan B, Barrett-Connor E Weight loss decreases follicle stimulating hormone in overweight postmenopausal women.Obesity (Silver Spring) 2015;23(1):228-33.
127. De Pergola G, Maldera S, Tartagni M, Pannacciulli N, Loverro G, Giorgino R. Inhibitory effect of obesity on gonadotropin,estradiol, and inhibin B levels in fertile women. Obesity (SilverSpring) 2006;14(11):1954-60.
128. Key TJ, Endogenous Hormones Breast Cancer Collaborative Group . Body mass index, serum sex hormones,and breast cancer risk in postmenopausal women. J Natl Cancer Inst 2003;95(16):1218-26.
129. Wang N, Kuang L, Han B, Li Q, Chen Y, Zhu C, Chen Y, Xia F, Cang Z, Zhu C, Lu M. Follicle-stimulating hormone associates with prediabetes and diabetes in postmenopausal women. Acta Diabetol 2016; 53(2):227-36.
130. Godsland IF. Estrogens and insulin secretion. Diabetologia 2005; 48(11):2213-20.
131. Akin F, Bastemir M, Alkis E. Effect of insulin sensitivity on SHBG levels in premenopausal versus postmenopausal obese women. Adv Ther 2007 ;24(6):1210-20.
132. Akin F, Bastemir M, Alkiş E, Kaptanoglu B. SHBG levels correlate with insulin resistance in postmenopausal women. Eur J Intern Med 2009;20(2):162-7.
133. Haffner SM, Dunn JF, Katz MS. Relationship of sex hormone-binding globulin to lipid, lipoprotein, glucose, and insulin concentrations in postmenopausal women. Metab Clin Exp 1992;41(3):278-84.
134. Lindstedt G, Lundberg PA, Lapidus L, Lundgren H, Bengtsson C, Björntorp P. Low sex-hormone-binding globulin concentration as independent risk factor for development of NIDDM: 12-yr follow-up of population study of women in Gothenburg, Sweden. Diabetes 1991;40(1):123-8.

135. Haffner SM, Valdez RA, Morales PA, Hazuda HP, Stern MP. Decreased sex hormone-binding globulin predicts noninsulin-dependent diabetes mellitus in women but not in men. J Clin Endocrinol Metab 1993;77(1):56-60.
136. Barros RP, Gabbi C, Morani A, Warner M, Gustafsson JA. Participation of ERα and ERβ in glucose homeostasis in skeletal muscle and white adipose tissue. Am J Physiol Endocrinol Metab 2009;297(1):E124-33.
137. Espeland MA, Hogan PE, Fineberg SE, Howard G, Schrott H, Waclawiw MA, Bush TL, Pepi Investigators. Effect of postmenopausal hormone therapy on glucose and insulin concentrations. Diabetes care 1998;21(10):1589-95.
138. Cagnacci A, Soldani R, Carriero PL, Paoletti AM, Fioretti P, Melis GB. Effects of low doses of transdermal 17 beta-estradiol on carbohydrate metabolism in postmenopausal women. J Clin Endocrinol Metab 1992;74(6):1396-400.
139. Godsland IF, Stevenson JC. Postmenopausal hormone replacement therapy and insulin metabolism: effects of route of administration and implications for heart disease and diabetes risk. Br J Clin Pract Suppl 1996;86:1-5.
140. Gupte AA, Pownall HJ, Hamilton DJ. Estrogen: an emerging regulator of insulin action and mitochondrial function. J Diabetes Res 2015;2015:916585
141. Nadal A, Alonso-Magdalena P, Soriano S, et al. The pancreatic beta-cell as a target of estrogens and xeno estrogens: implications for blood glucose homeostasis and diabetes. Mol Cell Endocrinol 2009 ;304(1-2):63-8.
142. Fukui M, Kitagawa Y, Kamiuchi K, et al. Association between serum estradiol concentrations and carotid atherosclerosis in men with type 2 diabetes mellitus. Metabolism 2008;57(2):285-9.
143. Nelson LR, Bulun SE. Estrogen production and action. J Am Acad Dermatol 2001;45(3 Suppl):S116-24
144. Szymczak J, Milewicz A, Thijssen JH, Blankenstein MA, Daroszewski J. Concentration of sex steroids in adipose tissue after menopause. Steroids 1998;63(5-6):319-2
145. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. Endocr Rev 2002;23(1):90-119
146. Sites CK, Calles-Escandon J, Brochu M, Butterfield M, Ashikaga T, Poehlman ET. Relation of regional fat distribution to insulin sensitivity in postmenopausal women. Fertil Steril 2000;73(1):61-5.

147. https://thyroidadvisor.com/estrogen-thyroid-relationship/
148. Žakula ZD, Đurica S, Radivojša SN, Milosavljević T, Ribarac-Stepić N. Interaction of estrogen and thyroid hormones in the nuclei of uterine tissue. Archives of Biological Sciences 2005;57(1):1-2.
149. Santin AP, Furlanetto TW. Role of estrogen in thyroid function and growth regulation. J Thyroid Res 2011;2011: 875125.
150. Chen GG, Vlantis AC, Zeng Q, van Hasselt CA. Regulation of cell growth by estrogen signaling and potential targets in thyroid cancer. Curr cancer drug targets 2008 ;8(5):367-77.

CHAPTER 7

SUMMARY AND CONCLUSION

7.1. SUMMARY :

- The purpose of the study was to know the relationship between thyroid hormones and glucose homeostasis in post menopausal women who are suffering from diabetes mellitus.
- We hypothesized that Thyroid hormone levels may be linked with menopause with special relation to diabetes. We also hypothesized that, such links may influence in differential regulation of glucose homeostasis.
- The objectives of the study were evaluated with a cross sectional study. Totally 200 participants have participated in the study.The participants were Pre Menopause Diabetic & Non-Diabetic aged between 25-45 years and Post Menopause Diabetic and Non-Diabetic women aged between 46-65 years included in the study. Based on the study design, they were categorized into four (4) groups and each group consists of 50 participants. They are

  Group I – Pre Menopause Non-Diabetic (n=50)

  Group II- Pre Menopause Diabetic (n=50)

  Group III – Post Menopause Non-Diabetic (n=50)

  Group IV- Post Menopause Diabetic (n=50)
- The following parameters tested for the study. Physical Anthropometric parameters includes Height, weight, Body surface area, Body mass index, Waist to Hip Ratio, and we found significant($p<0.05$) increase in all the parameters except height of post menopausal diabetic women.
- Physiological parameters includes Systolic Blood Pressure(SBP), Diastolic Blood Pressure, Pulse Pressure(PP), Mean Arterial Pressure(MAP), Pulse Rate(P.R) and we found there was a significant increase in SBP($p<0.000$) and decrease in DBP($p<0.001$) and increase in pulse pressure(P.P) in Post menopausal diabetic women and we found no change within the Mean Arterial Pressure(MAP) and Pulse rate(P.R)
- Haematological parameters include complete blood picture count (CBC) and we did not find any change except WBC count where it shows significant ($p<0.001$) increase in post menopausal diabetics.

- Blood Glucose profile includes Fasting Blood Sugar (FBS) and Glycosylated Haemoglobin(HbA1c). We found there is an increase in FBS of Pre menopausal Diabetic women compare to all other groups where as HbA1c remains same in both Pre and Post menopausal diabetics.
- Thyroid Hormonal Assay includes TSH, T_3 andT_4. We found there is an increase in T_3 andT_4 levels and decrease in TSH levels in post menopausal diabetics.
- Reproductive hormonal assay includes Lutenizing Hormone (LH) and Follicle Stimulating Hormone (FSH), Estradiol(E_2) and Progesterone(P_4). LH,FSH and E_2, P_4 values are showed to be decreased in post menopausal diabetics compared to non-diabetics.

7.2. CONCLUSION:

- Based on all these observations, we have concluded that the outcome of the present study enlightens the complex interaction between thyroid hormone and glucose regulatory hormones in post menopausal women.
- The Present need of the study is to establish a relationship between glucose regulation and thyroid hormones. Similarly the relationship also exists between thyroid hormones and reproductive hormones. Hence, a possible interaction of thyroid hormones and reproductive hormones on glucose regulation in post menopausal women may be expected.
- But, it is not completely understood whether regulation of blood glucose is influenced by thyroid hormones in case of altered glucose regulation (Diabetes Mellitus) on the post menopausal females.
- The current understanding of this complex scenario is, thyroid disorders deteriorates the glucose metabolism in post menopausal women suffering from diabetes mellitus. Long term coexistence of diabetes and thyroid disorder will lead to altered glucose homeostasis in post menopausal women. So monitoring of thyroid profile in post menopausal diabetic women would be a better therapy to prevent further complications in future.

7.3. LIMITATIONS OF THE STUDY

7.3.1. Study limitations:

- The study is confined only to female patients with limited sample size.
- Estimation of the lipid profile should have been done.

7.3.2. Future Prospective:

- The research further can also be extended by estimating the calcium and lipid profiles with more sample size.
- Study can also be done on males with andropause related thyroid disorders.

7.4. Public health importance

- The present study is a cross sectional study comprising euthyroid pre and post menopause diabetic and non-diabetic women. Though our results did not show any significant thyroid abnormality, it clearly represents that thyroid hormone levels within the normal range may also lead to the development of diabetes in pre and post menopausal women. So our study suggests that pre and post menopausal women especially diabetics should undergo screening of thyroid profile.

APPENDICES

B. L. D. E. U'S SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, BIJAPUR

RESEARCH INFORMED CONSENT FORM

Title of the Project:

"**Study on Relationship between Thyroid Hormones and Glucose Homeostasis Among Postmenopausal Diabetic women"**

Principal investigator's name: **Miss. Ch. Kalashilpa**

PhD scholar.

Name of the Guide: **Dr Sumangala M. Patil**

Professor,

Department of Physiology.

1: PURPOSE OF RESEARCH: I have been informed that this study will assess the relationship between thyroid Hormones and glucose homeostasis among Postmenopausal Diabetic women. This study will be useful academically as well as for clinically.

2: PROCEDURE: I understand that, the procedure of the study will involve recording of various physiological, Haematological, Biochemical and Hormonal parameters. The procedure will not interfere with any of my physiological parameters and they are non invasive.

3: RISK AND DISCOMFORTS: I understand determination of relationship between Thyroid Hormones and Glucose Homeostasis will not cause any discomfort to me and do not involve any risk to my Health.

4: BENEFITS: I understand that my participation in the study may not have a direct benefit to me but this may have a potential beneficial effect in the study of relations between thyroid and glucose homeostasis in future.

5: CONFIDENTIALITY: I understand that medical information produced by this study will become part of institutional records and will be subject to the confidentiality and privacy regulation of the said institute. Information of a sensitive personal nature will not be a part of medical record, but will be stored in investigators research file and

identified only by a code number. The code key connecting name two numbers will be kept in a separate secured location.

If the data are used for publication in the medical literature and for teaching purposes no names will be used and other identities such as photographs, audio and video tapes will be used only with my special written permission. I understand I may see the photographs and the video tapes and have the audio tapes before giving this permission.

6: REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time.

Concerned researcher is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study which might influence my continued participation. If during the study or later, I wish to discuss my participation in all concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital is available to talk with me. A copy of this consent form will be given to me to keep for careful re-reading.

7: REFUSAL OR WITHDRAWAL OF PARTICIPATION: I understand that my participation is voluntary and may refuse to participate or may withdraw my consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that researcher may terminate my participation in this study at any time after she/he has explained the reasons for doing so and had helped arrange for my continued care by my physician or physical therapist if this is appropriate.

8: INJURY STATEMENT: I understand that in unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, then medical treatment will be available to me, but no further compensation would be provided. I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

I have explained to _____ (Patient/Relevant guardian)

The purpose of the research, procedures required and the possible risk and benefits to the best of my ability.

Investigator/ UG (Guide) Date

I confirm that _____ (Name of the U.G. Guide /Chief researcher) has explained to me the purpose of research, the study procedure that I will undergo, and the possible risk and discomforts as well as benefits that I may experience. Alternative to my participation in the study have also been to give my consent from. Therefore I agree to give consent to participate as a subject and this research project.

Participant / Guardian Date:

Witness to signature Date:

Modified from Portney L.G, Watkins M.P., in Foundation of Clinical Research, Second Edition, New Jersey, Prentice Hall Health 2000. (APPENDIX – E)

PROFORMA FOR COLLECTION OF SAMPLE.

Name :

Age :

Sex :

Height : Weight: B.S.A: Waist-Hip ratio:

B.M.I :

History of Menopause:

History of Diabetes:

Smoking/ Non-smoking:

Alcoholic:

B.P:

Pulse rate:

FOLLOWING ARE THE INVESTIGATIONS ARE TO BE DONE

I. Determination of Complete Blood picture Count.

II. Estimation of blood glucose.

III. Glycosylated Haemoglobin ($HbA1_C$)

IV. Thyroid Hormone Profile(TSH,T_3,T_4)

V. Luteinizing Hormone (LH), Follicle stimulating hormone(FSH) analysis.

VI. Estrogen & Progesterone hormonal analysis

BLDE (DEEMED TO BE UNIVERSITY)

Annexure -I

PLAGARISM VERIFICATION CERTIFICATE

1. Name of the Student: KALASHILPA CHITTIKANNA Reg No. 14PHD004
2. Title of the Thesis: Study on relationship between thyroid hormones and glucose homeostasis among post menopausal diabetic women.
3. Department: Physiology
4. Name of the Guide & Designation: Dr. Sumangala. M. Patil, M.D, Professor
5. Name of the Co Guide & Designation: —

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

Software used... TURNITIN Date: 24/7/2018

Similarity Index(%): 17 (SEVENTEEN) Total word Count: 26467

The report is attached for the review by the Student and Guide.

The plagiarism report of the above thesis has been reviewed by the undersigned.

The similarity index is below accepted norms.

The similarity index is above accepted norms, because of following reasons:

.....

.....

.....

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

Dr. sumangala patil

Signature of the Guide
Name & Designation

Signature of Co-Guide
Name & Designation

ch. kalashilpa

Signature of Student

Prof.
Physiology
B.L.D.E.U`S Shri B.M.Patil Medical College, Hospital & R.C.
Vijayapur-586103.

Verified by (Signature)
Name & Designation

24/7/18

Librarian
B.L.D.E. University`s
Shri B.M. Patil Medical College
Bijapur.

B.L.D.E. UNIVERSITY

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act,1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

IEC Ref No-120/2015-16

April 10, 2015.

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on 16th March 2015 at 11 AM to scrutinize the Synopsis / Research projects of Postgraduate student / Undergraduate student / Faculty members of this University / college from ethical clearance point of view. After scrutiny the following original / corrected & revised version synopsis of the Thesis / Research project has been accorded Ethical Clearance.

Title "study on relationship between Thyriod Hormones & Glucose Homeostasis among Post menopausal Diabetic women."

Name of Ph.D./ P. G. / U. G. Student / Faculty member. Ch. Kalashilpa Department of Physiology.

Name of Guide : Dr.Sumangala.M.Patil.Professor Department of Physiology.

Dr. Sharada Metgud
Chairperson, I.E.C
BLDE University,
VIJAYAPUR – 586 103

Dr.G.V.Kulkarni
Secretary, I.E.C
BLDE University,
VIJAYAPUR – 586 103.

Note:-Kindly send Quarterly progress report to the Member Secretary.

Member Secretary,
Institutional Ethical Committee,
BLDE University, BIJAPUR.

Following documents were placed before Ethical Committee for Scrutinization:

- Copy of Synopsis / Research project
- Copy of informed consent form
- Any other relevant documents.

Institutional Ethical Committee
BLDE University, BIJAPUR

Smt. Bangaramma Sajjan Campus, Sholapur Road, Vijayapur – 586103, Karnataka, India.
University: Phone: +918352-262770, Fax: +918352-263303, Website: www.bldeuniversity.ac.in, E-mail: office@bldeuniversity.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, Website: www.bldeuniversity.ac.in, Emaibmpmc.principal@bldeuniversity.ac.in

PUBLICATIONS

1. Kalashilpa CH, Patil SM, Aithala M. Interaction Between Thyroid Hormones and Glucose Homeostasis in Post-Menopausal Diabetic and Non-Diabetic Women. Res J Pharm Biol Chem Sci. 2016; 7(5):2040-6. **(Indexed in Scopus)**

2. Kalashilpa CH, Sumangala Patil.Effect of Thyroid Hormones on Glucose Regulation in Pre & Post Menopausal Diabetic Women". Int J Pharm Bio Sci 2017 ; 8(2): (B) 66-71.**(Indexed in Scopus)**

ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Interaction Between Thyroid Hormones and Glucose Homeostasis in Post-Menopausal Diabetic and Non-Diabetic Women.

CH Kalashilpa[1]*, Sumangala M Patil[1], and Manjunatha Aithala[2].

[1]Department of Physiology, B.L.D.E.U Shri BM Patil Medical College, Bijapur, Karnataka, India.
[2]Department of Physiology, B.L.D.E.U Shri BM Patil Medical College, Bijapur, Karnataka, India.

ABSTRACT

To evaluate the interaction between Thyroid hormones and Glucose homeostasis in post menopausal Diabetic and Non-Diabetic women. Sixty women aged between 46-65 years were selected for the present study & further divided into 2 groups. 30 post menopausal diabetic women were compared with equal number of post menopausal Non-diabetic women. Anthropometric & Physiological parameters were taken. Complete blood picture count, Fasting blood glucose(FBS),Glycosylated haemoglobin(HbA1C), Oestradiol, progesterone, Follicle stimulating hormone(FSH), Luteinizing hormone(LH) levels were analyzed. Unpaired "t" test, is used for the analysis of data between Diabetic and Non-Diabetic group. $P<0.05$ considered statistically significant. In our study we found, there is significant increase in weight, BSA, BMI, where as significant decrease in DBP of diabetic group compared to Non-Diabetics & there is significant increase in FBS, HbA1C levels of Diabetic group & significant decrease in LH levels of Diabetics compared to Non-Diabetics. However T3 levels of Diabetic and Non-Diabetic group were almost same, where as T4, Oestradiol and progesterone levels of diabetic group non-significantly increased and FSH and TSH levels non-significantly decreased in Diabetics. There is frequent co-existence of thyroid dysfunction and diabetes mellitus among post menopausal diabetic women. So in conclusion, screening of all post menopausal diabetic women for thyroid disorders should be included in routine investigation for better diagnosis and prognosis of the patients.

Keywords: Thyroid hormones, Diabetes, Glucose regulatory hormones, Post Menopause, Glucose Homeostasis

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Menopause is the permanent cessation of menstruation due to loss of ovarian function. This transition is gradual which takes place over a period of time from the reproductive to non-reproductive phase of life. The transition from pre to post-menopause is often associated with the emergence of metabolic syndrome features. These risk factors may be due to the direct result of ovarian failure or an indirect result of the metabolic consequences of central fat redistribution with estrogen deficiency [1-3].

Diabetes and thyroid have showed to mutually influence each other and association between both the conditions have long been reported[4,5].Diabetic patients have higher prevalence of thyroid disorders compared with the normal population because patients with one organ-specific autoimmune disease are at high risk of developing other autoimmune disorders[6].The presence of thyroid disorders usually affects diabetes control.

Thyroid Hormones, namely Tri-iodothyronine (T3) and Thyroxin (T4); either or both of which may be elevated or reduced have both direct and indirect effects on blood glucose homeostasis. Elevated levels of free circulating thyroid hormone (hyperthyroidism) produce hyperglycemia by causing polyphagia, enhancing glucose absorption from the gastro-intestinal tract, accelerating insulin degradation and stimulating glycogenolysis. Reduced level of hormones(hypothyroidism) may cause hypoglycemia[7].The prevalence of thyroid disorders has been found to increase linearly with age and virtually all thyroid disorders are common in women[8].

In women, thyroid disorders are also associated with menopause. Menopause is the time of life when menstrual cycles ceases and is caused by reduced secretions of ovarian hormones, oestrogen and progesterone [9]. Menopause is also one of the most important period which favors weight gain and leading to obesity. Obesity can also be considered as a condition of exaggerated estrogen production. It has been demonstrated that the conversion of androgens to estrogen in peripheral tissues is significantly correlated with body weight and the amount of body fat [10]. There are many studies on thyroid hormones and diabetes and the relation between them. But very few studies are there on interaction of thyroid hormones and glucose regulatory hormones in postmenopausal diabetic and non-diabetic women.

So, Aim of the present study is to know the interaction between thyroid hormones and glucose regulatory hormones in post menopausal diabetic and non-diabetic women.

MATERIAL AND METHODS

The study was conducted at Shri B.M Patil Medical College, Hospital& Research center, BLDE University. 30 Post menopausal Non-diabetic women attending OPD, Department of OBG and 30 Post menopausal Diabetic women attending the out-patient clinic of Diabetes, aged between 46-65 years were selected for the study. Written informed consent was obtained from the subjects. Ethical clearance was obtained from the Research Ethical Committee of Shri B.M Patil medical college and Hospital, BLDE University.

Inclusion and Exclusion criteria

Post menopausal Diabetic and Non-Diabetic women aged 46-65 years old with resting blood pressure (BP<139/89) according to WHO hypertension guidelines and who were Non-smokers were included in this. For diabetic patients, duration of diabetes mellitus longer than one year or more have been included and where as the subjects with evidence of Hypertension (BP>139/89), with a history of alcohol intake, or they were taking any thyroid supplements, or they have undergone Hysterectomy, use of any oral contraceptives within the previous six months, and pregnant women were excluded from this study.

A detailed history of diabetes and menopause was taken from the subjects and complete physical examination was done at the time of recruitment. Menopause was confirmed by the criteria that women are not menstruating for a period of 12 consecutive months with no other abnormality noticed.

ISSN: 0975-8585

The following Physical Anthropometric & Physiological Parameters were assessed from the patients: Age, Height, weight, Body surface area, Body Mass Index, Waist to Hip Ratio, Blood pressure and Pulse rate. Height was measured in cm. Weight was measured nearest to 0.1 kg. Body mass Index was calculated by using the following formula:

$$\text{Body Mass Index (BMI)} = \frac{\text{Weight in kg}}{\text{Height in meter square}}$$

Body Surface Area (BSA) is calculated using Duboi's formula [11]

$$BSA\ (m^2) = 0.007184 \times \text{Height (cm)}^{0.725} \times \text{Weight (kg)}^{0.425}$$

Waist to Hip ratio was calculated by measuring waist at narrowest point under lowest rib and hips at the widest portion of buttocks using a tape and the ratio was calculated in cm by dividing waist measurement by hip measurement.

$$\text{Waist-Hip Ratio} = \frac{\text{Waist (cm)}}{\text{Hips (cm)}}$$

Blood pressure was measured by using mercury sphygmomanometer and Systolic Blood pressure (SBP, mmHg), Diastolic Blood pressure was recorded and Pulse Rate (beats per min, bpm) was also recorded.

Blood samples from patients were obtained at the morning hours after a 12 hour overnight fast for biochemical, Hormonal analysis. Haematological parameters like complete blood picture count (CBC) was analyzed by using SYSMEX XN-1000 Automated Haematology cell counter. Biochemical parameters like Fasting Blood Glucose(FBG) was analyzed by using Glucose oxidase-peroxidase method, with MISPO UNO(AGAPPE)-semi-auto analyzer, where as HbA1C was analyzed by Turbidimetric Inbition Immunoassay method by using fully auto analyzer(ROCHE COBAS C 311) and the hormonal analysis like thyroid profile(T3,T4,TSH) and Reproductive Hormonal profile (FSH, LH) were analyzed by the method ELFA(Enzyme linked fluorescent assay) by using Mini VIDAS, where as Estrogen and progesterone hormones were analyzed by the method C.L.I.A(Chemi Luminescent Immuno Assay).

Statistical analysis

It was done by using SPSS statistical software 16 version. Data was expressed as Mean±SD (standard deviation). Significance of difference between Diabetic group and Non-Diabetic group was determined by using student's unpaired (independent) sample "t" test. $P<0.05$ is considered statistically significant (two tailed).

RESULTS

Table 1 shows Comparison of Anthropometric and physiological parameters of Post Menopausal Diabetic and Non-diabetic women. There was no significant difference in height of both groups. Here it was shown that weight was significantly increased in Diabetic Group compared to Non-diabetic group. At the same time, Body surface area, Body Mass Index significantly increased in diabetic group where as Diastolic blood pressure (DBP) of diabetic group significantly decreased compared to Non-diabetic group, where as Waist to hip ratio, pulse rate, systolic blood pressure was non- significantly increased in diabetic group.

Table 2 shows comparison of Biochemical and Hormonal parameters of Post menopausal Diabetic and Non-diabetic women. Here it was shown that Fasting blood glucose(FBS) levels and Glycosylated hemoglobin(HbA1C) levels were significantly increased in diabetic group compared to non-diabetic group($P<0.0001$) where as Luteinizing hormonal(LH) level significantly decreased in diabetic group compared to non-diabetic group($P<0.007$) . However T3 levels of diabetic and non-diabetic group showed were almost same,where as T4, Oestradiol and progesterone levels of diabetic group non-significantly increased in diabetic group and Follicle stimulating hormone(FSH) and TSH levels non-significantly decreased in diabetic group.

ISSN: 0975-8585

Table 1: Anthropometric and physiological parameters of Post Menopausal Diabetic and Non-Diabetic women.

Parameter	Post menopause Diabetic (n=30)	Post menopause non-diabetic (n=30)	P Value
Height(cm)	152.29±5.55	150.16±6.83	0.19(NS)
Weight(kg)	59.56±7.35	54.10±8.48	0.009**(HS)
BSA(m^2)	1.55±0.11	1.47±0.12	0.01*(S)
BMI(kg/m^2)	25.64±2.45	23.95±3.16	0.02*(S)
Wasit to Hip ratio(cm)	1.00±0.12	0.95±0.10	0.06(NS)
Pulse rate(bpm)	74.06±3.87	74.00±4.51	0.08(NS)
SBP(mmhg)	128±11.90	123±10.22	0.08(NS)
DBP(mmhg)	75.33±3.83	78.20±5.71	0.02*(S)

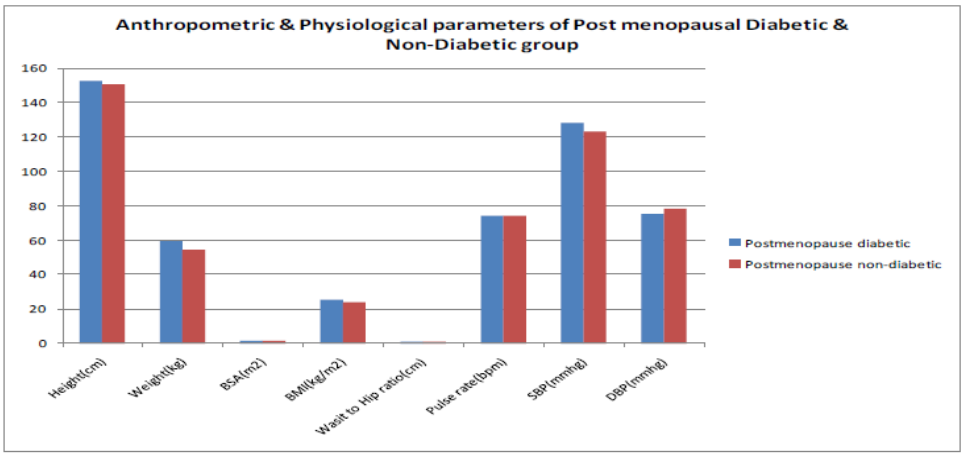
Data is presented as Mean±SD(standard deviation)
SBP-systolic blood pressure, DBP-Diastolic blood pressure, BMI-Body mass index,
BSA-Body surface area, bpm- beats per minute, mmhg-millimeters of mercury
P<0.05 considered significant(S). P>0.05 considered Non significant(NS)
P<0.001 considered Highly significant(HS).
*indicates level of significance. *P<0.05(S), **P<0.001(HS)

Table 2 : Biochemical and Hormonal parameters of Post Menopausal Diabetic and Non-Diabetic women.

Parameter	Post menopause Diabetic (n=30)	Post menopause non-diabetic (n=30)	P Value
Fasting blood glucose(mg/DL)	163.83±53.06	90.76±12.68	0.0001**(HS)
HbA1C(%)	8.14±2.16	5.24±0.76	0.0001**(HS)
T3(nmol/L)	1.71±0.75	1.71±0.36	0.96(NS)
T4(nmol/L)	96.74±15.31	93.77±19.07	0.50(NS)
TSH(mulU/ml)	2.37±1.40	2.54±2.30	0.73(NS)
FSH(IU/L)	56.62±27.34	71.46±33.21	0.06(NS)
LH(mIU/ml)	17.71±7.70	25.87±14.31	0.0079*(HS)
Oestradiol(E2)(pg/ml)	25.78±28.65	23.69±16.52	0.73(NS)
Progesterone(ng/ml)	0.62±2.40	0.24±0.17	0.38(NS)

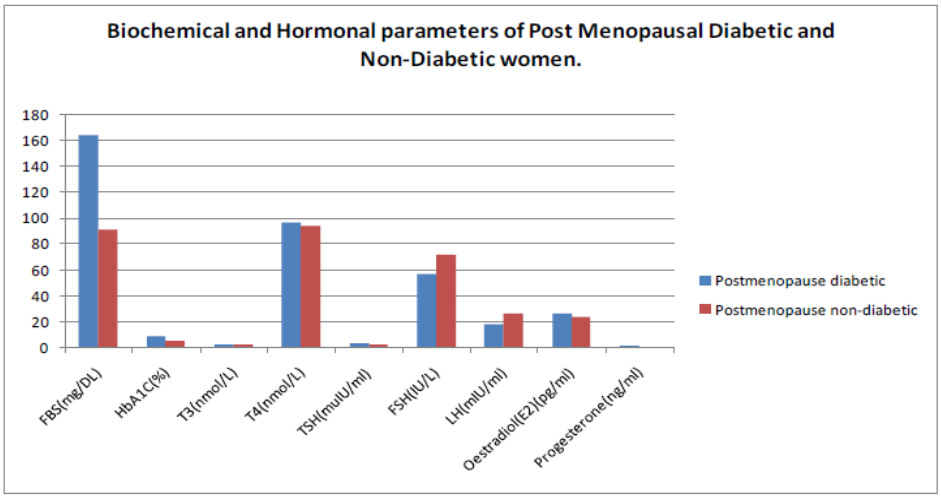
Data is presented as Mean±SD(standard deviation)
mg/DL- Milligrams per deciliter, nmol/L- Nanomoles per liter, muIU/ml- millimicrons-International units per milli liter,
IU/L-International unites per leter, mIU/ml-milli-International units per milli liter.
Pg/ml- Picograms per milliliter, ng/ml-nanograms per milli leter.
P<0.05 considered significant(S). P>0.05 considered Non significant(NS)
P<0.001 considered Highly significant(HS).
*indicates level of significance. *P<0.05(S), **P<0.001(HS)

Figure 1



ISSN: 0975-8585

Figure 2



DISCUSSION

Menopause in women leads to many physiological changes in the body. Here in our study, significant changes have been observed in post menopausal diabetic women compared to Non-diabetics. There was a significant increase in weight of the Post menopausal diabetic women. Obesity is one of the commonest cause of post menopausal diabetic women. Excess insulin causes polyphagia,so persons eat more to try to maintain a balance[12].women tend to gain weight and undergo an alteration to their fat distribution in mid-life[13,14].Obesity acts as a diabetogenic factor and leads to decrease in insulin receptors on the insulin responsive cells[15]. At the same time, there is a central redistribution of fat with a decrease in gluteo femoral fat and an increase in intra abdominal fat. Moreover, during climacteric, often appears an associated muscle mass loss. Likewise for weight gain, changes in body composition are related to aging. Different factors (diet - physical activity - GH secretion) could be involved. This tendency to visceral fat accumulation clearly favors the increased cardiovascular risk observed after menopause [16]. Changes in the body fat distribution at menopause may be due to decreased production of estrogen. Changes in body fat distribution with declining estrogen are likely due to changes in adipose tissue metabolism since estrogen is known to influence adipose tissue lipoprotein lipase (LPL) activity and lipolysis[17].

There was a significant increase in BSA, BMI in Diabetic group compared to Non-Diabetic group. This may be because of relationship between BMI, insulin resistance, blood glucose and HPG axis functions are complex. It is commonly accepted that the volume of fat mass increases with age results into the higher BMI noted during aging. Similar observation was reported by Forbes GB et al., [18]. However, we did not find any significant change in the waist to hip ratio between Diabetics and Non-diabetics.

There was a significant decrease in DBP of diabetic group compared to Non-diabetic group ($P<0.02$). It may be due to the age related decline in diastolic pressure is presumed to result from early recoil of the pressure wave, because of increasing arterial stiffness and lack of proper large artery compliance. Such shift of the reflection wave from the diastole to the systole increases systolic and decreases diastolic pressure. Here in this study we also found that SBP increased in Diabetic group compared to Non-diabetics, but it did not differ significantly. These observations were supported by Ronnback M et al., [19]. Pulse rate was almost similar in both the groups.

There was a significant Increase in Fasting blood glucose levels and HbA1C in Diabetic group. The increase in HbA1C during diabetes, may be due to the excess glucose present in blood reacts with hemoglobin.[20,21].There was a marked increase in HbA1C levels in diabetic patients, which could be due to excessive glycosylation of hemoglobin. Our finding is supported by Saha HR et al., [22].

There was a significant decrease in LH levels in diabetic group compared to non-diabetic group(P<0.007).At the same time There was a Non significant decrease in FSH levels in diabetic group. The decreased levels of these hormones may be due to increased level of estrogen and progesterone in diabetic group. It is well established that aging is associated with dramatic changes in gonadotropin secretion in healthy subjects. In women, after the initial elevation of serum gonadotropins that characterizes the menopause, a progressive decline in both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels occurs with age in the later post-menopausal years[23-27]. However there were no significant differences of estrogen and progesterone levels in both the groups. There were no significant differences in Thyroid hormones (T3,T4,TSH) of diabetic group compared to Non-diabetic group. T3 levels were almost similar in both groups. T4 levels Non-significantly increased in diabetic group where as TSH levels decreased in diabetic group compared to non-diabetic group.

The frequency of thyroid disorders rises with age and is higher women. In our study, TSH is low and T4 is high. Low levels of TSH may indicate "Hyperthyroidism". The occurrence of hyperthyroidism in patients with diabetes is greater than in general population. Thyroid hormones may influence glucose control through a variety of actions on intermediatory metabolism. Some of these effects become clinically relevant in patients with co-existent diabetes and hyperthyroidism. Excess Thyroid hormones promote hyperglycemia by facilitating glucose intestinal absorption, increasing insulin clearance, and enhancing glycogenolysis and gluconeogenesis also, Hyperthyroidism is associated with increased hepatic output, reduced insulin action and increased lypolysis in diabetics [28].

CONCLUSION

The interaction between thyroid hormones and glucose homeostasis among postmenopausal diabetic & Non-diabetic women is a typical phenomenon. The physiological basis and the complex interaction between thyroid hormones and glucose regulatory hormones and their relation with female reproductive hormones among postmenopausal diabetic women trigger the glycaemic control mechanism. So clinicians should be aware of the frequent co existence of thyroid dysfunction and diabetes mellitus among post menopausal diabetic women. Periodic thyroid screening should be targeted for post menopausal diabetic women. Recognition and prompt correction of thyroid dysfunction will optimize glycaemic control among postmenopausal diabetic women. So routine annual thyroid screening should be Mandatory.

This study will serve as a valuable guideline for physician to proceed for diagnosis and management of thyroid dysfunction in postmenopausal diabetic women. From our study, we found more prevalence of "Hyperthyroidism" among Postmenopausal diabetic women. So screening of all postmenopausal diabetic women for thyroid disorders should be included in routine investigation for better diagnosis and prognosis of the patients.

ACKNOWLWDGEMENT

We would like to thank all the patients and healthy subjects of Shri B.M. Patil Medical Hospital and Research Center, BLDE University, Vijayapur for participating in this study.

REFERENCES

[1] Carr MC. J Clin Endocrinol Metab 2003;6:2404-11.
[2] Fu CH, Yang CC, Lin CL, Kuo TB. Chin J Physiol 2008;2:100-5.
[3] Pellizzer AM, Straznicky NE, Lim S, Kamen PW, Krum H. Clin Exp Pharmacol Physiol 1999;8:656-60.
[4] Feely J, Isles TE. BMJ 1979; 6179:1678.
[5] Gray RS, Irvine WJ, Clarke BF. BMJ 1979; 6202: 1439
[6] Perros P, MC Crimmon RJ, Shaw G, Frier BM. Diabet med 1995; 7:622-627.
[7] Ghazali SM and Abbiyesuku FM. Niger J Physiol sci 2010;2:173-179.
[8] Der EM, Quayson SE, Clegg-Lamptey JN, Wiredu EK, Ephraim RKD, Gyasi RK. Journal of Medical and Biomedical Sciences 2013; 1: 1-7.
[9] Dr Heidi D Nelson, MD. THE LANCET 2008; 9614: 760-770.
[10] Siiteri PK. J Endocrinol 1981; 89: 119P—129P

[11] DuBois D, DuBois EF. Arch Int Med 1916; 17: 863-71.
[12] Rosenthal S. The type 2 diabetic women. Lincolnwood,Illinois:Lowell House. 1999.
[13] Wing RR, Malthews KA, Kuller LH, Meilahn EN, Plantinga PL. Arch Int Med 1991; 1:97-102.
[14] Svendesen OL, Hassager C,Christiansen C. Metabolism 1995;44:369-73.
[15] Satyanarayana U, Chakrapani U. Biochemistry, 3rd edition, 2006.
[16] Castelo-Branco C. Obstetrics and Gynaecology 2009;1-136
[17] Rebuffe-Scrive M, Eldh J, Hafstrom LO, Bjorntorp P. Metabolism 1986;9:792–797.
[18] Forbes GB, Reina JC. Metabolism. 1970; 9: 653-63.
[19] Ronnback M, Fagerudd J, Forsblom C, Pettersson-Fernholm K, Reunanen A, Groop PH. Finnish Diabetic Nephropathy(Finn Diane) Study Group age-related blood pressure pattern in type 1 diabetes.2004; 9:1076-1082.
[20] Gloria-Bottini F, Antonacci E, Bottini N, Ogana A, Borgiani P, et al. Hum Biol 2000;2: 287-294.
[21] Sampson MJ, Gopaul N, Davies IR, Hughes DA, Carrier MJ. Diabetes Care 2002; 3:537-541.
[22] Saha HR, Sarkar BC, Khan SA, Sana NK, Choudhury S. A Comparative study of Thyroid Hormone and Lipid Status in Diabetic and Non Diabetic Adults 2012;9:450.
[23] Kwekkeboom DJ, De Jong FH, Van Hemert AM, Vandenbroucke JP, Valkenburg HA &Lamberts SWJ. Journal of Clinical Endocrinology and Metabolism 1990 ;70: 944-950
[24] Rossmanith WG, Scherbaum WA & Lauritzen C. Neuroendocrinology 1991;3: 211–218.
[25] RossmanithWG. Exp Gerontol 1995; 3-4: 369–381.
[26] Lamberts SWJ, van den Beld AW & van der Lely AJ. Science 1997; 5337: 419–424.
[27] Santoro N, Banwell T, Tortoriello D, Lieman H, Adel T & Skurnick J. Am J Obstet Gynecol 1998;4:732–41
[28] Potenza M, Via MA, Yanagisawa RT. Endocr Pract 2009; 3: 254–62.

Int J Pharm Bio Sci 2017 Apr ; 8(2): (B) 66-71

Original Research Article | Physiology

International Journal of Pharma and Bio Sciences | ISSN 0975-6299

EFFECT OF THYROID HORMONES ON GLUCOSE REGULATION IN PRE AND POST MENOPAUSAL DIABETIC WOMEN

KALASHILPA CHITTIKANNA[1*], SUMANGALA M. PATIL[2]

[1] Department of Physiology, Shri B.M.Patil Medical College Hospital and Research Centre, BLDE University, Vijayapur-586103, Karnataka, India
[2]Department of Physiology, Shri B.M.Patil Medical College Hospital and Research Centre, BLDE University, Vijayapur- 586103, Karnataka, India

ABSTRACT

There is a mutual influence of thyroid and diabetes and the association between them reported long back. Prevalence of thyroid on diabetics is increasing worldwide. The presence of thyroid usually affects diabetic patients. Aim of the study is to evaluate the relationship between Thyroid hormones and Glucose Regulation in pre and post menopausal diabetic women. Total Sixty women, among them 30 pre menopausal diabetic aged between 25-45 years & 30 Post menopausal diabetic women aged between 46-65 years were selected for the present study. Anthropometric & Physiological parameters were taken. Complete blood count, Fasting blood glucose (FBS), Glycosylated hemoglobin (HbA1C), Thyroid hormone profile (T3, T4, TSH) levels were analyzed. In our study we found, there is significant increase in BSA, BMI, Waist to Hip ratio of Pre menopausal diabetic group compared to post menopausal diabetics where as significant increase in SBP of Post menopausal diabetic group compared to pre menopausal Diabetics & there is non-significant increase in HbA1C levels of post menopause Diabetic group. However, there is non-significant decrease in T3, T4 and significant increase in TSH levels of Pre menopause Diabetic group compared to post menopause diabetics. We found more prevalence of "Hypothyroidism" among pre menopausal diabetic women. So prompt recognition of thyroid dysfunction in premenopausal diabetic women and controlling the blood glucose level is necessary and also routine thyroid screening should be recommended for pre menopausal diabetic and post menopausal diabetic women to detect thyroid abnormalities in most of the patients in subclinical stage.

KEYWORDS : *Thyroid Hormones, Glucose Regulation, Pre Menopausal Diabetic, Post Menopausal Diabetic.*

KALASHILPA CHITTIKANNA [1*]

Department of Physiology, Shri B.M.Patil Medical College Hospital and Research Centre,BLDE University,Vijayapur-586103, Karnataka, India

Received on: 08-12-2016
Revised and Accepted on: 06-02-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.b66-71

INTRODUCTION

Thyroid diseases and diabetes mellitus are the two most common endocrine disorders encountered in clinical practice.[1,2]The physiological and biochemical interrelationship between insulin and influence of both insulin and iodothyronines on the metabolism of carbohydrates, proteins, and lipid have been recorded. [3, 4]such records indicate that iodothyronines are insulin antagonist with high levels being diabetogenic while absence of hormone inhibits the development of diabetes.[4]Diabetes mellitus is a common endocrine disorders rising in India and has reached approximately 20% in urban populations and approximately 10% in rural Population. [5] The first report showing the association between diabetes and thyroid dysfunction was published in 1979. Few studies also estimated much higher prevalence of thyroid dysfunction in diabetes.[6,7] Diabetes mellitus appears to influence thyroid function at two sites; firstly at the level of hypothalamic control of TSH release and secondly at the conversion of T4 to T3 in the peripheral tissue.[8] The term 'thyroid diabetes' was coined in the early literature to depict the influence of thyroid hormone alterations in the detoriation of glucose control. [9] Thyroid dysfunction may lead to hypothyroidism or hyperthyroidism in diabetic patients. In women thyroid disorders are also associated with pre menopause and post menopause. Thyroid hormones play an important role in normal reproductive function both through direct effects on the ovaries and indirectly by interacting with sex hormone binding proteins. Thyroid dysfunction can lead to menstrual irregularities and infertility.[10] Many studies were there on the thyroid and diabetes but very few studies were there on the relationship between thyroid hormones and glucose homeostasis among women. So our aim of the present study is to evaluate the relationship between thyroid hormones and glucose Regulation in pre menopausal diabetic and postmenopausal diabetic women.

MATERIALS AND METHODS

The study was conducted at Shri B.M Patil Medical College, Hospital& Research center, BLDE University. Total 60 women were selected. Among them 30 Pre menopausal diabetic women, aged between 25-45 years and 30 Post menopausal Diabetic women aged between 46-65 attending the out-patient clinic of Diabetes, were selected for the study. Written informed consent was obtained from the subjects. Ethical clearance was obtained from the Research Ethical Committee of Shri B.M Patil medical college and Hospital, BLDE University. A detailed history of diabetes taken from the subjects and pre menopause was confirmed by women having regular menstrual cycle every month and post menopause was confirmed by the women who were not menstruating for a period of 12 consecutive months and complete physical examination was done at the time of recruitment.

Inclusion criteria

Pre menopausal Diabetic & Post menopausal-Diabetic women with

i. Regular menstruation every month(for pre menopausal diabetic)
ii. Women not menstruating for a period of 12 consecutive months(for post menopausal diabetic)
iii. With Resting Blood pressure (BP<140/90)[11] (according to WHO guidelines)
iv. Non-smokers
v. Duration of Diabetes mellitus longer than one year or more(For Diabetic patients)

Exclusion criteria

Pre menopausal Diabetic & Post menopausal Diabetic women with

i. Evidence of Hypertension (BP>140/90)
ii. History of alcohol intake
iii. Intake of Thyroid supplements
iv. Hysterectomy
v. Use of any oral contraceptives within the previous six months
vi. Pregnant women

The following Physical Anthropometric & Physiological Parameters were assessed from the patients: Age, Height, weight, and Body surface area, Body Mass Index, Waist to Hip Ratio, Blood pressure and Pulse rate. Height was measured in cm. Weight was measured nearest to 0.1 kg. Body mass Index was calculated by using the following formula.

$$\text{Body Mass Index (BMI)} = \frac{\text{Weight in Kg}}{\text{Height in meter square}}$$

Body Surface Area (BSA) is calculated using Duboi's formula.[12]

Waist to Hip ratio was calculated by measuring waist at narrowest point under lowest rib and hips at the widest portion of buttocks using a tape and the ratio was calculated in cm by dividing waist measurement by hip measurement.

$$\text{Waist-Hip Ratio} = \frac{\text{Waist (cm)}}{\text{Hips (cm)}}$$

Blood pressure was measured by using mercury sphygmomanometer and Systolic Blood pressure (SBP, mmHg), Diastolic Blood pressure was recorded and Pulse Rate (beats per min, bpm) was also recorded. Blood samples from patients were obtained at the morning hours after a 12 hour overnight fast for biochemical, Hormonal analysis. Haematological parameters like complete blood picture count (CBC) was analyzed by using SYSMEX XN-1000 Automated Haematology cell counter. Biochemical parameters like

Fasting Blood Glucose (FBG) was analyzed by using Glucose oxidase-peroxidase method, with MISPO UNO (AGAPPE)-semi-auto analyzer, where as HbA1C was analyzed by Turbidimetric Inhibition Immunoassay method by using fully auto analyzer (ROCHE COBAS C 311) and the hormonal analysis like thyroid profile (T3, T4, TSH) were analyzed by the method ELFA (Enzyme linked fluorescent assay) by using Mini VIDAS.

Statistical analysis

It was done by using SPSS statistical software 16 version. Data was expressed as Mean±SD (standard deviation). Significance of difference between Diabetic group and Non-Diabetic group was determined by using student's unpaired (independent) sample "t" test. $P<0.05$ is considered statistically significant (two tailed).

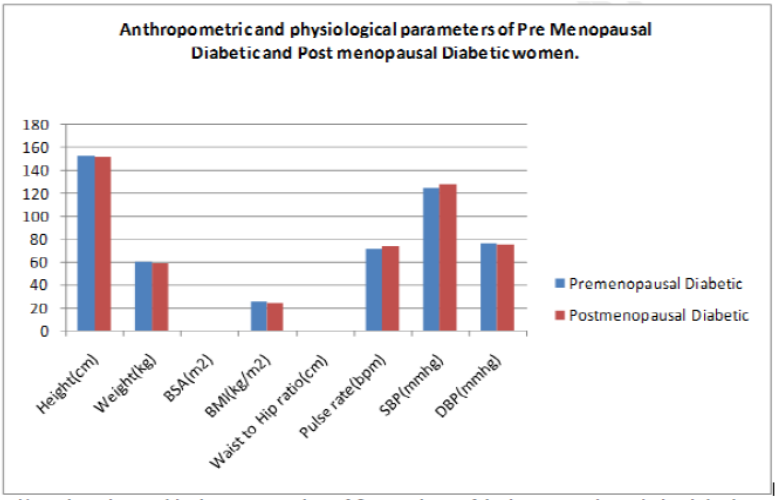
RESULTS

Table 1 shows Comparison of Anthropometric and physiological parameters of Pre and Post menopausal diabetic women. There was no significant difference in height and weight of both groups. But BSA, BMI, Waist to Hip Ratio of Pre menopause diabetic group is significantly more compared to Post menopause-Diabetic group. Pulse rate shows non-significant increase in post menopause diabetics, Whereas Systolic blood pressure (SBP) of post menopausal diabetic group significantly increased but diastolic blood pressure was non-significantly decreased in post menopausal diabetic group compared to Pre menopausal Diabetics. The same results were shown here in the form of graphical representation in Graph 1.

Table 1
Anthropometric and physiological parameters of pre menopausal diabetic and Post menopausal diabetic women

Parameter	Pre menopause Diabetic(n=30)	Post menopause Diabetic (n=30)	p value
Height(cm)	153.82±4.16	152.29±5.55	0.10
Weight(kg)	60.93±6.18	59.56±7.35	0.38
BSA(m^2)	1.58± 0.08	1.55±0.11	0.03*
BMI(kg/m^2)	25.93±2.83	25.64±2.45	0.000**
Waist to Hip ratio(cm)	1.07±0.21	1.00±0.12	0.000**
Pulse rate(bpm)	71.96±4.05	74.06±3.87	0.94
SBP(mmhg)	124.73±6.79	128±11.90	0.05*
DBP(mmhg)	76.53± 5.79	75.33±3.83	0.35

Data is presented as Mean±SD(standard deviation)
SBP-systolic blood pressure, DBP-Diastolic blood pressure, BMI-Body mass index, BSA-Body surface area, bpm- beats per minute, mmhg-millimeters of mercury
****indicates level of significance. *P<0.05(S), **P<0.001***



Here there is graphical representation of Comparison of Anthropometric and physiological parameters of Pre Menopausal Diabetic and Post menopausal diabetic women in Graph 1

Graph 1
Anthropometric and physiological parameters of Pre Menopausal Diabetic and Post Menopausal Diabetic women

Table 2
Biochemical and Hormonal parameters of Pre Menopausal Diabetic

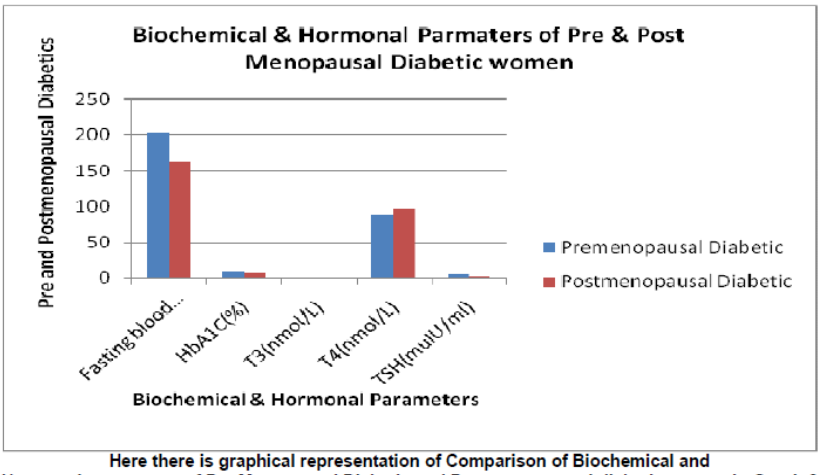
and Post Menopausal Diabetic women.

Parameter	Pre Menopause Diabetic (n=30)	Post menopause Diabetic (n=30)	p value
Fasting blood glucose(mg/DL)	204.1±76.90	163.83±53.06	0.18
HbA1C(%)	8.3±1.52	8.14±2.16	0.15
T3(nmol/L)	1.40±0.48	1.71±0.75	0.54
T4(nmol/L)	88.32±13.72	96.74±15.31	0.39
TSH(mulU/ml)	4.86±10.97	2.37±1.40	0.05*

Data is presented as Mean±SD(standard deviation)mg/DL- Milligrams per deciliter, nmol/L- Nanomoles per liter, mulU/ml- millimicrons-International units per milli liter,
****Indicates the level of significance. *P<0.05, **P<0.001***

Table 2 shows comparison of Biochemical and Hormonal parameters of Pre menopausal Diabetic and Post menopausal diabetic women. Fasting blood Sugar (FBS) levels non-significantly increased in Pre menopausal diabetic group. Glycosylated hemoglobin (HbA1C) levels were non-significantly increased in Post menopausal diabetic group .T3 and T4 levels Non-significantly decreased and TSH levels significantly increased in Pre menopausal Diabetic group compared to Post menopausal -Diabetic group . The same results were shown here in the form of graphical representation in Graph 2.

Graph 2
Biochemical and Hormonal parameters of Pre Menopausal Diabetic and Post Menopausal Diabetic women.



Here there is graphical representation of Comparison of Biochemical and Hormonal parameters of Pre Menopausal Diabetic and Post menopausal diabetic women in Graph 2

DISCUSSION

In our study, significant changes have been observed in Pre menopausal diabetic and Post menopausal diabetic women. There were no significant changes observed in height and weight of both groups but BSA, BMI, and Waist to Hip Ratio of Pre menopause diabetic group were significantly more compared to Post menopause-Diabetic group. It may be due to obesity which in turn may be associated with high BMI,BSA and waist hip ratio. Twin studies have shown important (up to 75%) genetic explanation to BMI.[13,14] With the exception of the rare mutations that cause severe morbid obesity, it seems that numerous genes, each with modest effect contribute to an individual's predisposition toward the more common forms of obesity.[15] The risk of diabetes increases by 9% for each kg gained in weight[16] and generally starts to increase at a BMI of 22 [17] and is 40 times higher at a BMI over 35. [18,19] Insulin resistance is widely recognized as a fundamental defect seen in obesity and type 2 diabetes. The development of diabetes is strongly associated with overweight and obesity. Over 90% of diabetics are overweight or obese [20] Weight gain and insulin resistance usually precede the onset of diabetes. Current theories indicate that diabetes develops when pancreatic beta cell output can no longer satisfy the demands imposed by increased insulin resistance.[21] Kissebah et al showed that regional body fat distribution was associated with glucose intolerance and hyperinsulinemia in premenopausal women.[22] There was significant increase in SBP and Non-significant decrease in DBP of Post menopausal diabetic group. It may be due to the age related decline in diastolic blood pressure. It is presumed to result from early recoil of the pressure wave, because of increasing arterial stiffness and lack of proper large artery compliance. Such shift of the reflection wave from the diastole to the systole increases systolic and decreases diastolic blood pressure. In our study, we also found that SBP increased in Post Menopausal-Diabetic group compared to Pre-menopausal diabetics. These observations were supported by Ronnback M et al. [23] There was no significant change in pulse rate. There was a non-significant Increase in HbA1C in Post

menopause Diabetic group. It may be due to the excess glucose present in blood reacts with hemoglobin. [24, 25] There was a marked increase in HbA1C levels in diabetic patients, which could be due to excessive glycosylation of hemoglobin. Our observations are supported by Saha HR et al. [26] In our study, T3 and T4 levels are non- significantly decreased and TSH levels were significantly increased in Pre menopause-Diabetic group compared to post menopause-diabetic group. Decrease in T3,T4 and increase in the TSH level may indicate "Hypothyroidism". The frequency of "Hypothyroidism" in pre-menopausal diabetic women is more than in general population. In pre menopausal diabetic women, Hypothyroidism may influence metabolic control through effects on glucose metabolism which include reductions in hepatic glucose output, gluconeogenesis and peripheral glucose utilization.[27] Glycaemic status may inturn influence thyroid function. A low T3 state is observed in patients with severe hyperglycaemia.[28] Celani et al. reported a high frequency of thyroid function abnormalities in acute hospital admissions with poorly controlled diabetes .[29]

CONCLUSION

From our study we found more prevalence of "Hypothyroidism" among pre menopausal diabetic women. So, prompt recognition of thyroid dysfunction in premenopausal diabetic women and controlling the blood glucose level is necessary to avoid further complications in future. So, routine thyroid screening should be included as a part of investigations in pre menopausal as well as post menopausal diabetic women. It will help in detection of thyroid abnormalities in subclinical stage itself.

ACKNOWLEDGEMENT

We thank all the Diabetic subjects of Pre & Post menopausal women of Shri B.M. Patil Medical Hospital and Research Center, BLDE University, Vijayapur for their participation in this study.

CONFLICT OF INTEREST

Conflict of Interest declared none.

REFERENCES

1. Feely J, Isles TE. Screening for thyroid dysfunction in diabetics. BMJ.1979Dec 1;2(6202):1439.
2. Gray RS, Irvine WJ, Clarke BF. Screening for thyroid dysfunction in diabetics. BMJ.1979 Dec 1;2(6202):1439.
3. Dias CM, Nogueira P, Rosa AN, De-Sa JV, Gouvea MF, Mannho-Falcos CM. Total cholesterol and high-density cholesterol in patients with insulin dependent diabetes mellitus. Acta Medica.1995; 8(11):619-28.
4. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harpers' Biochemistry, New York: McGraw-Hill , 2000:1
5. Ramachandran A, Snehalatha C. Current scenario of diabetes in India. Journal of diabetes. 2009 Mar 1;1(1):18-28.
6. Celani MF, Bonati ME, Stucci N. Prevalence of abnormal thyrotropin concentrations measured by a sensitive assay in patients with type 2 diabetes mellitus. Diabetes Res(Edinburgh, Scotland). 1993 Dec;27(1):15-25.
7. Udiong CE, Udoh AE, Etukudoh ME. Evaluation of thyroid function in diabetes mellitus in Calabar, Nigeria.IJCB 2007 Sep 1;22(2):74-8.
8. Shah SN. Thyroid disease in diabetes mellitus. J Assoc Physicians India1984 Dec;32(12):1057-9.
9. Güney E, Efe B, Akalın A, Kebapçı MN, Erenoğlu E. Thyroid disease in diabetes mellitus. Turk J Endocrinol Metab. 1999;3(3):119-22.
10. Poppe K, Glinoer D. Thyroid autoimmunity and hypothyroidism before and during pregnancy. Human reproduction update. 2003 Mar 1; 9(2):149-61.
11. World Health Organization, International Society of Hypertension Writing Group. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J Hypertension. 2003 Nov 1;21(11):1983-92.
12. DuBois D, DuBois EF. A formula to estimate the approximate surface area if height and weight be known. Arch Int Med. 1916; 17: 863-71.
13. Stunkard AJ, Harris JR, Pedersen NL, McClearn GE. The body-mass index of twins who have been reared apart. N Eng J med1990 May 24;322(21):1483-7.
14. Moll PP, Burns TL, Lauer RM. The genetic and environmental sources of body mass index variability: the Muscatine Ponderosity Family Study. Am.J.Hum.Genet 1991 Dec; 49(6):1243.
15. Damcott CM, Sack P, Shuldiner AR. The genetics of obesity. Endocrinol Metab Clin North Am 2003; 32:761-86.
16. Ford ES, Williamson DF, Liu S. Weight change and diabetes incidence: findings from a national cohort of US adults. Am J Epidemiol .1997 Aug 1;146(3):214-22.
17. Colditz GA, Willett WC, Stampfer MJ, Manson JE, Hennekens CH, Arky RA, Speizer FE. Weight as a risk factor for clinical diabetes in women. Am J Epidemiol. 1990 Sep 1;132(3):501-13.
18. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med.2001 Sep 13;345(11):790-7.
19. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. Jama. 1999 Oct 27;282(16):1523-9.
20. Albu J, Pi-Sunyer FX. Obesity and diabetes. In: Bray GA, Bouchard C, James WPT. Handbook of Obesity, Marcel Dekker New York, NY.1998:697-707.

21. DeFronzo RA. The triumvirate: β-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes. 1988;37(6):667-87.
22. Kissebah AH, Vydelingum N, Murray R, Evans DJ, Kalkhoff RK, Adams PW. Relation of Body Fat Distribution to Metabolic Complications of Obesity*. J Clin Endocrinol.Metab1982 Feb;54(2):254-60.
23. Rönnback M, Fagerudd J, Forsblom C, Pettersson-Fernholm K, Reunanen A, Groop PH, Finnish Diabetic Nephropathy (FinnDiane) Study Group. Altered age-related blood pressure pattern in type 1 diabetes. Circulation. 2004 Aug 31;110(9):1076-82.
24. Gloria-Bottini F, Antonacci E, Bottini N, Ogana A, Borgiani P, De Santis G, Lucarini N. RH blood groups and diabetic disorders: Is there an effect on glycosylated hemoglobin level?Hum Biol .2000 Apr 1:287-94.
25. Sampson MJ, Gopaul N, Davies IR, Hughes DA, Carrier MJ. Plasma F2 Isoprostanes Direct evidence of increased free radical damage during acute hyperglycemia in type 2 diabetes. Diabetes care. 2002 Mar 1;25(3):537-41.
26. Saha HR, Sarkar BC, Khan SA, Sana NK, Choudhury S. A Comparative study of Thyroid Hormone and Lipid Status in Diabetic and Non Diabetic Adults.2012;1:450.
27. Mouradian M, Abourizk N. Diabetes mellitus and thyroid disease. Diabetes Care 1983;6(5):512-20.
28. Naeije R, Golstein J, Clumeck N, Meinhold H, Wenzel KW, Vanhaelst L. A low T3 syndrome in diabetic ketoacidosis. Clin Endocrinol. 1978 Jun 1;8(6):467-72.
29. Celani MF, Bonati ME, Stucci N. Prevalence of abnormal thyrotropin concentrations measured by a sensitive assay in patients with type 2 diabetes mellitus. Diabetes Res(Edinburgh, Scotland). 1993 Dec;27(1):15-25.

PAPER PRESENTATIONS

Paper presentations at conferences:

Oral presentation:

At National Conference:

1) **Title:** Interaction between Thyroid Hormones and Glucose homeostasis in Post menopausal Diabetic and Non-Diabetic women.

   3rd ASSOPICON 14th- 17th September 2016, 'Physiology Decades Novelty of Vascular Sciences at BLDEU's Shri B.M Patil Medical College, Hospital & Research Center, Vijayapura.

Poster presentation:

At National conference:

1) **Title:** Role of Thyroid Hormones on Glucose homeostasis in Pre & Post Menopausal Diabetic women.

   63rd APPICON **,**10-14th October 2017, " Physiology Beyond Academics in the Era of Translational Research" at JIPMER, Puducherry

3rd Annual Conference of Association of Physiologists of India

ASSOPICON 2016

14th-17th September 2016

Theme : Physiology Decodes Novelty of Vascular Science

CERTIFICATE

This is to certify that

Dr/Mr/Miss Kalashilpa.Ch

Bearing Reg No. -- has partcipated as Delegate/Chairperson/Jury/Resource person/Presented Oral /Poster

Topic entitled Interaction between Thyroid Hormones and Glucose homeostasis in post menopausal diabetic and Non-Diabetic women.

In ASSOPICON-2016 held from 15th-17th September 2016,

Organized by Department of Physiology,

BLDE University's Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapur, Karnataka.

Karnataka Medical Council has granted 4 credit hours for Delegates/Faculty

vide letter No.: K.M.C./C.M.E./701/2016.

Dr. Manjunatha Aithala	Zonal Chairman	Dr. S. P. Guggarigoudar	Dr. G. K.Pal
Organizing Secretary	KMC CME Accreditation Committee	Organizing Chairman	General Secretary, ASSOPI

3rd Annual Conference of Association of Physiologists of India

Jawaharlal Institute of Postgraduate Medical Education and Research

(An Institution of National Importance under the Ministry of Health and Family Welfare, Govt. of India)

Puducherry

63rd Annual National Conference of Association of Physiologists and Pharmacologists of India

APPICON 2017

"Physiology Beyond Academics in the Era of Translational Research"

10th - 14th October 2017

Department Of Physiology

Certificate of Participation

This is to certify that Kalashilpa Chittikanna ..... has presented *Oral / Poster paper* titled "Role of Thyroid Hormones on Glucose homeostasis in Pre & Post Menopausal Diabetic Women ....."

in the 63rd Annual National Conference of Association of Physiologists and Pharmacologists of India organized by the Department of Physiology, JIPMER, Puducherry from 12th to 14th October 2017.

Dr. Vivek Kumar Sharma	Dr. Pravati Pal	Dr. G. S. Gaur	Dr. S. C. Parija
Organizing Secretary	Co-Organizing Chairman	Organizing Chairman	Director, JIPMER