SERUM THYROID PROFILE AND ANTI-THYROPEROXIDASE ANTIBODY LEVEL IN BENIGN AND MALIGNANT BREAST TUMORS

Dissertation submitted for

M.D. BIOCHEMISTRY BRANCH – XIII
DEGREE EXAMINATION

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI – 600 032
TAMIL NADU

APRIL 2011

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **''SERUM THYROID PROFILE AND ANTI-THYROPEROXIDASE ANTIBODY LEVEL IN BENIGN AND MALIGNANT BREAST TUMORS''** is the original bonafide work done by, **Dr.V.G.KARPAGHAVALLI,** Post Graduate Student, Institute of Biochemistry, Madras Medical College, Chennai under our direct supervision and guidance.

Dean
Government General Hospital
Madras Medical College,
Chennai – 600 003.

Director and Professor
Institute of Biochemistry
Madras Medical College
Chennai – 600 003.

SPECIAL ACKNOWLEDGEMENT

The author gratefully acknowledges and sincerely thanks Professor **Dr.J.Mohanasundaram M.D., Dip. NB, Ph.D., Dean,** Madras Medical College and Government Medical College, Chennai, for granting his permission to utilize the facilities of this Institution for the study.

ACKNOWLEDGEMENT

The author expresses her warmest respects and profound gratitude to **Dr.Pragna B.Dolia, M.D.,** Director and Professor, Institute of Biochemistry, Madras Medical College, Chennai, for her able guidance, constant encouragement , support and valuable time but for which this dissertation could not have been made possible.

The author is extremely thankful to **Dr.Gunaseelan, M.S** Professor and Head of Department of General Surgery, Government General Hospital, Chennai, for granting permission to obtain blood samples from the patients.

The author expresses her sincere gratitude to **Dr.M.Shyamraj M.D, Dr.K.Ramadevi M.D, Dr.A.Chitraa M.D** and **Dr.Amuthavalli. M.D,** Additional Professors, Institute of Biochemistry, Madras Medical College for their guidance and support.

The author expresses her warm respects and sincere thanks to **Dr.V.K.Ramadesikan, Dr.S.Sumathy, Dr.Poonguzhali Gopinath, Dr.C.Shanmuga Priya and Dr.V.Ananthan** Assistant Professors, Institute of Biochemistry, Madras Medical College for their guidance and constant encouragement.

The author expresses her special thanks to her colleagues for their immense help, constant encouragement and unconditional support throughout the study.

The author gratefully acknowledges the help rendered by **Mr.A.Venkatesan,** Statistician, Department of Evidence Based Medicine, Madras Medical College during the statistical analysis of the study.

The author is indebted to the patients and the persons from whom blood samples were collected for conducting the study.

Finally, the author expresses her special thanks to her husband **Mr.V.Senthil Kumar,** her daughter **S.K.Dhanasrie** and other family members, for the moral support and encouragement extended by them.

ABBREVIATION

Anti-TPO	-	anti-thyroperoxidase antibody
T_4	-	Thyroxine
T_3	-	Tri iodothyronine
fT_4	-	free thyroxine
fT_3	-	free tri iodothyronine
TSH	-	Thyroid stimulating hormone
TR	-	Thyroid hormone receptor
ER	-	Estrogen receptor
ERR	-	Estrogen receptor related receptor
FSH	-	Follicle stimulating hormone
LH	-	Leutinising hormone
GnRH	-	Gonodotrophin releasing hormone
PRL	-	Prolactin
BRCA	-	Breast Cancer gene
DNA	-	Deoxyribonucleic acid
AD	-	Autosomal dominant
FNAC	-	Fine needle aspiration cytology
TNM	-	Tumor Node Metastasis System
EGF	-	Epidermal Growth Factor

IGF	-	Insulin Like Growth Factor
NIS	-	Sodium Iodide Symporter
Tg	-	Thyroglobulin
TRE	-	Thyroid response element
RXR	-	Orphan receptor
TRAP	-	Thyroid receptor auxillary protein
TGFβ	-	Transfoming Growth Factor beta
MAPK	-	Mitogen associated protein kinase
PI3K	-	Phospho Inositide-3 Kinase
AITD	-	Autoimmune thyroid disease
6-IL	-	6-Iodo Lactone
PPARγ	-	Peroxisome Proliferators Activated Receptor gamma
ELISA	-	Enzyme Linked Immunosorbant Assay
SHBG	-	Steroid hormone binding globulin
HER2	-	Human epidermal growth factor receptor 2
MYC	-	Myelocytomatosis protein

INDEX

	Page No.
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	3
3. AIM OF THE STUDY	35
4. MATERIALS AND METHODS	36
5. STATISTICAL ANALYSIS	50
6. RESULTS	51
7. DISCUSSION	55
8. CONCLUSION	60
9. SCOPE FOR FURTHER STUDY	61
REFERENCES	

INTRODUCTION

Breast cancer, with its uncertain cause, has captured the attention of surgeons throughout the ages. Despite centuries of theoretical meandering and scientific inquiry, breast cancer remains one of the most dreaded of human diseases.[1]

Tumors or ulcers of breast have been reported as early as 1600 BC as reported in ancient Egyptian chronicles, the Papyrus. Currently carcinoma breast is the most common malignancy and the common cause of cancer deaths in women worldwide. In India carcinoma breast is the second most common malignancy in women.

Mammography and ultrasonography are being used for screening of breast cancer. Cancer that is detected early can potentially be cured when the tumor is small enough to be completely removed surgically. Unfortunately, most cancers do not produce any symptoms until the tumors are either too large to be removed surgically or cancerous cells have metastasized. Hence there is a need to detect cancer at an early stage.

Tumor markers are used for the detection of risk, population screening, diagnosis, staging and prognosis. It can also predict the response to therapy, monitor treatment, detect the presence of occult metastatic disease, and monitor the course of the disease. Several tumor markers are in use nowadays, which include alpha fetoprotein, carcinoembryonic antigen, human chorionic gonadotropin, prostate specific antigen and relatively less specific markers like lactate dehydrogenase, alkaline phosphatase, ferritin and gamma glutamyl transpeptidase.

There may be re-expression of certain fetal antigen in breast cancer tissues that may lead to development of autoantibodies in patients.[2]

Breast cancer and thyroid disease predominantly affect females and that both have a postmenopausal peak incidence has inevitably resulted in a search for an association between the two diseases.[3,4]

The thyroid hormones are important regulators of growth, development, and metabolism in higher animals and humans.[5] Thyroid hormones have a role in the normal development of breast.[6]

Where an association between thyroid disease and breast cancer was shown hypothyroidism frequently exists. The presence of circulating TPO antibodies in asymptomatic individuals has been implicated as conferring an increased risk for future hypothyroidism.[7]

This study was done to measure the levels of TSH , free T_4 ,freeT_3 and anti TPO antibody levels in patients with newly diagnosed carcinoma breast and benign breast tumors to understand antiproliferative role of thyroid hormones in breast tumors and to find if there is any correlation between of anti TPO antibodies level and breast neoplasia.

REVIEW OF LITERATURE

EMBRYOLOGY AND FUNCTIONAL ANATOMY OF THE BREAST

Embryology

Breast starts to develop from ventral band of thickened ectoderm at 5-6 wks. Each breast develops when an ingrowth of ectoderm forms a primary tissue bud in the mesenchyme. The primary bud, in turn, initiates the development of 15 to 20 secondary buds. Epithelial cords develop from the secondary buds and extend into the surrounding mesenchyme. The breast remains undeveloped in the female until puberty, when it enlarges in response to ovarian estrogen and progesterone, which initiate proliferation of the epithelial and connective tissue elements. However, the breasts remain incompletely developed until pregnancy occurs.[1]

Functional Anatomy

The breast is composed of 15 to 20 lobes, each composed of several lobules. Fibrous bands of connective tissue travel through the breast (suspensory ligaments of Cooper), insert perpendicularly into the dermis, and provide structural support.[1]

Nipple–areola Complex

The epidermis of the nipple–areola complex is pigmented and is variably corrugated. The areola contains sebaceous glands, sweat glands, and accessory glands, which produce small elevations on the surface of the areola (Montgomery tubercles).

Inactive and Active Breast

Each lobe of the breast terminates in a major (lactiferous) duct (2 to 4 mm in diameter), which opens through a constricted orifice (0.4 to 0.7 mm in diameter) into

the ampulla of the nipple. Immediately below the nipple–areola complex, each major duct has a dilated portion (lactiferous sinus), which is lined with stratified squamous epithelium. Major ducts are lined with two layers of cuboidal cells, while minor ducts are lined with a single layer of columnar or cuboidal cells. Myoepithelial cells of ectodermal origin reside between the epithelial cells in the basal lamina and contain myofibrils. In the inactive breast, the epithelium is sparse and consists primarily of ductal epithelium.

PHYSIOLOGY OF THE BREAST

Breast Development and Function

Breast development and functions are initiated by a variety of hormonal stimuli, including estrogen, progesterone, thyroid hormone, prolactin, oxytocin, cortisol and growth hormone. Estrogen initiates ductal development, while progesterone and thyroid hormones are responsible for differentiation of epithelium and for lobular development. Prolactin is the primary hormonal stimulus for lactogenesis in late pregnancy and the postpartum period. It upregulates hormone receptors and stimulates epithelial development. **Figure -1** depicts the secretion of neurotrophic hormones from the hypothalamus, which is responsible for regulation of the secretion of the hormones that affect the breast tissues. The gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) regulate the release of estrogen and progesterone from the ovaries. In turn, the release of LH and FSH from the basophilic cells of the anterior pituitary is regulated by the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Negative feedback effects of circulating estrogen and progesterone regulate the secretion of LH, FSH, and GnRH. These hormones are responsible for the development, function, and maintenance of breast tissues.[1]

COMMON BENIGN DISORDERS AND DISEASES OF THE BREAST[1]

Early Reproductive Years

Fibroadenomas are seen predominantly in younger women age 15 to 25 years. Fibroadenomas usually grow to 1 or 2 cm in diameter and then are stable, but may grow to a larger size. Small fibroadenomas (1 cm in size or less) are considered normal, while larger fibroadenomas (up to 3 cm) are disorders and giant fibroadenomas (larger than 3 cm) are disease. Similarly, multiple fibroadenomas (more than five lesions in one breast) are very uncommon and are considered as disease. The precise etiology of adolescent breast hypertrophy is unknown. A spectrum of changes from limited to massive stromal hyperplasia (gigantomastia) is seen. Nipple inversion is a disorder of development of the major ducts, which prevents normal protrusion of the nipple. Mammary duct fistulas arise when nipple inversion predisposes to major duct obstruction, leading to recurrent subareolar abscess and mammary duct fistula.[1]

Later Reproductive Years

Cyclical mastalgia and nodularity are usually associated with premenstrual enlargement of the breast and are regarded as normal. Painful nodularity that persists for more than 1 week of the menstrual cycle is considered a disorder. In epithelial hyperplasia of pregnancy, papillary projections sometimes give rise to bilateral bloody nipple discharge.

PAGE'S CLASSIFICATION OF BENIGN BREAST DISORDERS[1]

Nonproliferative disorders of the breast

- Cysts and apocrine metaplasia
- Duct ectasia
- Calcifications
- Fibroadenoma and related lesions

Proliferative breast disorders without atypia

- Sclerosing adenosis
- Radial and complex sclerosing lesions
- Ductal epithelial hyperplasia
- Intraductal papillomas

Atypical proliferative lesions

- Atypical Lobular Hyperplasia
- Atypical Ductal Hyperplasia

EPIDEMIOLOGY AND NATURAL HISTORY OF BREAST CANCER

Epidemiology

Breast cancer is the most common cancer in women and is the leading cause of death from cancer for women aged 40 to 44 years. It accounts for 33% of all female cancers and is responsible for 20% of the cancer-related deaths in women.[1]

The Primary Breast Cancer

More than 80% of breast cancers show productive fibrosis that involves the epithelial and stromal tissues. With growth of the cancer and invasion of the surrounding breast tissues, the accompanying desmoplastic response entraps and shortens the suspensory ligaments of Cooper to produce a characteristic skin retraction. Localized edema (peau d'orange) develops when drainage of lymph fluid from the skin is disrupted. With continued growth, cancer cells invade the skin and eventually ulceration occurs. As new areas of skin are invaded, small satellite nodules appear near the primary ulceration. The size of the primary breast cancer correlates with disease-free and overall survival, but there is a close association between cancer

size and axillary lymph node involvement. In general, up to 20% of breast cancer recurrences are locoregional, more than 60% are distant, and 20% are both locoregional and distant.

HISTOPATHOLOGY OF BREAST CANCER

Carcinoma in Situ

Cancer cells are in situ or invasive depending on whether or not they invade through the basement membrane.[1]

- **Lobular Carcinoma in Situ**
- **Ductal Carcinoma in Situ**

Invasive Breast Carcinoma

Invasive breast cancers have been described as lobular or ductal in origin. Eighty percent of invasive breast cancers are described as invasive ductal carcinoma of no special type (NST). These cancers generally have a worse prognosis than special-type cancers. Foote and Stewart originally proposed the following classification for invasive breast cancer.[1]

I. Pagets' disease of the nipple.

II. Invasive ductal carcinoma

- Adenocarcinoma with productive fibrosis (scirrhous, simplex, NST) 80%
- Medullary carcinoma 4%
- Mucinous (colloid) carcinoma 2%
- Papillary carcinoma 2%
- Tubular carcinoma (and ICC) 2%

III. Invasive lobular carcinoma 10%

IV. Rare cancers (adenoid cystic, squamous cell, apocrine)

RISK FACTORS FOR BREAST CANCER

Hormonal Risk Factors

Increased exposure to estrogen is associated with an increased risk for developing breast cancer, whereas reducing exposure is thought to be protective. Correspondingly, factors that increase the number of menstrual cycles, such as early menarche, nulliparity, and late menopause, are associated with increased risk. Moderate levels of exercise and a longer lactation period, factors that decrease the total number of menstrual cycles, are protective. Finally, there is an association between obesity and increased breast cancer risk. Because the major source of estrogen in postmenopausal women is the conversion of androstenedione to estrone by adipose tissue, obesity is associated with a long-term increase in estrogen exposure.[1]

Nonhormonal Risk Factors

Nonhormonal risk factors include radiation exposure. Young women who receive mantle radiation therapy for Hodgkin's lymphoma have a breast cancer risk that is 75 times greater than that of age-matched control subjects. Survivors of the atomic bomb blasts in Japan during World War II have a very high incidence of breast cancer, likely because of somatic mutations induced by the radiation exposure. In both circumstances, radiation exposure during adolescence, a period of active breast development, magnifies the deleterious effect. Studies also suggest that the amount and duration of alcohol consumption are associated with an increased breast cancer risk. Alcohol consumption is known to increase serum levels of estradiol. Finally,

evidence suggests that chronic consumption of foods with a high fat content contributes to an increased risk of breast cancer by increasing serum estrogen levels.

Risk-Assessment Models

Two risk-assessment models are currently used to predict the risk of breast cancer. From the Breast Cancer Detection Demonstration Project, a mammography screening program conducted in the 1970s, Gail and colleagues developed the most frequently used model, which incorporates age at menarche, the number of breast biopsies, age at first live birth, and the number of first-degree relatives with breast cancer.[8,9]

GENETICS OF BREAST CANCER

GENETIC PREDISPOSITION

Percent Incidence of Sporadic, Familial, and Hereditary Breast Cancer

Sporadic breast cancer 65–75%

Familial breast cancer 20–30%

Hereditary breast cancer 5-10%

- BRCA-1 35-45%
- BRCA-2 25-35%
- p53 (Li-Fraumeni syndrome) 1%
- PTEN (Cowden disease) <1%
- Unknown 20%

BRCA- Breast Cancer gene

Five to 10% of breast cancers are caused by inheritance of germline mutations such as BRCA-1 and BRCA-2, which are inherited in an autosomal dominant fashion with varying penetrance. Both BRCA-1 and BRCA-2 function as tumor-suppressor genes, and for each gene, loss of both alleles is required for the initiation of cancer. Breast cancer in these families appears as an autosomal dominant trait with high penetrance. Approximately 50% of children of carriers inherit the trait.

BRCA-1

BRCA-1 gene is located in chromosome 17q at position 21.In general, BRCA-1–associated breast cancers are invasive ductal carcinomas, are poorly differentiated, and are hormone receptor–negative. BRCA-1 associated breast cancers have a number of distinguishing clinical features, such as an early age of onset when compared with sporadic cases; a higher prevalence of bilateral breast cancer; and the presence of associated cancers in some affected individuals, specifically ovarian cancer and possibly colon and prostate cancers.[10-14]

BRCA-2

BRCA-2 is located on chromosome 13q and spans a genomic region of about 70 kb of DNA. Like BRCA-1, it is postulated to play a role in DNA damage response pathways. BRCA-2 messenger RNA also is expressed at high levels in late G1 and S phases of the cell cycle. The kinetics of BRCA-2 protein regulation in the cell cycle is similar to that of BRCA-1 protein, suggesting that these genes are coregulated. Breast cancer in BRCA-2 families is an autosomal dominant trait and has a high penetrance.. BRCA-2–associated breast cancers are invasive ductal carcinomas, which are more

likely to be well differentiated and to express hormone receptors than BRCA-1–associated breast cancer.[10-14]

EPIGENETICS OF BREAST CANCER

In addition to inherited mutations that occur in hereditary breast cancer, sporadic breast cancers exhibit ‘epigenetic’’ mechanisms for inactivating several important DNA repair genes including BRCA1, ATM, CHK2 and p53. Epigenetics describes chromatin and DNA modifications that alter gene expression but do not involve changes in the under lying DNA sequences.[15]

In cancer, the main epigenetic mechanisms underlying abnormal gene expression include aberrant CpG-island-promoter methylation and alteration in the histone modification (deacetylation and methylation). These abnormalities can be reversed by inhibitors of both DNA methyl transferases and histone deacetylases.[15]

Both inherited and sporadic breast cancers can exhibit variable ER-α expression.ER-α is an epigenetically regulated gene that undergoes promoter methylation in a significant proportion of breast cancers.[16]

GROWTH FACTOR RECEPTOR PATHWAYS[17]

Growth factor receptors play an essential role in initiating both proliferative and cell survival pathways in the breast as well as other epithelia. In the breast cancer biology, the EGFRs and insulin-like growth factor receptors have been studied extensively. These receptors have a cytoplasmic tyrosine kinase-containing domain that can activate downstream signaling cascades. Growth factor receptors can be constitutively activated by excessive ligand levels, activating mutations, or gene

amplifications/over expression that ultimately leads to inappropriate kinase activity and growth promoting second messenger activation.

EGFR (HER1, Erb1) and HER2 (EGFR2 or ErbB2) appear to be particularly relevant receptors in breast cancer biology. At the molecular level, HER2 amplification is associated with deregulation of G1/S phase cell cycle control via up-regulation of cyclins D1, E, and cdk6, as well as p27 deregulation.

In addition to activation of EGFR pathway, signaling via insulin-like growth factor-1 and receptor can result in phosphorylation and activation of a variety of oncogenic kinases including PI3-K and HER2. IGF-1R is the primary response mediator of IGF and is expressed in all epithelial cell types. Elevated IGF-1 levels have been implicated in breast cancer risk.

HORMONAL REGULATION[17]

The degree of exposure to estrogen is a well established risk factor for developing ER-positive breast cancer. Estrogen is a steroid hormone that has a profound proliferative effect on normal human mammary epithelium through its activation of ER-α, a classic nuclear receptor.

Estrogen exerts its action through both ‘genomic’ and ‘nongenomic’ mechanisms. Genomic action refers to the transcriptional regulation of specific target genes by the ligand bound and dimerised ER-α. Activated ER-α dimers direct selective gene expression through binding to regulatory regions known as estrogen response elements. These regions direct the estrogen mediated transcription of several growth-promoting genes including cyclinD1 and MYC.

Nongenomic actions of ERα are extremely rapid and result from hormone-dependent activation of membrane-bound and /or cytosolic ERs. These nonnuclear ER actions result in rapid phosphorylation and activation of important growth regulatory kinases like EGFR, IGF-1R etc.

This ‘cross-talk’ between ER-α and growth factor receptors are bidirectional. Constitutive HER2, for example can increase ER-α signaling to a point where it is unresponsive to antiestrogen treatments. (Figure: 2)

PI3-K PATHWAY[17]

The PI3-K pathway is activated in response to a number of events that result in increased breast cancer cell growth and proliferation. Activating mutations of PI3-K may be an important contributing factor to mammary tumor progression.

Activation of the PI3-K pathway, results in 3-phosphoionositide-dependent kinase-mediated activation of several known kinases including AKT1, AKT2. Activated AKT1 appears to be antiapoptotic but also plays an anti-invasive role in tumor formation.

DIAGNOSING BREAST CANCER

In 33% of breast cancer cases, the woman discovers a lump in her breast. Other less frequent presenting signs and symptoms of breast cancer include (1) breast enlargement or asymmetry (2) nipple changes, retraction, or discharge (3) ulceration or erythema of the skin of the breast (4) an axillary mass and (5) musculoskeletal discomfort. However, up to 50% of women presenting with breast complaints have no physical signs of breast pathology. Breast pain usually is associated with benign disease.[1]

I. CLINICAL EXAMINATION

- **Inspection**
- **Palpation**

II. IMAGING TECHNIQUES

- **Mammography**

In screening mammography, the craniocaudal (CC) view and the mediolateral oblique (MLO) views of the breast are obtained. Mammography is also used to guide interventional procedures, including needle localization and needle biopsy. Specific mammography features like asymmetric thickening of breast tissues, and clustered microcalcifications are suggestive of breast cancer.[1]

- **Ultrasonography**

Ultrasonography is an important method of resolving equivocal mammography findings, defining cystic masses, and demonstrating the echogenic qualities of specific solid abnormalities. Ultrasonography is used to guide fine-needle aspiration biopsy, core-needle biopsy, and needle localization of breast lesions

- **Magnetic Resonance Imaging**

There is current interest in using MRI to screen the breasts of high-risk women and of women with a newly diagnosed breast cancer.

III. BREAST BIOPSY

- **Nonpalpable Lesions**

Image-guided breast biopsies are frequently required to diagnose nonpalpable lesions. Ultrasound localization techniques are employed when a mass is present,

while stereotactic techniques are used when no mass is present (microcalcifications only).[1]

- **Palpable Lesions :**

FNA biopsy of a palpable breast mass is performed in an outpatient setting.

BREAST CANCER STAGING

The clinical stage of breast cancer is determined primarily through physical examination of the skin, breast tissue, and lymph nodes (axillary, supraclavicular, and cervical). Mammography, chest x-ray, and intraoperative findings (primary cancer size, chest wall invasion) also provide necessary staging information. Pathologic staging combines clinical stage data with findings from pathologic examination of the resected primary breast cancer and axillary lymph nodes. A frequently used staging system is the TNM (tumor, nodes, and metastasis) system. The American Joint Committee on Cancer (AJCC) has modified the TNM system for breast cancer. [(18)]

TNM SYSTEM OF STAGING

Primary tumor (T) Definitions for classifying the primary tumor (T) are the same for clinical and for pathologic classification. If the measurement is made by physical examination, the examiner will use the major headings (T1, T2, or T3); if other measurements, such as mammographic or pathologic measurements, are used, the subsets of T1 can be used. Tumors should be measured to the nearest 0.1-cm increment

- TX Primary tumor cannot be assessed
- T0 No evidence of primary tumor
- Tis Carcinoma in situ

- T1(mic) Microinvasion 0.1 cm or less in greatest dimension
- T1 Tumor 2 cm or less in greatest dimension
- T2 Tumor more than 2 cm but not more than 5 cm in greatest dimension
- T3 Tumor more than 5 cm in greatest dimension
- T4 Tumor of any size with direct extension to (a) chest wall or (b) skin or (c) both or (d)Inflammatory carcinoma

Regional lymph nodes—Clinical (N)

- NX Regional lymph nodes cannot be assessed (e.g., previously removed)
- N0 No regional lymph node metastasis
- N1 Metastasis to movable ipsilateral axillary lymph node(s)
- N2 Metastases in (a)ipsilateral axillary lymph nodes fixed or matted, or in clinically apparent (b)ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastasis.
- N3 Metastasis in (a)ipsilateral infraclavicular lymph node(s) with or without axillary lymph node involvement, or in clinically apparent (b) ipsilateral internal mammary lymph node(s) and in the presence of clinically evident axillary lymph node metastasis; or metastasis in (c) ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement.

Distant metastasis (M)

- MX Distant metastasis cannot be assessed
- M0 No distant metastasis
- M1 Distant metastasis

ROLE OF THYROID HORMONE IN BREAST DEVELOPMENT

It is well known that thyroid hormone is a major physiological regulator of mammalian development through specific effects on the rate of cell differentiation and gene expression.[19]

Thyroid hormones and cognate nuclear receptors are involved in cell growth and differentiation of many cell types.[20]

Growing and developing breasts require the coordinated action of several hormones such as estrogen (E2), progesterone, thyroid hormones, adrenal steroids, insulin, and prolactin.[21]

Estrogen is considered to be a potent mitogen for the normal mammary gland, whereas thyroid hormones appear to stimulate lobular development, contributing to the differentiation of normal breast tissue.[23] About one third of breast cancers maintain estrogen dependence for growth. The concentration of ER in malignant breast tissues is an indicator of their hormonal dependence.[22]

Consistent with the proposal that thyroid hormones act on the breast, TR have been described in breast cancer.[24] There are reports on interference between estradiol and thyroid hormones. Studies suggested a cross talk between ER and TR in neuroendocrine tissues leading to inhibition of estrogenic effects by thyroid hormone.[25]

The biological activity of thyroid hormones and E2 is only manifested in cells expressing thyroid hormone (TR) and estrogen receptors (ER), respectively. These receptors belong to the nuclear receptor superfamily. They share a common mechanism of action whereby hormone-receptor complexes bind to cis acting DNA elements and enhance or repress transcription of target genes.[22.]

The half-site of the consensus sequence of nucleotide bases constituting the thyroid hormone response element is identical to the ER response element half-site. TR has been shown to bind to ER response elements in addition to their cognate response elements.[26]

The T_3 receptor binds to estrogen response elements with high affinity. The T_3 receptor binds to estrogen response elements in a transcriptionally inactive form competing with estrogen receptor binding. It thus inhibits estrogen-dependent transactivation resulting in a net decrease in gene expression.

Recent reports showed that TRs and T_3 are involved in carcinogenesis and influence processes of differentiation, proliferation, apoptosis, and metastasis. Majority of currently available data suggest the suppressive role of T_3 /TRs in carcinogenesis.[27]

ANTICANCER EFFECTS OF T_3 /TRS IN HUMAN CELLS

Hypothyroidism affects tumor growth and invasiveness differentially. Increasing evidence shows that loss of expression and/or function of the thyroid hormone receptors (TRs) could result in a selective advantage for tumor development.[28]

Increased aggressiveness was also observed with parental cells that do not express TRs. These results show that changes in the stromal cells secondary to host hypothyroidism can modulate tumor progression and metastatic growth independently of the presence of TRs on the tumor cells.[28]

The vast majority of studies on antitumoral activity of TRs are focused on TRβ1 isoform. This could suggest that among the two isoforms, TRβ1 is the one that controls expression of protein regulators engaged in majority of pathways contributing to protection against cancer. Different biological functions of TRs result probably from subtle changes in expression of TR regulated genes. This is in agreement with the hypothesis of procancerous effects of disturbed expression of TRs. Aberrantly expressed TRs may lead to deregulation of proteins controlling cell cycle, such as E_2F1.[29, 30]

Kamiya Y et al found that mutations of both α and β TRs leads to severe improperties in its function, including disturbances in binding of T_3, DNA and coregulatory proteins. E_2F1 is a transcription factor controlling G1 to S phase transition whose expression is negatively regulated by TRs.[31-34]

Yen et al[35] found that T_3 inhibits proliferation of hepatoma HepG2-TR cell line (Fig. 3). In these cells T_3 stimulates the activity of promoter and expression of TGF-β. Upregulation of TGF-β results in repression of cell cycle regulating proteins: cdk2, Cyclin E and ppRb (hyperphosphorylated retinoblastoma protein), and contributes to inhibition of cell proliferation.

The antitumor role of T_3 in liver cancers is also supported by the observation that hypothyroidism is a possible risk factor for hepatocellular carcinoma in patients with no known underlying cause of liver disease.[36]

This is in agreement with experiments in rats showing that although T_3 treatment induces liver proliferation, it also leads to loss of hepatocellular carcinoma nodules, possibly due to redifferentiation of nodular hepatocytes.[37] It is known that tumor microenvironment may influence the activity of tumor suppressors to result in switch into their oncogenic function.[38]

TRβ1 mediates inhibition of Na2-β neuroblastoma cell line proliferation and induction of morphological differentiation by an arrest in G0/G1. TR expression leads to the suppression of ras oncogene mediated tumor formation in vivo in nude mice (Fig. 4). The expression of Ha-rasval12 oncogene activates transcription of cyclin D1 via kinase Rsk2 mediated mechanism, leading to enhanced proliferation.[39, 40]

T_3 inhibits Rsk2 activity, expression of both TRα1 and TRβ1 and reduces transforming ability of ras oncogene. TRβ1, however, exerts stronger antiproliferative effect and is able to inhibit ras-mediated transformation even in the absence of T_3 and to totally abolish tumor formation by ras-transformed cells in nude mice.

Martinez-Iglesias et al[41] showed that re-expression of TRβ1 in cells that have lost TR expression leads to tumor growth retardation, partial mesenchymal to epithelial transition and strongly suppresses invasiveness, extravasations and metastasis formation in nude mice. (Fig-5) TRβ1 expression suppresses expression of IGFR and EGFR, the receptors of key growth factors activating signaling pathways of ERK and PI3K. TRβ1 also reduces expression of other downstream elements of these signaling pathways, and blocks TGF-β dependent activation of MAPK and PI3K. In consequence, the expression of genes involved in metastatic growth is repressed. Interestingly, experiments in nude mice suggest that TRs play diverse roles at different stages of tumorigenesis. TRs deficiency inhibits benign tumor formation at early stages of carcinogenesis but increases malignant transformation at later stages.[41]

The same group showed also that hypothyroidism in mice affects invasiveness and formation of metastasis independently of the cellular expression of TRβ1.[28]

PHYLOGENETIC SIMILARITY BETWEEN THYROID AND BREAST

Some endocrine stimuli from thyroid products, thought to exert a simultaneous action on the breast and the various thyroid antibodies, could also interact with receptors on breast tumors. This was postulated to be responsible for the coincidence of mammary and thyroid gland disorders.[42, 43]

The possible interactions between thyroid gland and breast tissue are based on the common property of the mammary and thyroid epithelial cell to concentrate iodine by a membrane active transport mechanism as well as on the presence of TSH receptors in mammary gland.[7, 44]

Thyroid cells phylogenetically derived from primitive iodide-concentrating gastroenteric cells, during evolution, migrated and specialized in uptake and storage of iodine, in order to adapt the organisms from iodine-rich sea to iodine-deficient land. Mammary cells are also derived from primitive iodide-concentrating ectoderm. Stomach, breast and thyroid share an important iodide-concentrating ability and an efficient peroxidase activity, which transfers electrons from iodides to the oxygen of hydrogen peroxide and so protects the cells from damage caused by lipid peroxidation.[45]

THYROPEROXIDASE

Thyroperoxidase belongs to a family of heme peroxidases along with human myeloperoxidase, eosinophil peroxidase and lactoperoxidase. All share some

structural similarity, with 14 out of the 15 cysteines present in each of the four enzymes located at identical positions.[99]

Thyroid Peroxidase (TPO) is a key enzyme in the synthesis of thyroid hormone and is a major thyroid microsomal antigen corresponding to anti-microsomal autoantibodies in thyroid autoimmune diseases. TPO's primary role is the oxidation of iodide and coupling of iodine with organic molecules like proteins and lipids. TPO is stimulated by TSH, which upregulates gene expression.

STRUCTURE OF TPO

Thyroperoxidase is an auto-antigen with a mosaic structure made of nuclear and mitochondrial gene modules. In humans, thyroperoxidase is encoded by the TPO gene. The structural gene for human TPO was located on 2q and is about 150 kb long and consists of 17 exons and 16 introns. The full-length transcript is 3048 bp codes for a molecule, which consists of 933 amino acid residues. Comparison of the cDNA sequences from TPO clones and microsomal antigen clones provides definite proof that the microsomal antigen is TPO.[46,99]

Increasing diversity of human thyroperoxidase generated by alternate splicing has been reported in normal thyroid tissues.[47,48] The functional significance of the newly described spliced mRNA variants still remains to be elucidated.

The TPO extracellular region comprises a large myeloperoxidase-like domain, linked to the plasma membrane by two smaller domains with homology to complement control protein (CCP) and epidermal growth factor (EGF), respectively. These data, together with findings from other studies, localize the TPO autoantibody IDR to the junction of the CCP-like domain and the much larger myeloperoxidase-like domain on TPO.[49] Yet another domain presenting a significant homology with a

putative heme-binding region of cytochrome C oxidase polypeptide raises the possibility that a mitochondrial gene module has contributed a piece to the evolution of a typical nuclear mosaic gene.[99]

SYNTHESIS OF THYROPEROXIDASE

TPO is synthesized on polysomes, gets inserted in the membrane of the endoplasmic reticulum and undergoes core glycosylation. TPO is then transported to the Golgi where it is subjected to terminal glycosylation and packaged into transport vesicles along with thyroglobulin. These vesicles fuse with the apical plasma membrane, a process stimulated by TSH. TPO delivered at the apical pole of thyrocytes, exposes its catalytic site with the attached heme in the thyroid follicular lumen. TPO activity is restricted to the apical membrane, but most of the thyroid TPO is intracellular, being located in the perinuclear part of the endoplasmic reticulum. Most of this intracellular protein is incompletely or improperly folded; it contains only high mannose-type carbohydrate units, while the membrane TPO has complex carbohydrate units. Glycosylation is essential for enzymatic activity. Chronic TSH stimulation increases the amount of TPO and its targeting at the apical membrane.[50]

ANTI-THYROPEROXIDASE ANTIBODY

Thyroid peroxidase (TPO) autoantibody epitopes are largely restricted to an immunodominant region (IDR) on the extracellular region of the native molecule.[51]

Thyroperoxidase Antibodies (TPO) activate complement and are thought to be involved in thyroid dysfunction and the pathogenesis of hypothyroidism.

TPO autoantibodies, the hallmark of human autoimmune thyroid disease, are of IgG class and are associated with thyroid destruction and hypothyroidism.TPO

autoantibody V region genes are not unique; H chain V genes are usually mutated, while L chain V genes are sometimes in germ-line conformation. Finally, TPO autoantibody epitopic fingerprints are distinct for individual sera that are not associated with hypothyroidism, but have been conserved over time. Evidence for conservation as well as inheritance of the fingerprints in some families, together with VH gene polymorphisms, may provide insight into the genetic basis of human autoimmune thyroid disease.[52]

In patients with subclinical hypothyroidism, the presence of TPO antibodies is associated with an increased risk of developing overt hypothyroidism. Many clinical endocrinologists use the TPO antibody test as a diagnostic tool in deciding whether to treat a patient with subclinical hypothyroidism.

H_2O_2 GENERATING SYSTEM

Peroxidase requires H_2O_2 for its oxidative function. H_2O_2 is produced at the apical plasma membrane by an enzyme, thyroid NADPH oxidase that requires both calcium and NADPH.[53]

SODIUM IODIDE SYMPORTER – Common Antigen of Thyroid and Breast

The sodium iodide symporter (NIS) is a plasma basolateral membrane protein that actively transports iodide to the thyroid follicular cells as the first step of thyroid hormone biosynthesis. NIS also mediates active iodide transport in other human tissues including salivary glands, lactating mammary gland and gastric mucosa.[54, 55]

The ability of the breast to express sodium–iodide symporter[56-58] and to take up significant amounts of iodide has led to studies of the potential for use of ^{131}I

ablative therapy in breast cancer, analogous to that employed in the treatment of hyperthyroidism or thyroid cancer.

The plasma membrane of the mammary gland epithelium contains a NIS protein with a molecular mass different from that of thyroid NIS (~75 kDa vs. ~90 kDa). In the mammary gland, NIS is processed differently after translation and subjected to regulation by lactogenic stimuli.[58.] It has been reported that over 80% of human breast cancer samples expressed this symporter. As it is absent in normal non-lactating tissue, NIS may represent a marker for breast malignancy and even a possible target for radioiodine therapy.[57]

Radio iodide an effective therapy for thyroid cancer, exploits the thyroid specific expression of sodium iodide symporter (NIS) gene, which allow rapid internalization of iodide into thyroid cells. Transfection of NSCLC cells with both Human NIS and TPO genes resulted in increased radioiodine uptake and retention and increased tumor cell apoptosis.[59]

IODINE- AN ANTIOXIDANT AND ANTIPROLIFERATIVE AGENT

Iodine is essential to maintaining the normalcy of the thyroid and the breast. An iodine-deficient state renders the rat thyroid and the breast susceptible to physiological changes and leads to atypia, dysplasia, and hyperplasia.[60]

Lactating breast tissue concentrates a significant amount of iodide as a result of stimulation of NIS expression. The trapped iodide is secreted in milk and provides iodine for thyroid hormone synthesis to the developing infant. The lactating breast can concentrate iodide to a similar degree as that seen in the thyroid, producing milk which is iodine rich. In the mammary gland, iodination of tyrosyl residues on casein

and other milk proteins correlate with peroxidase activity. Iodide organification may occur even in inactive breast tissues.[57]

Inorganic iodine enters the body primarily as iodide, I-. There are evidences that dietary iodine may affect breast cancer risk.[97] Venturi.S.et al have hypothesized that dietary iodine deficiency is associated with the development of some gastric and mammary cancers, as it is well-known for thyroid cancer.[45]

Geographical variations in the incidence of breast cancer have been attributed to differences in dietary iodine intake, and an effect of iodine on the breast has been postulated.[61,62]

Molecular iodine (I_2) results in a decrease of rat breast hyperplasia and perilobular/ ductal fibrosis. The beneficial effect of molecular iodine has also been documented in the human fibrocystic breast condition and in cyclic mastalgia.[63, 64]

ANTIOXIDANT ACTION OF IODINE

Oxidation of iodide to iodine (organification) is an important step in thyroid hormonogenesis. Iodine deficiency may produce conditions of oxidative stress with high TSH producing a high level of H_2O_2, which in the absence of iodide is not being used to form thyroid hormones. The cytotoxic actions of excess iodide in thyroid cells may depend on the formation of free radicals and can be attributed to both necrotic and apoptotic mechanisms with necrosis predominating in goiter development and apoptosis during iodide induced involution.[65]

Oxidants, which inactivate many enzymes, are a feature of lipid peroxidation and DNA damage, and have been shown to be associated with carcinogenesis in the breast. On the other hand, increased serum levels of antioxidants have been associated

with reduction in breast cancer risk .There is also some evidence that iodide itself may act as an antioxidant.[66]

IODINE INDUCES APOPTOSIS IN TUMOR CELLS

Molecular iodine (I_2) is known to inhibit the induction and promotion of N-methyl-n-nitrosourea-induced mammary carcinogenesis, to regress 7, 12 dimethylbenz (a) anthracene -induced breast tumors in rat, and has also been shown to have beneficial effects in fibrocystic human breast disease. Cytotoxicity of iodine on cultured human breast cancer cell lines showed iodine induced apoptosis in the entire cell lines tested. Iodine-induced apoptosis was independent of caspases. Iodine dissipated mitochondrial membrane potential, exhibited antioxidant activity, and caused depletion in total cellular thiol content. Iodine treatment induces the translocation of apoptosis-inducing factor from mitochondria to the nucleus, and treatment of N-acetyl-L-cysteine prior to iodine exposure restored basal thiol content, ROS levels, and completely inhibited nuclear translocation of apoptosis-inducing factor and subsequently cell death, indicating that thiol depletion may play an important role in iodine induced cell death.[67]

Iodine, in conjunction with medroxy progesterone acetate, and an iodine-rich seaweed diet are shown to regress 7, 12-dimethylbenz (a) anthracene-induced rat breast tumors, and this effect has been corroborated by high tumor tissue iodine content and induction of apoptosis at the tumor site.[67]

Iodide excess is known to induce apoptosis in the thyroid cells in vitro and also in sodium iodide symporter and thyroperoxidase transfected non-small cell lung carcinoma cells. Sodium iodide symporter facilitates iodide transport, and thyroperoxidase oxidizes iodide (I^-) to iodine (I_2), which is important for its organification. Propyl-thiouracil, an inhibitor of peroxidase, completely abolishes the

cell death-inducing effect of iodide in thyroid cells, establishing I_2 as the mediator of apoptosis.[67]

6- IODO LACTONES- AN IODINATED ANTIPROLIFERATIVE AGENT

Delta-iodolactone are synthesized from arachidonic acid, iodine, and H_2O_2 catalyzed by peroxidases. Thus, iodolactone formation is not limited to the thyroid gland but can occur in any organ that contains peroxidases (e.g., the breast).

The delta-iodolactones of arachidonic acid inhibits the EGF (Epidermal Growth Factor) receptor. When the EGF receptor is inhibited, the metabolism of arachidonic acid induced by EGF (and TGF-α) is inhibited. This inhibition suppresses goiter formation and can induce involution (shrinking the goiter).The delta-iodolactone of eicosapentaenoic acid exerts an antiproliferative effect in the nanomolar range. Neither arachidonic acid nor other iodolactones (besides delta-iodolactones) have this antiproliferative effect. Anti-proliferative effect of iodine requires oxidation of I^- to I_2 because inhibitors of TPO or I– trapping cause reversal of this inhibitory effect. Such inhibitory actions of iodo-compounds on cell proliferation might play a role in the breast as well.[68]

The antiproliferative properties of I_2 and the arachidonic acid (AA) derivative 6-iodolactone (6-IL) in both thyroid and mammary glands act via the cellular pathways activated by these molecules and causing cell cycle arrest and apoptosis in normal (MCF-12F) and cancerous (MCF-7) breast cells.[69]

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA MEDIATED ACTION OF IODINE

A complex between 6-iodolactone and the peroxisome proliferator-activated receptor type gamma may mediate the antineoplastic effect of iodine in mammary cancer. Molecular iodine exhibits potent antiproliferative and apoptotic effects in mammary cancer models. In the human breast cancer cell line MCF-7, iodine treatment generates iodine-containing lipids similar to 6-iodo-5-hydroxy-eicosatrienoic acid and the 6-iodolactone (6-IL) derivative of arachidonic acid (AA), and it significantly decreases cellular proliferation and induces caspase-dependent apoptosis. Several studies have shown that arachidonic acid is a natural ligand of the peroxisome proliferator-activated receptors (PPARs), which are nuclear transcription factors thought to participate in regulating cancer cell proliferation as shown by:

- 6-IL specific and high affinity binding to PPAR proteins.
- 6-IL induced activation of both transfected (by transactivation assays) and endogenous peroxisome proliferator response elements, and
- 6-IL supplementation increases PPAR gamma and decreases PPAR alpha expression. These results implicate PPARs in a molecular mechanism by which I_2, through formation of 6-IL, inhibits the growth of human breast cancer cells.[69]

ASSOCIATION BETWEEN THYROID DYSFUNCTION AND BREAST TUMORS

The fact that both breast cancer and thyroid disease predominantly affect females and that both have a postmenopausal peak incidence has inevitably resulted in a search for an association between the two diseases.[3,4]

One of the earliest reports on the association of breast cancer with thyroid disease was that from Beatson in 1896, who used oopherectomy and thyroid extract to treat breast cancer.[71]

The relationship between breast cancer and thyroid disease is a matter of controversy. Some studies suggested an association between breast carcinoma and thyroid diseases, including thyroid carcinoma.[73-76] Where an association between thyroid disease and breast cancer was shown to exist, hypothyroidism was the most frequently observed finding. In fact, many reports considered hyperthyroidism to be protective against breast cancer because progression of such cancers was more frequently observed when the hyperthyroidism was treated.[3]

Smyth et al demonstrated that a direct relationship between thyroid enlargement and breast carcinoma exists. Thyroid volume was assessed by ultrasound in 200 patients with breast cancer and 354 with benign breast disease. Results were compared to appropriate female control groups showed a significantly greater volume in the breast cancer group than controls.[72]

Thus almost every form of thyroid disease, including nodular hyperplasia, hyperthyroidism and thyroid cancer, has been identified in association with breast cancer.[61]

Martínez-Iglesias O et al reported that hypothyroidism might be a possible risk factor for liver and breast cancer in humans.[28]

Even though many studies have shown that thyroid diseases are common in women with breast cancer, other reports have not confirmed this association.[77]

ROLE OF AUTOIMMUNE THYROID DISEASE IN BREAST TUMORS

Shering SG et al reported that the prevalence of hyperthyroidism and hypothyroidism in patients with breast cancer versus those without breast cancer were very similar. 45.5% of breast cancer patients had thyroid enlargement as compared with only 10.5% of controls and, antithyroid peroxidase autoantibodies were twice more common in breast cancer patients than in controls.[80]

Rasmusson B et al also reported a higher frequency of thyroid peroxidase antibodies (TPO antibody) in breast carcinoma patients, compared with control groups.[81]

These findings have led to the investigation of the relationship between breast cancer and autoimmune thyroid diseases (AITDs). Such a relationship is not a new observation, and some authors have reported a higher prevalance of AITDs among breast cancer patients than in age-matched control individuals.[7, 84, 85]

In a study conducted by Ito and Maruchi,[77,82] those investigators reported that there was an increase in risk for breast cancer among patients with Hashimoto's thyroiditis.

Turken and coworkers describe an association between breast cancer and autoimmune thyroid disease (AITD), showing not only an increased prevalence of thyroid peroxidase (TPO) antibodies in patients with breast cancer but also a significantly increased rate of goiter as compared with control individuals.[61,82]

The percentage of positive TGB-antibody and TPO-antibody serum levels was higher in women with breast cancer as compared to those with colorectal cancer and the control group. The results of the study support the controversial theory that there is an increased prevalence of autoimmune thyroiditis in women with breast cancer.[86]

It has been proposed that the immune response might be directed both by tumor and by thyroid tissue, or that the tumor and thyroid tissue share common properties, as they both express TPO and the sodium iodide symporter gene.[87, 88.]

Mittra et al showed that with the use of specific immunoassays for TPO and thyroglobulin antibodies, an increased level of TPO has been demonstrated in breast cancer.[61]

ANTITUMOR CYTOTOXICITY - ANOTHER HYPOTHESIS

Although high TPO level has been shown to be a very important factor in antibody-dependent cell cytotoxicity in the thyroid, and there may be a possible association between autoimmune thyroiditis and the immune system, there is no agreement on the significance of its association with breast cancer.[61,77]

The mechanisms through which TPO-antibody positivity or reduced/enlarged thyroid volumes might influence breast carcinoma progression remain a matter of conjecture. Rodien PM et al observed an association of TPO-antibody positivity with improved outcome only when thyroid volume was in the intermediate range (10.1–18.8 mL). It shows that thyroidal involvement, whether in terms of volume or antibody positivity, was effected via different pathways.[88]

Sera from patients with cancer often react against their own tumor cells. These so-called tumor-specific antibodies have been shown to occur in a diversity of malignant conditions, demonstrated by means of cytotoxic techniques or immunofluorescence.[2]

Apparently the surface of the cancer cell contains antigens that are normal fetal or adult components but which become exposed to a greater degree or in a higher concentration than in normal cells and so induce the formation of these antibodies.[2]

These "non-tumor specific" antibodies have been studied in detail in patients with breast cancer by two groups of workers with somewhat conflicting results. Wasserman and his colleagues observed a higher incidence of serum mitochondrial and anti nuclear antibodies in breast cancer patients at mastectomy than in matched controls. They noted that the patients who later had recurrence of their disease showed a significantly higher incidence of multiple antibodies compared with those who remained well. They proposed that in patients with breast cancer the increased incidence of autoantibodies might reflect not only a change in the cell membrane but also tissue damage due to the presence of cancer or disordered immunological reactivity associated with deficient tumor surveillance. These theories might also explain the prognostic significance of the autoantibodies.[89]

In the case of thyroid autoantibodies being associated with better disease outcome in breast cancer, renal carcinoma or melanoma, it is possible that the immune response to thyroid and tumor tissue might be similarly regulated in that it might be directed against both tumor and thyroid antigens. Another possibility is that tumor and thyroid share the same antigens because expression of the sodium–iodide symporter has been demonstrated in both thyroid and breast tissues.[56-58]

Smyth PPA et al reported that the presence of TPO antibodies is associated with a significant improvement in both disease-free and overall outcome in breast cancer patients, and that the magnitude of this prognostic effect was of a similar order of magnitude to well established prognostic indices for breast cancer such as axillary nodal status or tumour size.[77]

Absence of ER expression and TPOAb were related to a higher mortality rate. ER+ and TPOAb+ are positive prognostic parameters in BC and the absence of any relationship between them seems to propose an independent role on the prognosis of BC patients.[90]

TREATMENT INDUCED ANTITUMOR CYTOTOXICITY

The association of thyroid antibody positivity, sometimes with transient thyroid dysfunction, has been reported in the course of immunotherapy with recombinant cytokines interleukin-2 and interferon-gamma for various cancers.[91,92] Thyroid antibody related hypothyroidism has been suggested as being associated with a favorable tumour response to such therapies.

Thus, we have the anomalous situation in which the presence of TPO antibodies, while being associated with breast cancer, also appears to confer prognostic benefits. These findings have led to the investigation of the relationship between breast tumors and autoimmune thyroid diseases (AITDs).

AIM OF THE STUDY

Thyroid hormone plays an important role in the development of breast .Thyroid hormone antagonizes the proliferative effect of estrogen both by increasing the level of SHBG thereby decreases free estrogen level and by acting at the receptor level.

The aim of the study is:

- To determine thyroid profile and anti - TPO antibody level in benign and malignant breast tumors.
- To analyze possible association between thyroid hormone levels and breast tumors.
- To analyze possible association between anti - TPO antibody levels and breast tumors.
- Whether anti -TPO antibody can be used as a tumor marker in diagnosis of patients with breast cancer.

MATERIALS AND METHODS

The study was conducted during the period January 2010 and April 2010. It was carried out in three groups, namely apparently healthy female controls as group-1, females with newly diagnosed benign breast tumors as group-2 and females with newly diagnosed malignant breast cancer as group-3.

Controls

The group comprised of 30 apparently healthy female subjects in the age group between 32 to 65 years attending the general Out patient Department of Madras Medical College, Chennai.

Cases

The group comprised of:

- 28 women with newly diagnosed benign breast tumor in the age group between 33 to 65 years and
- 30 women with newly diagnosed malignant breast cancer in the age group between 32 to 65 years.

All breast tumors were proved by **FNAC.**

- Benign breast tumours comprised of:- Fibro adenoma, Fibroadenosis and Breast cyst
- Malignant breast tumors comprised of:- Ductal carcinoma of the breast

Inclusion criteria for cases

- Newly diagnosed patients with benign and malignant breast tumors.

Exclusion criteria: for cases and controls

- History of thyroid disorder.
- Patients with breast tumor undergoing treatment.

Sample collection

5 mL of venous blood was collected in plain tube from all cases and controls. The sample was centrifuged at 2000 rpm for 20 minutes and serum was aliquoted in separate plain tube and stored at -20 degrees till analysis .The sera was analysed for TSH, FreeT_4, FreeT_3 and anti –TPO antibody levels.

BIOCHEMICAL MARKERS

Determination of TSH level in serum

TSH level was measured by ELISA method with an open system automated ELISA analyser (Triturus analyzer) and manufacturer's reagent kits.

Methodology

The TSH quantitative kit (ERBA THYROKIT TSH) is a solid phase enzyme linked immunosorbent assay.

Principle: **Immunoenzymometric assay**

The essential reagents required for a immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and

distinct epitope recognition, in excess and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a micro plate well through the interaction of anti-TSH coated on the well and exogenously added monoclonal anti-TSH antibody.

Upon mixing a reaction takes place between fixed monoclonal antibody, the enzyme-labeled second antibody and a serum containing the native antigen. The reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a sandwich complex.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Kit contents

A. Thyrotropin Standards: 0.4 mL/each

Six vials of references for TSH Antigen at levels of 0(A), 0.25(B), 0.75(C), 2(D), 5(E), 15(F) mIU/mL

B. Thyrotropin control-0.4 mL/vial

C. Enzyme-conjugate (ready to use)

D. Coated Micro plate -96 wells

E . Wash solution concentrate-25 mL(40 fold)

F. TMB-substrate (12 mL)

G. Stop solution (12 mL)Sulphuric acid 0.25 mol/L

Procedure

Preparation of wash solution

Dilute contents of wash concentrate to1000 mL with distilled water in a suitable storage container

Assay procedure

Before proceeding with the assay, bring all reagents, serum references to room temperature.

Secure the coated wells in the holder. Mark data sheet with sample identification.

25 µL of reference, control and serum samples are dispensed into the appropriate well.

Incubate 10 minutes at room temperature.

100 µL of enzyme conjugate is dispensed into the wells.

Swirl the micro plate gently for 10 seconds to mix and cover .Incubate for 90 minutes at room temperature.

Discard the contents of the micro plate by decantation or aspiration.

Add 300µL of wash buffer, decant or aspirate. repeat 4 additional times .

100µL of TMB substrate is dispensed into all wells.

Incubate at room temperature for 20 minutes.

Add 100 µL of stop solution to all the wells. mix gently for 15-20 seconds .

Measure the absorbance at 450 nm in a micro plate reader.

The results should be read within 5 minutes of adding stop solution.

Standard curve

Plot the mean value of absorbance of the standards against concentration. Draw the best –fit curve through the plotted points.

Calculation of results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentration expressed in mIU/L

Reference interval

TSH - 0.5 to 5 mIU/L

DETERMINATION OF FREET$_4$ LEVEL IN SERUM

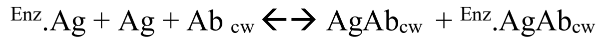
FreeT$_4$ level was measured by ELISA method with an open system automated ELISA analyzer (Triturus analyzer) and manufacturer's reagent kits.

Methodology: the free T$_4$ quantitative kit (DIAMETRA) is a solid phase enzyme linked immunosorbent assay.

Principle

The essential reagents required for a solid phase enzyme linked immunosorbent assay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native free antigen, a competition reaction results between the native free antigen and enzyme –antigen conjugate for a limted number of unsolubilized binding sites.

The interaction is illustrated by the following equation:



$^{Enz}.Ag + Ag + Ab_{cw} \leftrightarrow AgAb_{cw} + {}^{Enz}.AgAb_{cw}$

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Kit contents

Free T_4 standards 6x (1 vials = 1ml)

The serum are human serum reference for free T4 at app concentrations of 0,0.3, 0.95, 2.1, 3.6 and 7.0ng/dL.

Conjugate (12mL)

T4-HRP conjugate

Coated Microplate

Anti-T_4 IgG adsorbed on microplate wells-96 wells.

TMB-substrate (12 mL)

H_2O_2-TMB 0.25gr/L

Stop solution (12 mL)

Sulphuric acid 0.15 mol/L

Conc.wash solution (20 mL)

NaCl 9 gr/L ; Tween20 1gr/L

Preparation of wash solution

Dilute contents of wash concentrate to1000 mL with distilled water in a suitable storage container.

Assay procedure

Before proceeding with the assay, bring all reagents, serum references to room temperature.

Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.

50 µL of reference and serum samples are dispensed into the appropriate well.

100 µL of enzyme conjugate is dispensed into the wells.

Swirl the micro plate gently for 20 -30 seconds to mix and cover .Incubate for 60 minutes at room temperature.

Discard the contents of the micro plate by decantation or aspiration.

Add 300µL of wash buffer, decant or aspirate. Repeat 2 additional times

100µL of TMB substrate is dispensed into all wells.

Incubate at room temperature for 15 minutes.

Add 100 µL of stop solution to all the wells. mix gently for 15-20 seconds .

Measure the absorbance at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a micro plate reader.

The results should be read within 30 minutes of adding stop solution.

Standard curve

Plot the mean value of absorbance of the standards against concentration .Draw the best –fit curve through the plotted points.

Calculation of results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentration expressed in ng/dL

Reference interval

Adult 0.8 – 2.0 (ng/dL)

Pregnancy 0.8 – 2.2 (ng/dL).

DETERMINATION OF FREE T_3 LEVEL IN SERUM

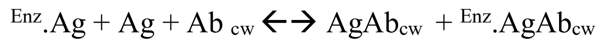
Free T_3 level was measured by ELISA method with an open system automated ELISA analyzer (Triturus analyzer) and manufacturer's reagent kits.

Methodology: the free T_3 quantitative kit (DIAMETRA) is a solid phase enzyme linked immunosorbent assay.

Principle

Competitive Enzyme Immunoassay – Analogue Method for Free T3.The essential reagents required for a solid phase enzyme linked immunosorbent assay include immobilized T_3 antibody, enzyme T_3- conjugate and native T_3 antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native free antigen, a competition reaction results between the native free antigen and enzyme –antigen conjugate for a limited number of unsolubilized binding sites.

The interaction is illustrated by the following equation



$^{Enz}.Ag + Ag + Ab_{cw} \leftrightarrow AgAb_{cw} + ^{Enz}.AgAb_{cw}$

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Kit contents

Free T_3 standards 6x (1 vials = 1ml)

The serum are human serum reference for free T_3 at app concentrations of 0, 0.4, 1.2, 4.5, 8 and 18 pg/mL.

Conjugate (12mL)

T_3-HRP conjugate

Coated Microplate :-

Anti- **T_3** IgG adsorbed on microplate wells-96 wells.

TMB-substrate (12 mL)

H_2O_2-TMB 0.25gr/L

Stop solution (12 mL)

Sulphuric acid 0.15 mol/L

Conc.wash solution (20 mL)

NaCl 9 gr/L ; Tween20 1gr/L

Preparation of wash solution

Dilute contents of wash concentrate to1000 mL with distilled water in a suitable storage container

Assay procedure

Before proceeding with the assay, bring all reagents, serum references to room temperature.

Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.

50 µL of reference and serum samples are dispensed into the appropriate well.

100 µL of enzyme conjugate is dispensed into the wells.

Swirl the micro plate gently for 20 -30 seconds to mix and cover .Incubate 60 minutes at room temperature.

Discard the contents of the micro plate by decantation or aspiration.

Add 300µL of wash buffer, decant or aspirate. Repeat 2 additional times

100µL of TMB substrate is dispensed into all wells.

Incubate at room temperature for 15 minutes.

Add 100 µL of stop solution to all the wells. Mix gently for 15-20 seconds .

Measure the absorbance at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a micro plate reader.

The results should be read within 30 minutes of adding stop solution.

Standard curve

Plot the mean value of absorbance of the standards against concentration. Draw the best –fit curve through the plotted points.

Calculation of results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentration expressed in pg/mL.

Reference interval

Adult 1.4 - 4.2 (pg/mL)

Pregnancy 1.8 – 4.2 (pg/mL).

DETERMINATION OF ANTI-TPO IN SERUM

Methodology

Immunoenzymatic colorimetric method for quantitative determination of anti-TPO concentration in serum and plasma.

Principle

This test is based on two–site sandwich enzyme immunoassay principle. Tested specimen is placed into the micro wells coated by the antigen. Antibodies from the specimen bind coated antigen on the micro well surface. Unbound material is removed by washing procedure. Second antibodies directed towards human Ig and labeled with peroxidase enzyme, are then added into the micro wells. After subsequent washing procedure, the remaining enzymatic activity bound to the micro well surface is detected and quantified by addition of chromogen substrate mixture,

stop solution and photometry at 450 nm.Optical density in the micro well is directly related to the quantity of specific antibodies in the specimen.

Kit contents

Anti-TPO standards 6 X(1 vial = 1.2 mL)

Std0 (0), std 1(5), std 2(10), std 3(20), std 4(80) and std 5(320) [AU/mL]

Controls

Negative and Positive

Sample Diluent :- 100mL

Conjugate- 15 mL

Anti h-IgG conjugated with peroxidase, BSA 0.1%, Proclin < 0.003%

Coated Microplate -96 wells

Thyroperoxidase adsorbed on microwells surface.

TMB –Substrate -15 mL

3,3’ 5,5’ – tetramethylbenzidine 0.26 g/L, hydrogen peroxide 0.05%.

Conc. Wash solution -20 mL

Phosphate buffer 0.2M, proclin < 0.002%

Stop solution -15 mL

Sulfuric acid 0.15 M

Preparation of wash solution

Dilute the contents of concentrated wash solution with distilled water to final volume to 1000mL

Preparation of sample

All serum samples prediluted with sample diluents 1 : 100 .

10µL of sample diluted with 1000µL of sample diluents.

Assay procedure

Allow all reagents to attain room temperature.

Secure the coated wells in the holder. Mark data sheet with sample identification.

100µL of reference, control and diluted serum samples are dispensed into the appropriate well.

Incubate 30 minutes at room temperature.

Discard the contents of the micro plate by decantation; wash the wells three times with 300µL of wash buffer.

100 µL of enzyme conjugate is dispensed into the wells.

Swirl the micro plate gently for 10 seconds to mix and cover Incubate 30 minutes at room temperature.

Discard the contents of the micro plate by decantation, wash the wells three times with 300µL of wash buffer.

100µL of TMB substrate is dispensed into all wells.

Incubate at room temperature for 15 minutes.

Add 100 µL of stop solution to all the wells. Mix gently for 15-20 seconds

Measure the absorbance at 450 nm in a micro plate reader.

The results should be read within 5 minutes of adding stop solution.

Standard curve

Plot the mean value of absorbance of the standards against log concentration of the calibrators. Draw the best –fit curve through the plotted points.

Calculation of results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentration expressed in AU/mL.

Reference interval

Anti-TPO (AU/mL)

- Normal: **< 20**
- Elevated: ≥20

STATISTICAL ANALYSIS

1) **MEAN**
2) **STANDARD DEVIATION**
3) **ONEWAY ANOVA**
4) **MULTIPLE COMPARISON USING BONFERRONI T-TEST**
5) **ROC CURVE**
6) **ODDS RATIO**
7) **CHI SQUARE TEST**

RESULTS

The mean age of the controls was 48.1± 9.7 years, of benign breast tumor cases was 48.07± 7.8 years and of malignant breast tumor cases was 51.03± 9.5 years.

The biochemical parameters namely TSH, fT_3, fT_4 and anti TPO antibody level along with the biological parameters such as age, parity, age at first child birth , duration of exposure to endogenous estrogen and family history of breast cancer obtained from the study population are given in Master Chart : for 30 controls, 28 benign breast tumor cases and 30 malignant breast tumor cases.

Table - 1: Shows the comparison of biological risk factors among cases and controls. The statistical significance was determined by oneway ANOVA and Chi-square test. There was no significant difference between the controls, benign and malignant breast tumor cases with respect to age, parity, age at first childbirth, family history of breast cancer and duration of exposure to endogenous estrogen.

Table – 2: Shows the comparison of TSH among controls, benign and malignant breast tumors.The mean value and standard deviation were: for controls 2.59±0.89 mIU/L, for benign 2.76±0.68 mIU/L and for malignant breast tumors 3.17±0.62 mIU/L respectively.

Figure 6 : Shows the comparison of TSH among controls, benign and malignant breast tumors.

Figure 7: Shows the distribution of TSH values among controls and benign and malignant breast tumors patients.

The TSH values was significantly high among the breast cancer patients when compared to the controls with a p-value = 0.01. Though the mean TSH value of the

benign breast tumor patients was higher than the controls it was not significant. (p-value = 1.0).

Table -3: Shows the comparison of fT_4 among controls, benign and malignant breast tumors. The mean value and standard deviation were: for controls 1.61 ± 0.46 ng/dL ,for benign 1.43 ± 0.33 ng/dL and for malignant breast tumors 1.00 ± 0.28 ng/dL respectively.

Figure 8: Shows the comparison of fT_4 among controls, benign and malignant breast tumors.

Figure 9: Shows the distribution of fT_4 values among controls and benign and malignant breast tumors patients.

The levels of free T_4 was significantly low among the breast cancer patients when compared to the controls and the benign breast tumor patients. (p-value- 0.001). The benign breast tumor patients had a mean fT_4 value lower than the controls, but was not significant. (p-value = 0.19).

Table -4: Shows the comparison of fT_3 among controls, benign and malignant breast tumors.

The mean value and standard deviation were: for controls 2.89 ± 0.85 pg/mL, for benign 2.66 ± 0.70 pg/mL and for malignant breast tumors 2.05 ± 0.73 pg/mL respectively.

Figure 10: Shows the comparison of fT_3 among controls, benign and malignant breast tumors.

Figure 11: shows the distribution of fT_3 values among controls and benign and malignant breast tumors patients.

The levels of free T_3 was significantly low among the breast cancer patients when compared to the controls and the benign breast tumor patients. (p-value = 0.001).The benign breast tumor patients had mean fT_3 value lower than the controls, but was not significant. (p-value = 0.756)

Table -5: Shows the comparison of anti-TPO among controls, benign and malignant breast tumors. The mean value and standard deviation were: for controls 11.11 ± 10.2 AU/mL, for benign 12.98 ± 6.84AU/mL and for malignant breast tumors 67.77 ± 89.41 AU/mL respectively.

Figure 12: Shows the comparison of anti-TPO among controls, benign and malignant breast tumors.

Figure 13: Shows the distribution of anti-TPO values among controls and benign and malignant breast tumors patients.

The levels of anti TPO antibodies were significantly high among the breast cancer patients when compared to the controls and the benign breast tumor patients. (p-value = 0.001). The benign breast tumor patients had mean anti-TPO value higher than the controls, but was not significant. (p-value- 1.0)

Table 6: Shows frequency of cancer patients by their staging and their respective anti-TPO values. On comparing the anti-TPO values among the various stages of breast cancer there was an increase in the anti-TPO levels with increasing progression of cancer.

Figure 14: shows the ROC curve for the anti-TPO values and a diagnostic cutoff of 20.350AU/mL was made which had a sensitivity of 76% and specificity of 83%. So anti-TPO antibody level can be included in the risk assessment profile of breast cancer.

Table 7: Shows the odds' ratio calculation with anti-TPO cut-off value of 20.35AU/mL.

O.R value = 13.14 ; 95% confidence interval of 3.8 to 45.0.

DISCUSSION

Breast cancer is an extremely heterogeneous disease caused by interactions of both inherited and environmental risk factors that lead to progressive accumulation of genetic and epigenetic changes in tumor suppressor genes of the breast epithelial cells.[1]

Excess proliferation of breast epithelial cells caused by unopposed estrogen and various mitogenic growth factors like EGF and IGF by stimulating the growth factor signaling pathway leads to development and progression of breast cancer. Thyroid hormone by causing differentiation of breast epithelial cells antagonizes the proliferative effect of estrogen and mitogenic growth factors.

We evaluated the association between thyroid dysfunction including autoimmune thyroid disease and breast tumors in this study.

The TSH values were significantly high among the breast cancer patients when compared to the controls proving the association of thyroid hypofunction and the resultant increase in TSH in breast cancer patients.

The levels of free T_4 and free T_3 were significantly low among the breast cancer patients when compared to the controls and the benign breast tumor patients.

The low freeT_4 and freeT_3 further substantiates that there is association between hypothyroidism and breast cancer.

Although conflicting results have been documented and hyperthyroidism has been described as risk for breast cancer, the results of this study however showed the presence of hypothyroidism being associated with development of breast cancer. [(61)]

Similar association has been shown by Goldman et al who found that hypothyroidism was the most frequently observed finding when thyroid disease and breast cancer coexisted. In fact, many reports considered hyperthyroidism to be protective against breast cancer because progression of such cancers was more frequently observed when the hyperthyroidism was treated.[3, 28]

The nonsignificant p-value of TSH, free T_4 and free T_3 between benign breast tumor patients and controls suggest a possibility that pathogenesis of benign breast neoplasia involves other unidentified mechanism not involving thyroid hormones.

The levels of anti TPO antibodies were significantly high among the breast cancer patients when compared to the controls and benign breast tumor patients, which show the association between autoimmune thyroid disease and breast cancer.

The mean anti -TPO value of benign breast tumor patients was not significant when compared to the controls.

Though the anti-TPO values among the various stages of breast cancer showed an increase with increasing progression of cancer, it carried less credit when the groups were substratified since the sample size was not adequate.

The findings in the present study confirmed reports of Rasmusson B et al who showed a higher TPO.Ab positivity in women with breast carcinoma.[81]

The tendency towards thyroid hypofunction, evident in the skewed distribution of serum TSH values in breast carcinoma patients associated with TPO.Ab positivity, is consistent with previous reports showing an association between thyroid disease and breast carcinoma, in which hypothyroidism was most frequently observed finding.[95, 96]

The finding of higher serum TSH values, even within the reference range, together with the presence of TPO.Ab, may indicate susceptibility to future hypothyroidism.[7]

The thyroid hormones are important regulators of growth, development, and metabolism in higher animals and humans. A pathophysiological role of hypothyroidism is supported by invivo data indicating a role of thyroid hormones in mammary gland development by stimulating ductal branching and alveolar budding.[5]

The observed association between hypothyroidism and breast carcinoma may be due to the biological effect of T_3 at the cellular level through either a direct interaction with thyroid receptor or a modulation of the TSH receptor.[93]

TRs are widely distributed in mammalian tissues, but transformed or immortalized cells in general express very low levels of TR. TR can also alter expression of genes that do not contain a hormone response element through positive or negative interference of other transcription factors and signaling pathways.

Hypothyroidism is a common disorder with an incidence of approximately 3-4% of symptomatic disease in general population that increases to up to 13-14% among individuals aged more than 65 years. Hypothyroidism may be an independent risk factor for development of breast cancer.[93]

The association between TPO.Ab positivity and breast carcinoma, suggests the presence of subclinical autoimmune thyroid disease in a proportion of patients with breast carcinoma.[77]

Because thyroid hormone has an antiproliferative effect on mammary epithelial cells, it was hypothesized that presence of excess antiTPO antibodies by

inhibiting TPO causes hypothyroidism which in turn poses a risk of developing breast cancers.

Another intriguing possible role may be ascribed to iodide, based on its protective antioxidant mechanism. This theory has been postulated based on the capacity of breast tissue to transport and concentrate iodide.[97]

One area in which thyroid and breast functions overlap is in the uptake and utilization of iodide. Both organs require a method of oxidizing I^- to I_2 (organification) in order to produce iodoproteins and iodolipids like 6 iodolactones. This involves the presence of H_2O_2 as an oxidizing agent catalyzed by TPO in the thyroid and by lactoperoxidases in the breast.[97]

Iodolipids like 6-iodolactones which acts via peroxisomal proliferator activator receptor – gamma (PPAR-γ) and prevents proliferation of mammary epithelial cells.

Crosstalk between PPARs and TRs may also be mediated indirectly, such as a reciprocal regulation of PPAR expression by TRs or vice versa. The TR action may also be regulated indirectly by PPARs, e.g. via its modulating the intermediate enzyme genes, such as deiodinase-2 involved in T_3 metabolism.[94]

The possibility of an autoimmune-mediated mechanism being responsible for inhibition of iodide uptake in the thyroid has been previously reported.

Also since breast and thyroid have a common phylogenetic origin, it is possible for an increased expression of TPO antigen in the breast cancer cells similar to expression of sodium iodide symporter.[56-58] The raise in the anti TPO antibody level can be a part of antitumor immunity directed against breast cancer.

The question of whether the presence of TPO antibodies in serum of patients with breast cancer is breast specific or part of a generalized immunogenic response needs to be explored. Also requiring study are the involvement of iodide transport in the breast and additional roles for iodinated compounds within the mammary glands, with their accompanying benefit of providing a new therapeutic pathway for radioiodine ablative therapy.

CONCLUSION

We evaluated the association between thyroid dysfunction including autoimmune thyroid disease and breast tumors in this study.

In the study TSH was elevated in the breast cancer patients when compared to the controls. The fT_4, fT_3 were lowered and Anti-TPO antibody was elevated in breast cancer patients when compared to benign breast tumors and controls. Anti-TPO antibody by decreasing the synthesis of thyroid hormones and iodo-lipids increases the risk of developing breast cancer.

To conclude:

- There is an association between hypothyroidism and breast cancer.
- There is no association between hypothyroidism and benign breast tumors.
- There is an association between autoimmune thyroid disease and breast cancer and
- Anti –TPO antibody can be included in the risk assessment profile of breast cancer.

SCOPE FOR FURTHER STUDY

- Thyroid receptor status of breast cancer tissue can be studied and a possible association between TR and ER status can be investigated.
- Thyroid receptor polymorphism studies can be done on breast cancer patients.
- TPO mRNA expression in breast tumors can be assessed.
- Tissue iodine determination in breast tumor tissue can be estimated.

REFERENCES

1. Chapter 16, The Breast, Schwartz's principles of surgery. Eighth edition.

2. Mittra I, Perrin J, Kumaoka S. Thyroid and other autoantibodies in British and Japanese women: an epidemiological study of breast carcinoma. Br Med J 1976;1:257–9

3. Goldman ME: Thyroid diseases and breast cancer. Epidemiol Rev 1990; 12:16-28.

4. Smyth PPA: The thyroid and breast cancer: a significant association? [Editorial]. Ann Med 1997; 29:189-*191*

5. Susana García-Silva and Ana Aranda* The Thyroid Hormone Receptor Is a Suppressor of *ras*-Mediated Transcription, Proliferation, and Transformation. Molecular And Cellular Biology, Sept. 2004, p. 7514–7523 Vol. 24, No. 17

6. Selliti et al nuclear thyroid hormone receptors in C3H/HeN mouse mammary glands and spontaneous tumors. Cancer Res 1983;43:1030-1038.

7. Giani C, Fierabracci P, Bonacci R. Relationship between breast cancer and thyroid disease. J Clin Endocrinol Metab 1996;81:990-4

8. Claus EB, Risch N, Thompson WD: Autosomal dominant inheritance of early-onset breast cancer: Implications for risk prediction. *Cancer* 73:643, 1994. [PMID: 8299086]

9. Domchek SM, Eisen A, Calzone K, et al: Application of breast cancer risk prediction models in clinical practice. *J Clin Oncol* 21:593, 2003. [PMID: 12586794]

10. Wooster R, Weber BL: Breast and ovarian cancer. *N Engl J Med* 348:2339, 2003. [PMID: 12788999]

11. Gowen LC, Avrutskaya AV, Latour AM, et al: BRCA1 required for transcription-coupled repair of oxidative DNA damage. *Science* 281:1009, 1998. [PMID: 9703501]

12. Roa BB, Boyd AA, Bolcik K, et al: Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. *Nat Genet* 14:185, 1996. [PMID: 8841191]

13. Ford D, Easton DF, Stratton M, et al: Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 62:676, 1998. [PMID: 9497246]

14. Rosen EM, Fan S, Pestell RG, Goldberg ID: BRCA1 gene in breast cancer. J Cell Physiol 196:19, 2003. [PMID: 12767038]

15. Bird A. Perceptions of Epigenetics. Nature 2007;447(7143);396

16. Giacinti L.et al. Epigenetic information and estrogen receptor alpha expression in breast cancer. Oncologist 2006; 11 :(1)1.

17. Chapter 43, Cancer of The Breast, Textbook of Cancer.pg 1602-1604.

18. Yeatman TJ, Bland KI: Assessment and designation of breast cancer stage, in Bland KI, Copeland EM III (eds): The Breast: Comprehensive Management of Benign Malignant Diseases. Philadelphia: WB Saunders, 1998, p 400.

19. Paul M. Yen Physiological and Molecular Basis of Thyroid Hormone Action Physiological Reviews Vol. 81, No. 3, July 2001

20. Nogurira and Brentai,1996; Bassett and Williams, 2003; Jones et al, 2003), Cell Growth Effects of Triiodothyronine and Expression of Thyroid Hormone Receptor in Prostate Carcinoma Cells Journal of Andrology, Vol. 26, No. 3, May/June 2005

21. Lai LC (2002). Role of steroid hormones and growth factors in breast cancer. Clinical Chemistry and Laboratory Medicine, 10: 969-974.

22. Jensen EV, Cheng G, Saji S, Mäkelä S, Noorden SV, Wahlström T,Warner M, Coombes RC & Gustafsson J (2001). Estrogen receptorsand proliferation markers in primary and recurrent breast cancer.Proceedings of the National Academy of Sciences, USA, 98: 15197-15202.

23. Neville MC, McFadden TB & Forsyth I (2002). Hormonal regulation of mammary differentiation and milk secretion. Journal of Mammary Gland Biology and Neoplasia, 1: 49-66.

24. Silva JM, et al (2002). Expression of thyroid hormone receptor/erbA genes is altered in human breast cancer. Oncogene, 21: 4307-4316.

25. Morgan MA, Dellovade TL & Pfaff W (2000). Effect of thyroid hormone administration on estrogen-induced sex behavior in female mice. Hormones and Behavior, 37: 15-22.

26. Glass CK & Holoway JM (1990). Regulation of gene expression by the thyroid hormone receptor. Biochimica et Biophysica Acta, 1032:157-176

27. Thyroid Hormone Receptors And Cancer .Agnieszka Piekielko-Witkowska,Hot thyroidology

28. Martínez-Iglesias O, García-Silva S, Regadera J, Aranda A. Hypothyroidism enhances tumor invasiveness and metastasis development. PLoS One 4:e6428, 2009.

29. Chan IH.,Isoform-specific transcriptional activity of overlapping target genes that respond to thyroid hormone receptors alpha 1 and beta 1.Mol endocrinol 23 :1758-1775,2009

30. Turowska O et al .Overexpression of E2F 1in clear cell renal carcinoma: a potential impact of erroneous regulation by thyroid hormone nuclear receptors.Thyroid 17:1039-1048,2007.

31. Kamiya Y et al.Expression of mutant thyroid hormone nuclear receptors is associated with human renal clear cell carcinoma. Carcinogenesis 23:25-33, 2002.

32. Puzianowska-Kuznicka M. Expression of thyroid hormone receptors is disturbed in human renal clear cell carcinoma .Cancer lett 155:145-152.2000.

33. Rosan MD,Thyroid hormone receptor mutation found in renal clear cell carcinomas alter corepressor release and reveal helix 12 as key determinant of corepressor specificity. Mol Endocrinol 23:1183-1192, 2009.

34. Nygard M, Hormone-dependent repression of E2F-1 gene by thyroid receptors. Mol Endocrinol 17:79-92.2003.

35. Yen CC Mediation of the inhibitory effect of thyroid hormone on proliferation of hepatoma cells by transforming growth factor-beta. J Mol Endocrinol 36:9-21, 2006.

36. Reddy A, Clin Gastroenterol Hepatol. 2007 Jan;5(1):118-23.Hypothyroidism: a possible risk factor for liver cancer in patients with no known underlying cause of liver disease.

37. Ledda-Columbano GM, Cell proliferation induced by triiodothyronine in rat liver is associated with nodule regression and reduction of hepatocellular carcinomas.Cancer Res. 2000 Feb 1;60 (3):603-9.

38. Eccles SA, Paon L. Breast cancer metastasis: when, where, how? Lancet 365:1006-1007, 2005.

39. Lebel, J. M., J. H. Dussault, and J. Puymirat. Overexpression of the beta 1 thyroid receptor induces differentiation in neuro-2a cells. Proc Natl Acad Sci USA 91:2644–2648, 1994.

40. García-Silva S, Aranda A. The thyroid hormone receptor is a suppressor of ras-mediated transcription, proliferation, and transformation. Mol Cell Biol 24:7514-7523, 2004.

41. Martínez-Iglesias O, Thyroid hormone receptor beta1 acts as a potent suppressor of tumor invasiveness and metastasis. Cancer Res 69:501-509, 2009.

42. Aksoy H, Goiter survey in Van province and thyroid functions in people older than 60. XXI. National Endocrinology and Metabolism Disease Congress. 29th September – 3rd October 1998, Istanbul, Abstract book, P:13.

43. Ron E, Multiple primary breast and thyroid cancer. Br J Cancer 1984;49: 87-90.

44. Davies TF. The thyrotrophin receptors spread themselves around. J Clin Endocrinol Metabol 1994; 79: 1232-38.

45. Venturi S, Role of iodine in evolution and carcinogenesis of thyroid, breast and stomach . Adv Clin Path. 2000 Jan;4(1):11-7.

46. Kotani T, Ohtaki S. Thyroid peroxidase 1990 Apr;38(4):442-5.Central Laboratory for Clinical Investigation, Miyazaki Medical College Hospital

47. Elisei R, Vassart G & Ludgate M. Demonstration of the alternatively spliced form of thyroid peroxidase in normal thyroid. Journal of Clinical Endocrinolgy and Metabolism 1991 72 700–702.

48. Niccoli P, Fayadat L, Panneels V, Lanet J & Franc JL. Journal of Biological Chemistry 1997 272 29487–29492.

49. Localization of the thyroid peroxidase autoantibody immunodominant region to a junctional region containing portions of the domains homologous to complement control protein and myeloperoxidase 2002 Oct 25;277(43):40189-95. Epub 2002 Aug 7.

50. Ericson LE, Johanson V, Molne J, Nilsson M, Ofverholm T: Intracellular transport and cell surface expression of thyroperoxidase. In: Thyroperoxidase and Thyroid Autoimmunity.Eds: Carayon P, Ruf J. John Libbey Eurotext, London, 1990, pp 107-116.

51. Pichurin PN ,Evidence that the complement control protein-epidermal growth factor-like domain of thyroid peroxidase lies on the fringe of the immunodominant region recognized by autoantibodies. 2002 Dec;12(12):1085-95.

52. McLachlan Genetic and epitopic analysis of thyroid peroxidase (TPO) autoantibodies: markers of the human thyroid autoimmune response. Clin Exp Immunol. 1995 Aug;101(2): 200-6.

53. Dupuy C et al: Thyroid NADPH-dependent H2O2 generating system: mechanism of H2O2 formation and regulation by Ca2+. In: Thyroperoxidase and Thyroid Autoimmunity. Eds: Carayon P, Ruf J. John Libbey Eurotext, London, 1990, pp 95-102.

54. Elisei R, Vivaldi A, Pacini F. Biology and clinical application of the NIS gene. Tumori 2003;89(5):523-8

55. Spitzweg C. The sodium-iodide symporter. Pathophysiologic, diagnostic and therapeutic significance. Internist 2003;44(4):396-402

56. Kilbane MT, et al: Tissue iodine content and serum mediated 125I uptake blocking activity in breast cancer. J Clin Endocrinol Metab 2000, 85:1245-1250.

57. Tazebay UH, et al: The mammary gland iodide transporter is expressed during lactation and in breast cancer. Nat Med 2000, 6:871-878.

58. Wapnir IL, et al: Immunohistochemical profile of the sodium/iodide symporter in thyroid, breast,and other carcinomas using high density tissue microarrays and conventional sections. J Clin Endocrinol Metab 2003, 88:1880-1888.

59. Min Huang et al, Ectopic expression of thyroperoxidase gene augments radioiodide uptake and retention mediated by the sodium iodide symporter in non-small cell lung cancer.Cancer Gene Ther .2001 August ;8(8): 612 -618.

60. Eskin, B. A., Shuman, R., Krouse, T., and Merion, J. A. (1975) Cancer Res.35, 2332–2339.

61. Turken O,et al: Breast cancer in association with thyroid disorders. Breast Cancer Res 2003, 5:R110-R113

62. Ghent, W. R., Eskin, B. A., Low, D. A., and Hill, L. P. (1993) Can. J. Surg. 36, 453–460

63. Kessler, J. H.et al (2004) Breast J. 10, 328–336

64. Smyth PP. Role of iodine in antioxidant defence in thyroid and breast disease. Biofactors. 2003;19(3-4):121-30.

65. Polat MF,et al.Oxidant/antioxidant status in blood of patients with malignant breast tumors and benign breast diseases. Cell Biochem Funct 2002, 20:327-331.

66. Ashutosh Shrivastava et al Molecular Iodine Induces Caspase-independent Apoptosis in Human Breast Carcinoma Cells Involving the Mitochondria-mediated Pathway ,the Journal of biological chemistry vol. 281, no. 28, pp. 19762–19771, July 14, 2006

67. Funahashi, H., Imai, T., Tanaka, Y., Tsukamura, K., Hayakawa, Y., Kikumori,Y., Takahashi, M., and Narita, T. (1999) Jpn. J. Cancer Res. 90, 922–927.

68. Dugrillon A: Iodolactones and iodoaldehydes-mediators of iodine in thyroid autoregulation. Exp Clin Endocrinol Diabetes 1996, Suppl 4:41-45.

69. Endocr Relat Cancer. 2008 Dec;15(4):1003-11. Epub 2008 Sep 30.Signaling pathways involved in the antiproliferative effect of molecular iodine in normal and tumoral breast cells: evidence that 6-iodolactone mediates apoptotic effects. Arroyo-Helguera.

70. Prostaglandins and other lipid mediators 2009 Jun; 89(1-2):34-42. Epub 2009 Apr 10.

71. Beatson GT: on the treatment of inoperable cases of carcinoma of mamma: suggestions for a new method of treatment, with illustrative cases. Lancet 1896, ii;104-110.

72. Smyth PPA, Smith DF, et al A direct relationship between thyroid enlargement and breast carcinoma. J Clin Endocrinol Metab 1996;81:937–41.

73. Vassilopoulou-Sellin R, Palmer L, Taylor S, Cooksley CS. Incidence of breast carcinoma in women with thyroid carcinoma. Cancer 1999;85(3):696-705

74. Hrafnkelsson J, Tulinius H, Jonasson JG, Olafsdottir G, Sigvaldason H. Papillary thyroid carcinoma in Iceland. A study of the occurrence in families and the coexistence of other primary tumours. Acta Oncol 1989;28:785-8

75. McTiernan A, Weiss NS, Daling JR. Incidence of thyroid cancer in women in relation to known or suspected risk factors for breast cancer. Cancer 1987; 47:292-5.

76. Glass CK & Holoway JM (1990). Regulation of gene expression by the thyroid hormone receptor. Biochimica et Biophysica Acta, 1032:157-176.

77. Smyth PP, Shering SG, Kilbane MT, Murray MJ, McDermott EW, Smith DF, O'Higgins NJ. Serum thyroid peroxidase autoantibodies, thyroid volume, and outcome in breast carcinoma. J Clin Endocrinol Metab 1998;83:2711-6

78. McThernan A, Weiss NS, Daling JR: Incidence of thyroid cancer in women in relation to known or suspected risk factors for breast cancer. Cancer Res 1987, 47:292-294. PubMed Abstract.

79. Ron E, Curtis R, Hooffman DA, Flannery JT: Multiple primary breast and thyroid cancer. Br J Cancer 1984, 49:87-90. PubMed Abstract

80. Shering SG et al Thyroid disorders and breast cancer Eur J Cancer Prev 1996 Dec;5(6):504-6.

81. Rasmusson B, et al. Thyroid function in patients with breast carcinoma. Eur J Cancer Clin Oncol 1987; 23: 553-6

82. Ito K, Maruchi N. Breast carcinoma in patients with Hashimoto's thyroiditis. Lancet 1975; 2:1119–21.

83. Gogas J, Kouskos E, Tseleni-Balafouta S, Markopoulos C, Revenas K, Gogas G, Kostakis A: Autoimmune thyroid disease in women with breast carcinoma. Eur J Surg Oncol 2001, 27:626-630. PubMed Abstract | Publisher Full Text.

84. Myhil J, Reeve TS, Hales IB: Thyroid function in breast cancer. Acta Endocrinol 1966, 51:290-300. PubMed Abstract.

85. Jiskra J,et al Autoimmune thyroid diseases in women with breast cancer and colorectal cancer. Physiol res 2004;53(6):693-702.

86. Smyth PPA. Autoimmune thyroid disease and breast cancer: a chance association. J Endocrinol Invest 2000;23:42-3

87. Spitzweg C, Joba W, Eisenmenger W, Heufelder A. Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complementary deoxyribonucleic acids from salivary gland, mammary gland and gastric mucosa. J Clin Endocrinol Metab 1998;83:1746-51

88. Rodien PM, et al.1996 Antibody dependent cell-mediated cytotoxicity in autoimmune thyroid disease:relationship to antithyroperoxidase antibodies . J Clin Endocrinol Metab. 81:2595-2600.

89. Wasserman, J, Glas, U, and Blomgren, H, Clinical and Experimental Immunology, 1975,19, 417

90. Prognostic value of anti-thyroperoxidase antibodies in high malignancy degree breast cancer Emilio Fiore[1]et al , European Society of Endocrinology.

91. Weijl NI, et al Hypothyroidism during immunotherapy with interleukin-2 is associated with antithyroid antibodies and response to treatment. J Clin Oncol 1993, 11:1376-1383.

92. Franzke A, et al: Autoimmunity resulting from cytokine treatment predicts long-term survival in patients with metastatic renal cell cancer. J Clin Oncol 1999, 7:529-533.

93. Massimo Cristafonilli et al Thyroid Hormone and Breast Carcinoma .online publication Feb 2005 in Wiley InterScience, American Cancer Society.

94. Changxue Lu and Sheue-Yann Cheng. Review Thyroid hormone receptors regulate adipogenesis and carcinogenesis via crosstalk signaling with peroxisome proliferator-activated receptors .Journal of Molecular Endocrinology (2010) 44, 143–154

95. Stoll BA.1965 Breast carcinoma and hypothyroidism.Carcinoma.18: 1431-1436.

96 Takatani O,et al1989 Relationship between the levels of serum thyroid hormones, estrogen status and the risk of breast carcinoma genesis in Japanese women. Cancer Res. 9:3109-3112

97. Peter PA Smyth.The thyroid , iodine,and Breast cancer.Breast Cancer Res 2003,5:235-238.

98. Marie-Madelaine et al Primary structure of bovine lactoperoxidase, a fourth member of a mammalian heme peroxidase family. Eur J Biochem .733-739(1991).

99. Libert F et al. Thyroperoxidase ,an autoantigen with a mosaic structure made of nuclear and mitochondrial gene modules. EMBO J. 1987 Dec 20;6 (13):4193-6.

MASTER CHART- BENIGN BREAST TUMOUR PATIENTS

S.No	Age yrs	endogenous estrogenexposure in yrs	parity	age at first childbirth yrs	Premenopause /menopause	family H/O breast cancer	TSH mIU/L	fT4 ng/dL	fT3 pg/mL	anti-TPO ab AU/mL
1	60	28	2	20	m	n	2.51	1.46	2.94	10.75
2	43	21	1	24	m	n	2.44	1.47	2.98	10
3	51	32	0	NA	m	n	2.48	1.46	2.97	10
4	49	30	1	22	m	n	3.74	1.01	2.06	22.4
5	53	31	2	21	m	n	3.12	1.43	2.46	7.2
6	48	28	2	19	m	n	2.78	1.38	2.79	13.2
7	35	20	3	19	p	n	2.98	1.12	2.22	11.2
8	51	31	2	25	m	y	2.82	1.37	2.79	13.25
9	50	31	3	25	m	n	2.78	1.47	2.88	11.5
10	61	32	2	20	m	n	2.72	1.44	2.9	11
11	58	33	3	20	m	n	2.22	1.57	3.12	7.5
12	52	34	2	22	m	n	2.62	1.38	3.74	7.2
13	51	30	2	23	m	n	2.98	1.31	2.26	7.3
14	65	30	5	25	m	n	1.32	2.06	4.14	8.76
15	44	29	3	18	p	n	3.89	0.61	1.24	27.2
16	49	29	2	23	m	n	2.94	1.11	2.24	9.75
17	48	34	3	18	p	n	2.4	1.4	2.82	13
18	50	30	2	20	m	n	1.96	2.11	3.38	6.2
19	47	30	3	25	p	n	2.76	1.63	2.74	10.6
20	48	35	0	NA	p	n	2.01	1.68	3.41	5.5
21	45	31	2	21	p	n	2.42	1.42	2.82	12.6
22	47	30	2	27	p	n	2.26	1.43	2.98	8.5
23	52	32	4	20	m	n	2.38	1.42	2.88	14.5
24	47	30	2	20	p	n	2.2	1.51	3.02	7.8
25	36	25	2	20	p	n	2.82	1.41	2.6	16.6
26	38	25	3	19	p	n	3.2	1.86	1.16	26.2
27	33	21	1	25	p	n	4.71	1.82	1.66	21.9
28	35	21	3	17	p	n	3.89	0.69	1.4	31.9
mean	48.07	29.04					2.76	1.43	2.66	12.98
S.D	7.83	4.12					0.68	0.33	0.70	6.84

MASTER CHART - CONTROLS

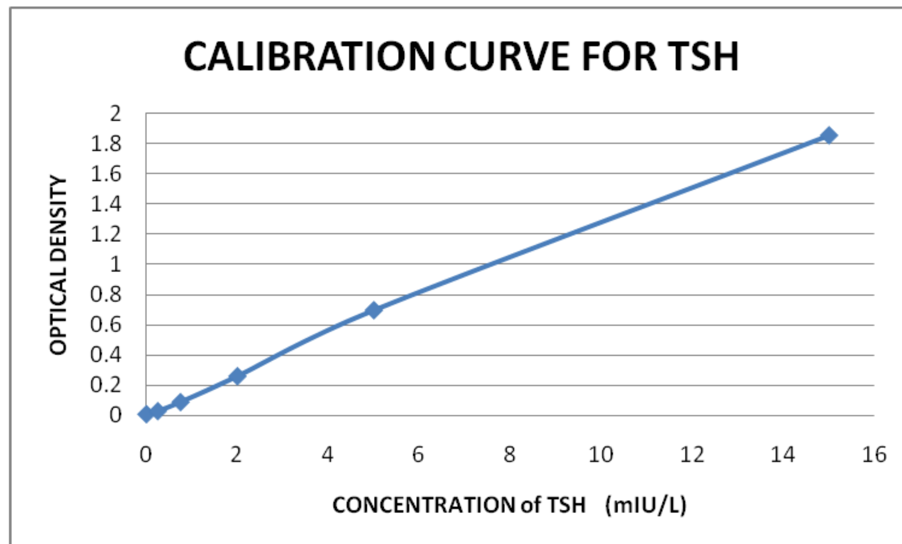
S.No	Age yrs	Endogenous estrogenexposure in years	parity	age at first childbirth yrs	premenopause/ menopause	family H/O breast cancer	TSH mIU/L	fT4 ng/dL	fT3 pg/mL	anti-TPO antibody AU/mL
1	62	33	4	24	m	n	1.82	1.97	3.67	3.5
2	45	32	3	20	p	n	1.72	2.01	3.72	5
3	48	34	3	23	m	n	1.14	2.01	4.02	1
4	63	35	4	21	m	n	3.79	0.71	1.32	38.3
5	60	32	3	20	m	n	1.88	1.75	3.34	9
6	40	27	3	21	p	n	1.94	2.11	3.52	5.5
7	64	32	1	26	m	n	1.74	2.11	3.71	6.25
8	38	24	1	32	p	n	1.72	2.2	3.8	2
9	32	20	1	18	p	n	1.92	1.87	3.52	8
10	39	25	3	24	p	n	3.51	1.57	2.16	0.5
11	58	36	2	20	m	n	4.51	1.86	0.89	22.2
12	44	32	3	20	p	n	2.04	2.02	3.5	6
13	50	32	4	20	m	n	2.08	1.86	3.42	6.6
14	54	31	0	NA	m	n	1.02	2.07	3.79	3.2
15	37	25	2	18	p	n	3.16	1.65	2.84	13.4
16	38	25	2	19	p	n	3.38	0.62	2.02	20.3
17	55	33	3	18	m	n	3.26	1.32	2.52	17.5
18	55	34	4	20	m	n	3.62	0.78	1.45	37.6
19	50	32	2	20	m	n	3.58	0.97	1.72	23.7
20	65	35	4	20	m	n	2.52	1.13	3.04	1
21	48	32	3	20	m	n	2.35	1.28	2.74	1.5
22	50	31	0	NA	m	n	1.88	1.99	3.65	4
23	38	24	2	20	p	n	3.62	1.29	2.28	18.3
24	40	26	2	20	p	n	3.52	0.99	1.81	21.1
25	45	30	3	20	m	n	2.72	2.03	3.54	5.55
26	48	34	4	20	p	n	3.46	1.28	2.24	18.3
27	49	33	2	22	m	n	3.11	1.71	2.82	13.6
28	32	20	2	24	p	n	2.01	1.77	3.32	0.5
29	37	24	3	25	p	n	2.76	1.63	3.14	11
30	59	32	4	19	m	n	2.01	1.73	3.4	9
Mean	48.1	29.83					2.59	1.61	2.89	11.11
SD	9.78	4.56					0.89	0.46	0.85	10.21

MASTER CHART-MALIGNANT BREAST CANCER PATIENTS

S.No	Age In yrs	Endogenous Estrogen exposure yrs	parity	age at first childbirth in yrs	Premenopause /menopause	family H/O breast cancer	clincal stage	TSH mIU/L	fT4 ng/dL	fT3 pg/mL	anti-TPO antibody AU/mL
1	32	20	2	17	p	n	II A	3.7	0.79	1.44	41.5
2	47	34	4	20	m	n	III B	3.78	0.8	1.32	322
3	47	29	3	20	m	n	II B	2.19	1.5	2.98	12.2
4	31	19	2	15	p	n	III B	3.52	0.78	1.46	38.25
5	51	33	3	20	m	n	III B	3.5	0.8	1.38	58.5
6	65	32	4	25	m	n	III B	3.46	0.8	2.24	31.5
7	35	23	2	20	p	n	II B	3.68	0.81	1.48	58.2
8	51	30	2	25	m	n	II B	1.59	1.18	3.9	18.5
9	65	34	5	16	m	n	III B	3.4	0.83	2.25	31.2
10	55	33	1	27	m	n	III B	3.66	0.82	1.56	38.5
11	61	28	2	18	m	n	II B	3.1	0.98	2.02	22.3
12	55	33	5	20	m	n	III B	3.48	0.83	1.52	37.5
13	50	28	2	22	m	n	III B	3.22	0.84	1.82	33
14	43	29	3	16	m	n	II B	3.5	0.81	1.4	44
15	65	36	3	25	m	n	III B	3.7	0.9	1.35	52
16	48	33	0	NA	m	n	III B	2.55	1.39	2.86	16
17	47	35	2	26	p	n	II A	3.5	0.98	1.92	24
18	50	27	3	20	m	n	IV	3.42	0.84	2.32	301
19	41	28	3	20	p	n	III A	3.51	0.88	1.41	148
20	51	33	1	35	m	n	III B	3.42	0.87	1.43	134
21	50	33	4	22	m	y	II B	3.69	0.82	1.42	48.75
22	63	30	2	25	m	n	II B	3.34	0.98	1.39	46.75
23	51	28	2	21	m	n	III B	2.36	1.48	2.92	13.6
24	65	31	4	20	m	n	III B	3.49	0.83	2.02	41
25	43	30	3	19	p	n	III B	1.8	1.73	3.42	10.6
26	65	31	2	18	m	n	III B	3.12	0.98	2.42	23
27	45	30	0	NA	m	n	III B	3.62	0.94	1.34	323
28	56	32	4	18	m	n	III B	2.18	1.51	3.12	12
29	52	34	0	NA	m	n	III A	3.34	0.82	2.32	35.75
30	51	32	3	20	m	n	III B	2.32	1.5	2.94	16.5
mean	51.03	30.27						3.17	1.00	2.05	67.77
S.D	9.51	4.03						0.62	0.28	0.73	89.41

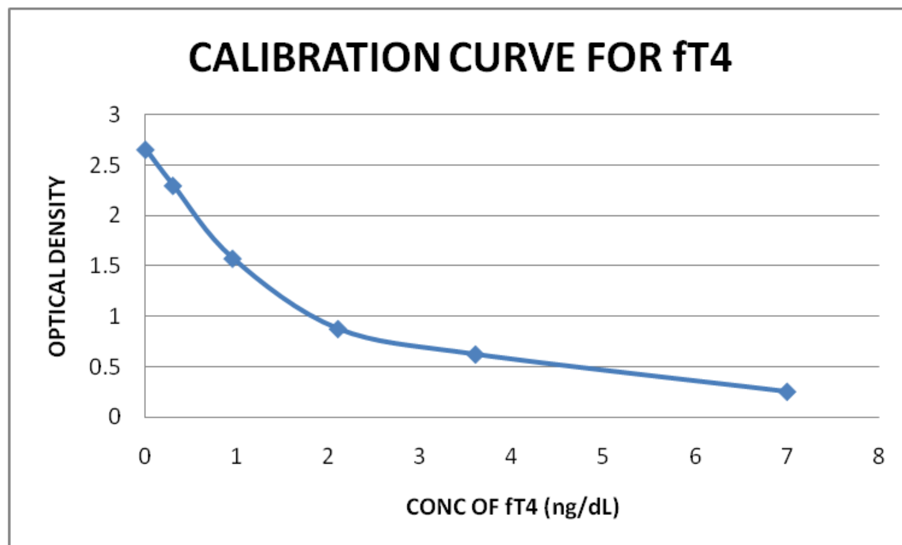
TSH CALIBRATION

S.No	Concentration of TSH (mIU/L)	Optical Density
1	0	0.011
2	0.25	0.031
3	0.75	0.091
4	2	0.262
5	5	0.698
6	15	1.853



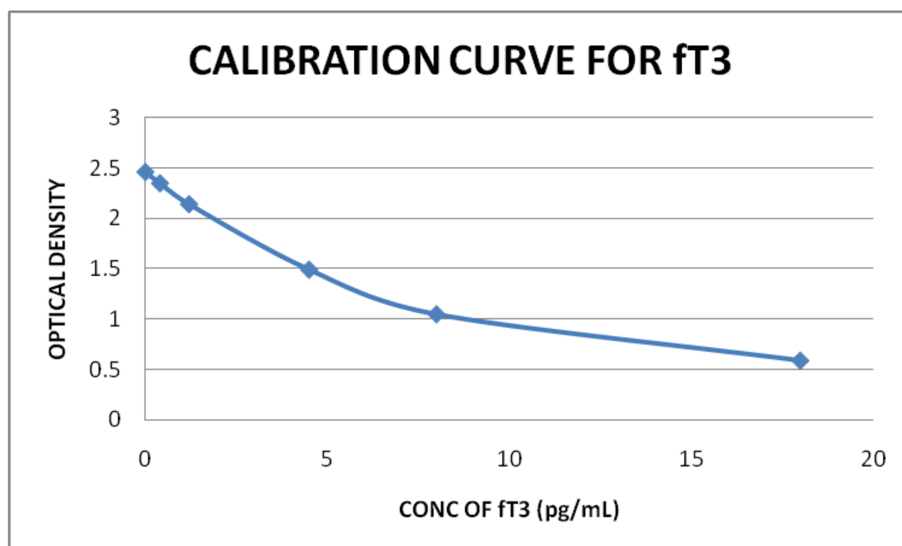
FreeT$_4$ CALIBRATION

S .No	Concentration of fT4(ng/dL)	OD
1	0	2.656
2	0.3	2.298
3	0.95	1.574
4	2.1	0.875
5	3.6	0.6224
6	7	0.2519



FreeT$_3$ CALIBRATION

S. No	Concentration of fT3(pg/mL)	OD
1	0	2.457
2	0.4	2.346
3	1.2	2.139
4	4.5	1.491
5	8	1.049
6	18	0.59



Anti-TPO CALIBRATION

S.No	Conc of Anti-TPO(AU/mL)	log conc ANTI-TPO	OD
1	0	0	0.012
2	5	0.6989	0.123
3	10	1	0.21
4	20	1.301	0.395
5	80	1.903	1.221
6	320	2.505	2.474

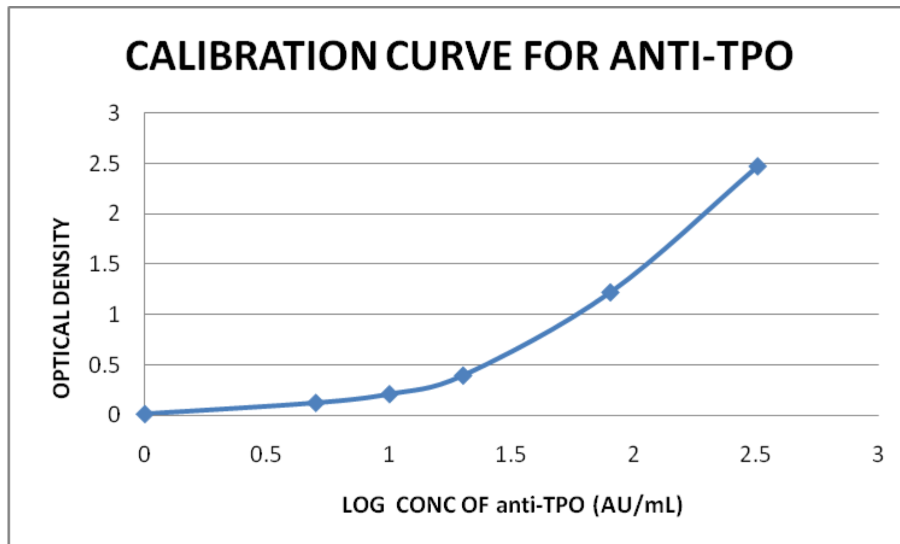
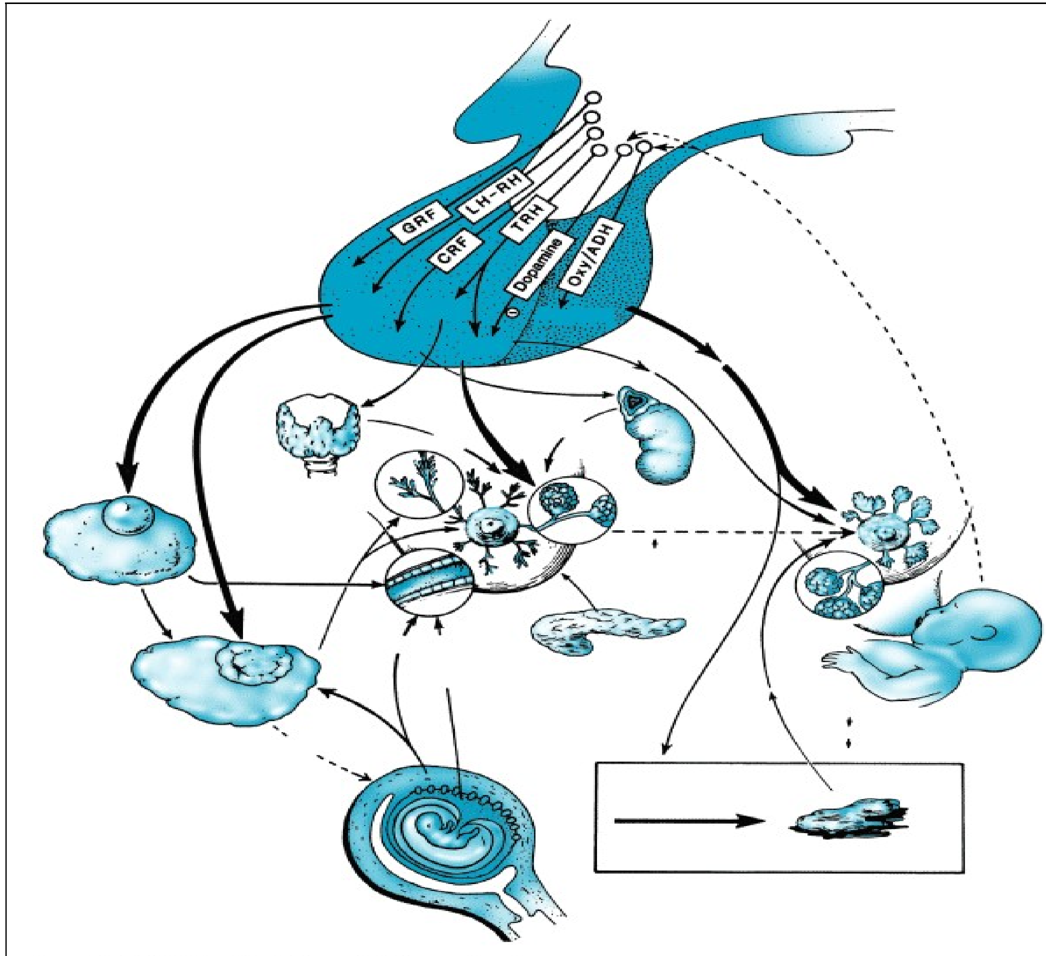


Figure-1. HORMONES INVOLVED IN THE DEVELOPMENT OF BREAST



Courtesy:-Schwartz's principles of surgery

Figure 2: ESTROGEN AND GROWTH FACTOR PATHWAY IN THE DEVELOPMENT OF BREAST CANCER

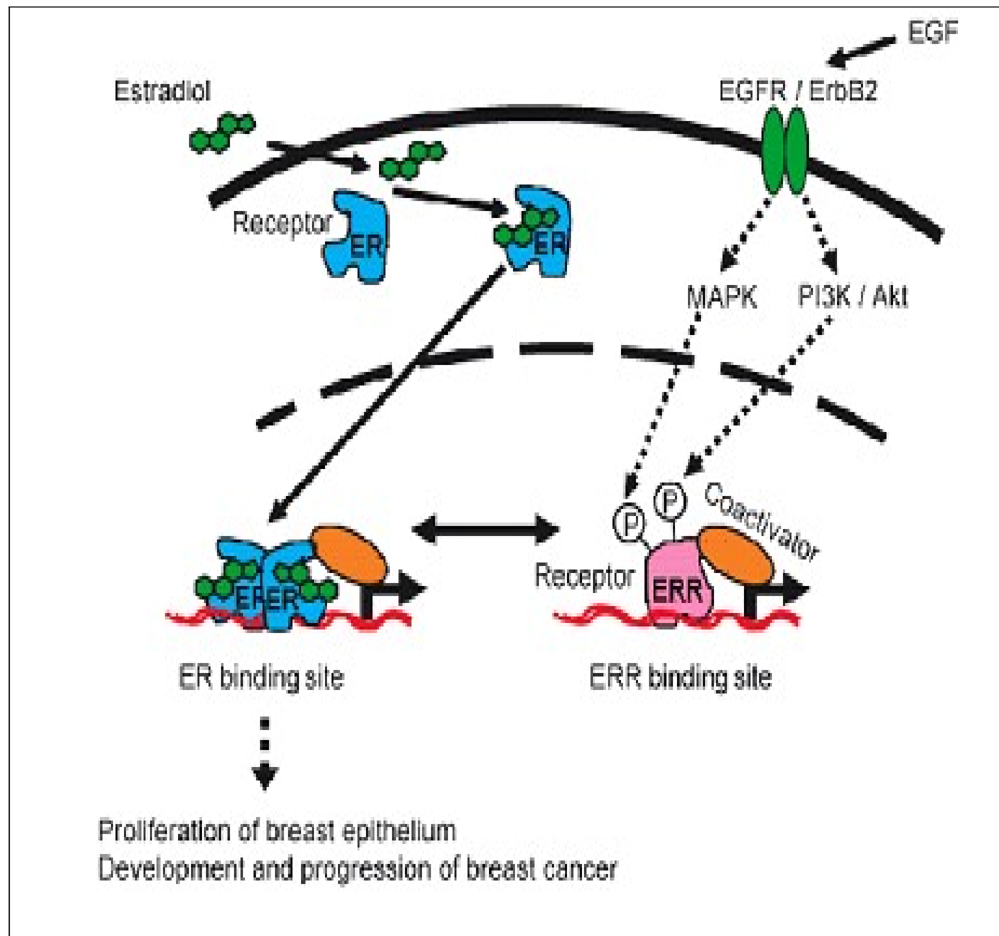
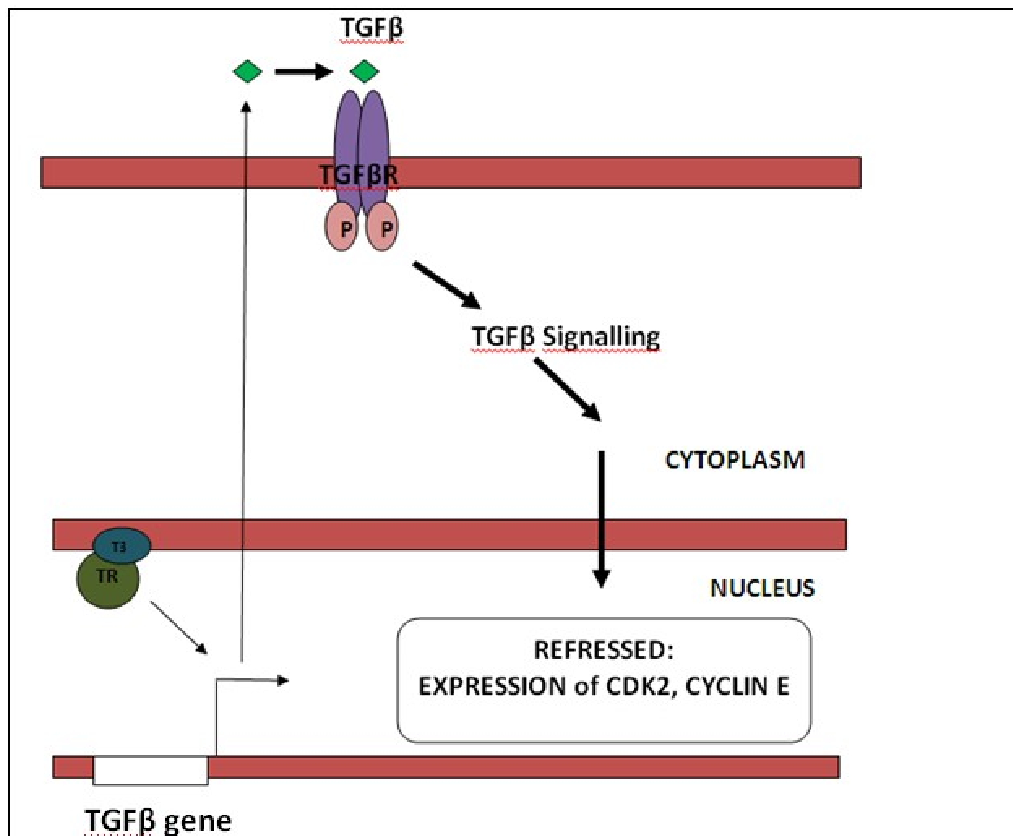
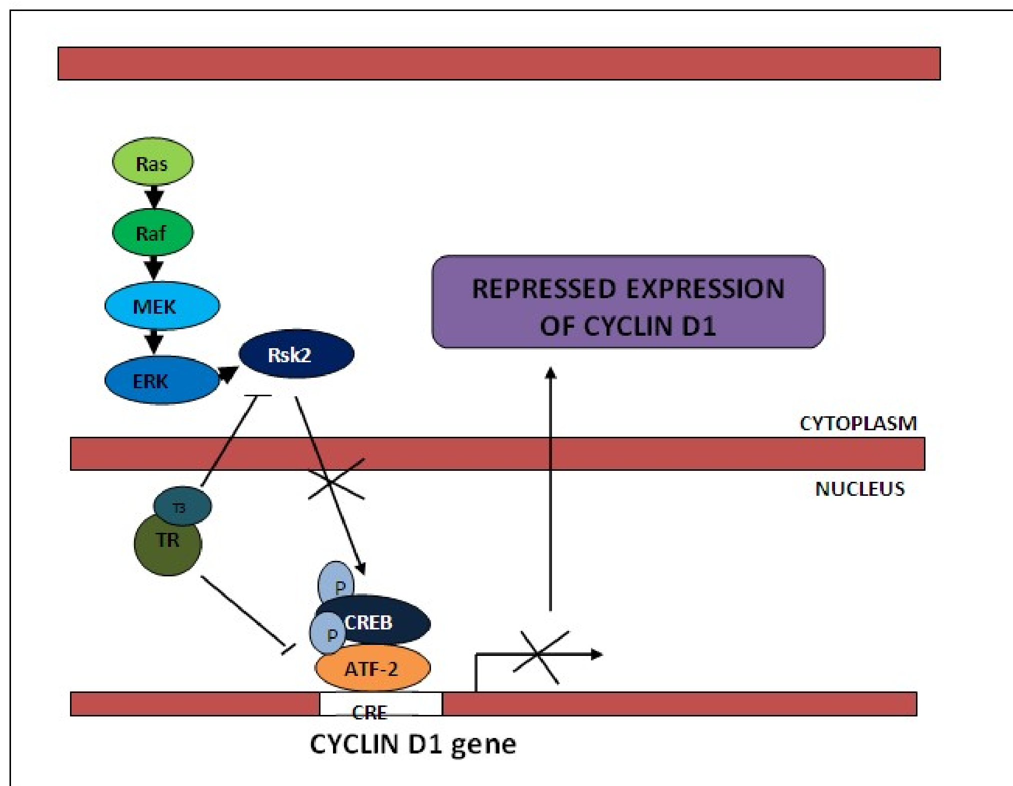


Figure 3: ANTIPROLIFERATIVE EFFECT OF T_3/TR STIMULATED EXPRESSION OF TGFB IN HEPATOMA CELL LINE.



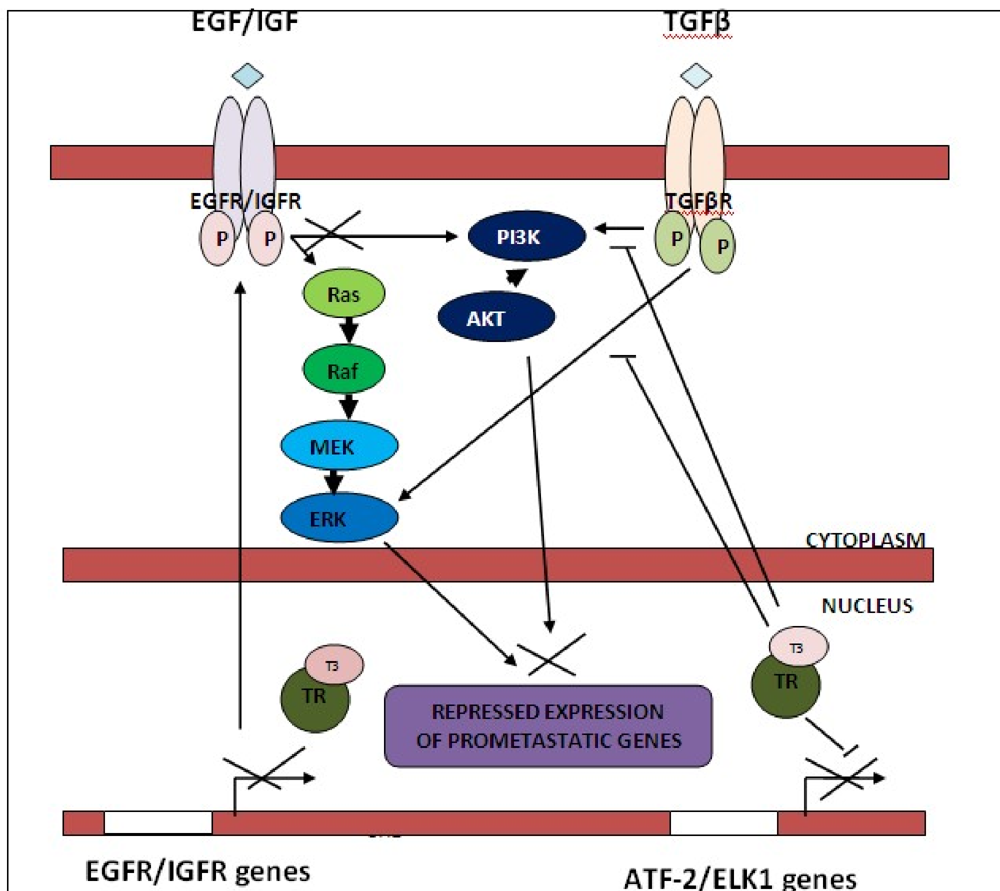
Courtesy:-Hot thyroidology.

Figure-4: T_3/TR MEDIATED INHIBITION OF RAS-INDUCED PROLIFERATION IN NEUROBLASTOMA CELL LINE



Courtesy:-Hot thyroidology

Figure-5: T_3/TR MEDIATED REPRESSION OF PROMETASTATIC GENES IN BREAST AND HEPATOMA CELL LINES



Courtesy:-Hot thyroidology.

FIG:7 : DISTRIBUTION OF TSH VALUES AMONG CASES AND CONTROLS

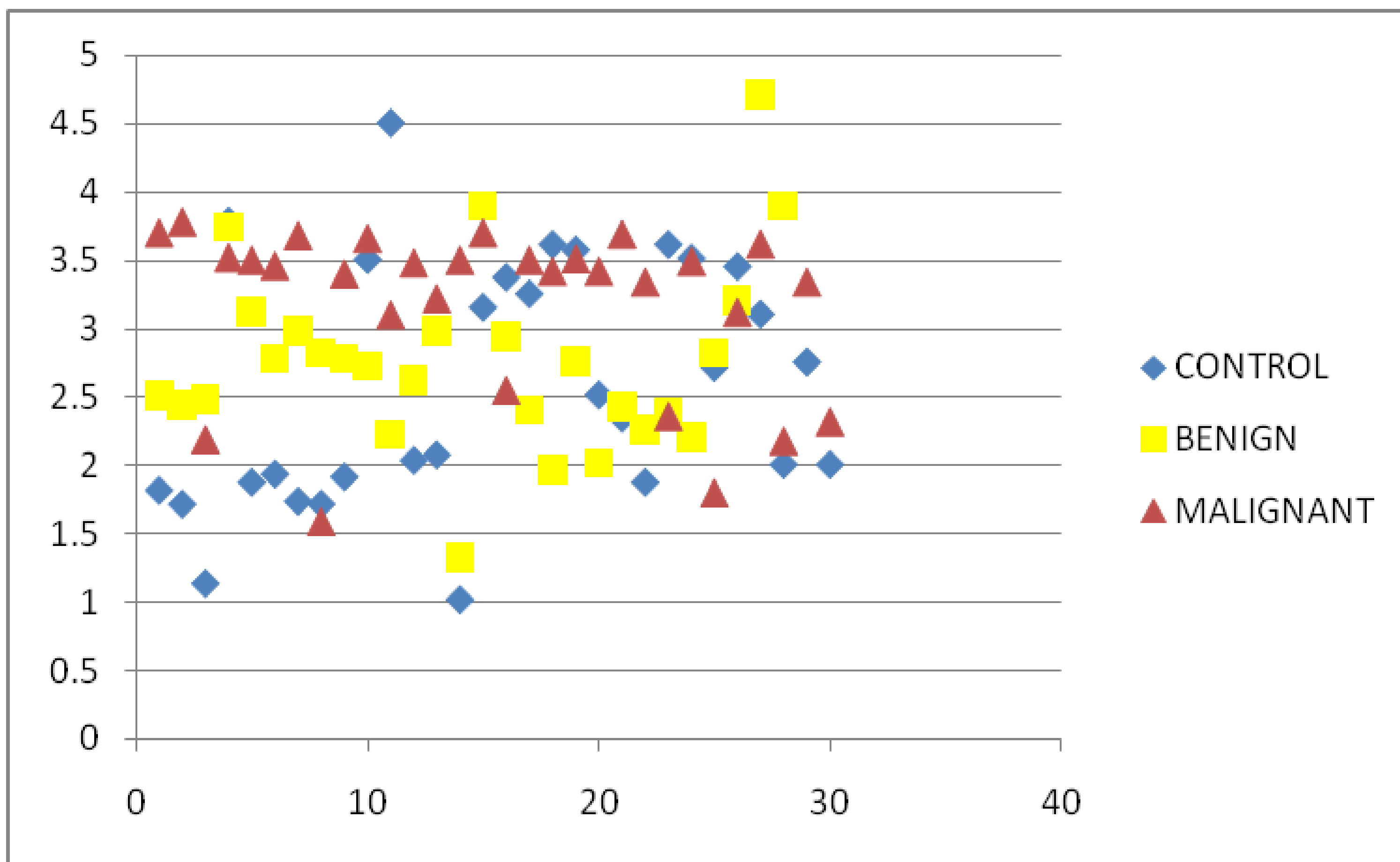


FIG:9 : DISTRIBUTION OF FT_4 VALUES AMONG CASES AND CONTROLS

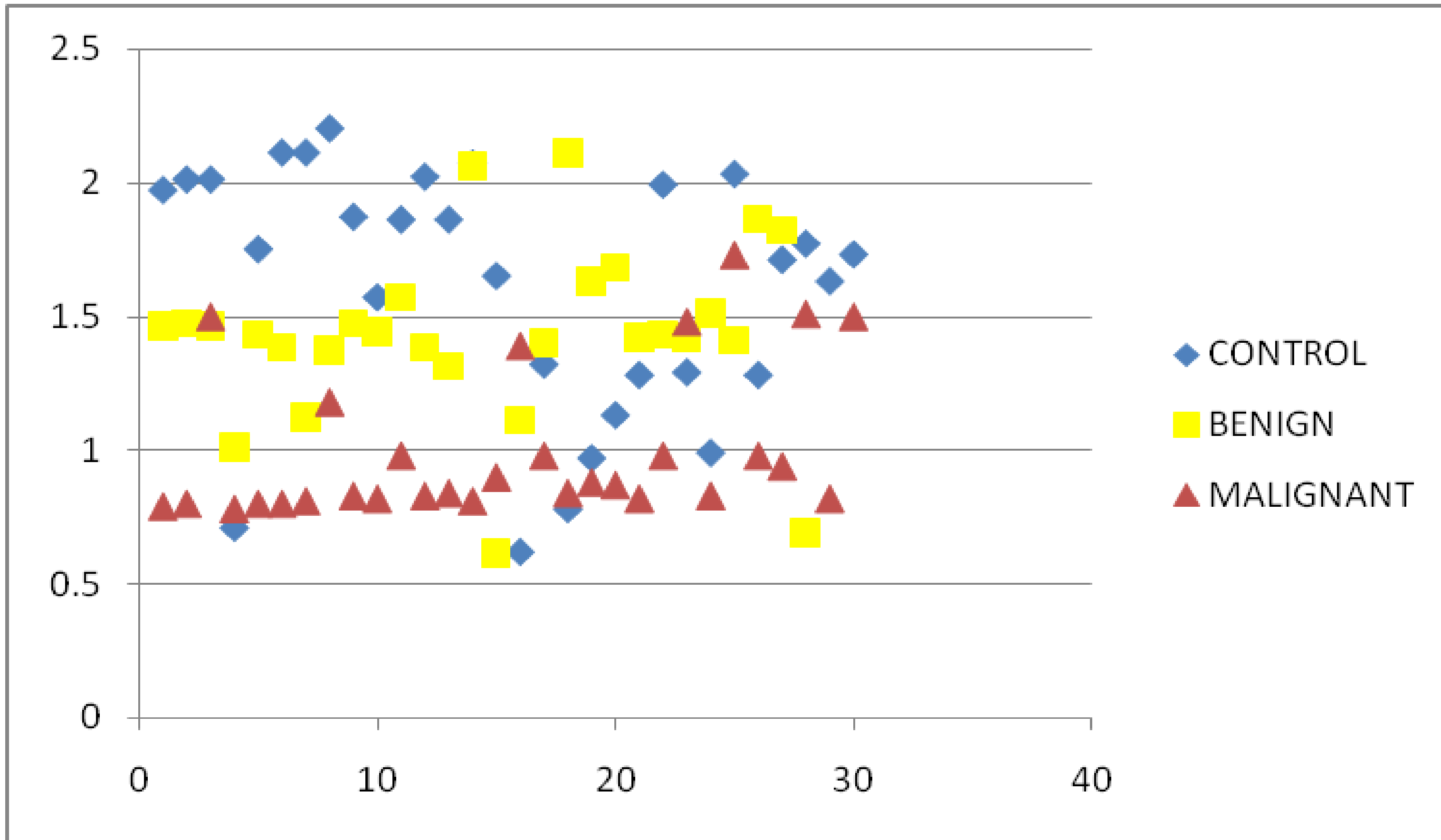


FIG:11 : DISTRIBUTION OF FT_3 VALUES AMONG CASES AND CONTROLS

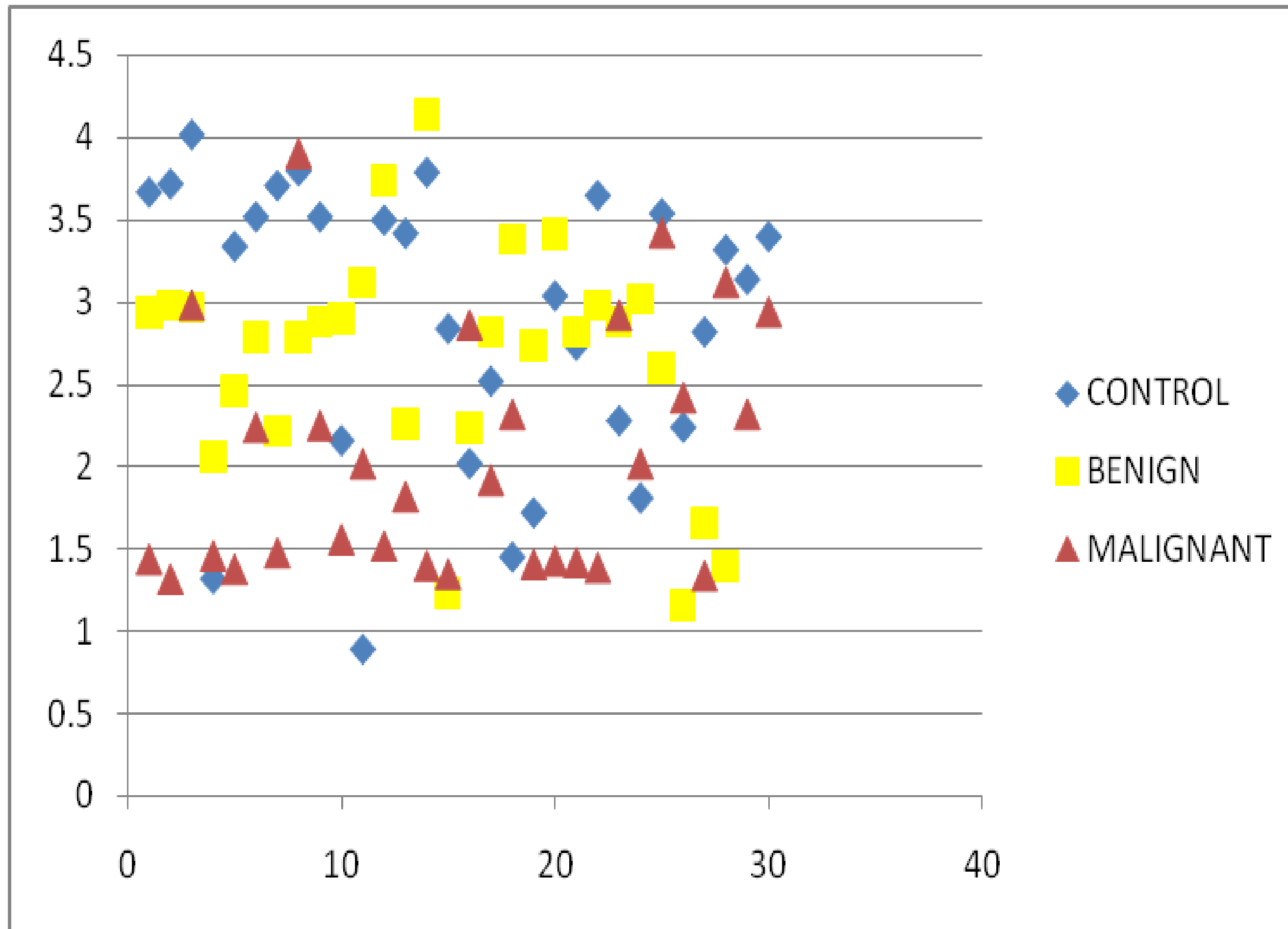


FIG:13 : DISTRIBUTION OF ANTITPO VALUES AMONG CASES AND CONTROLS

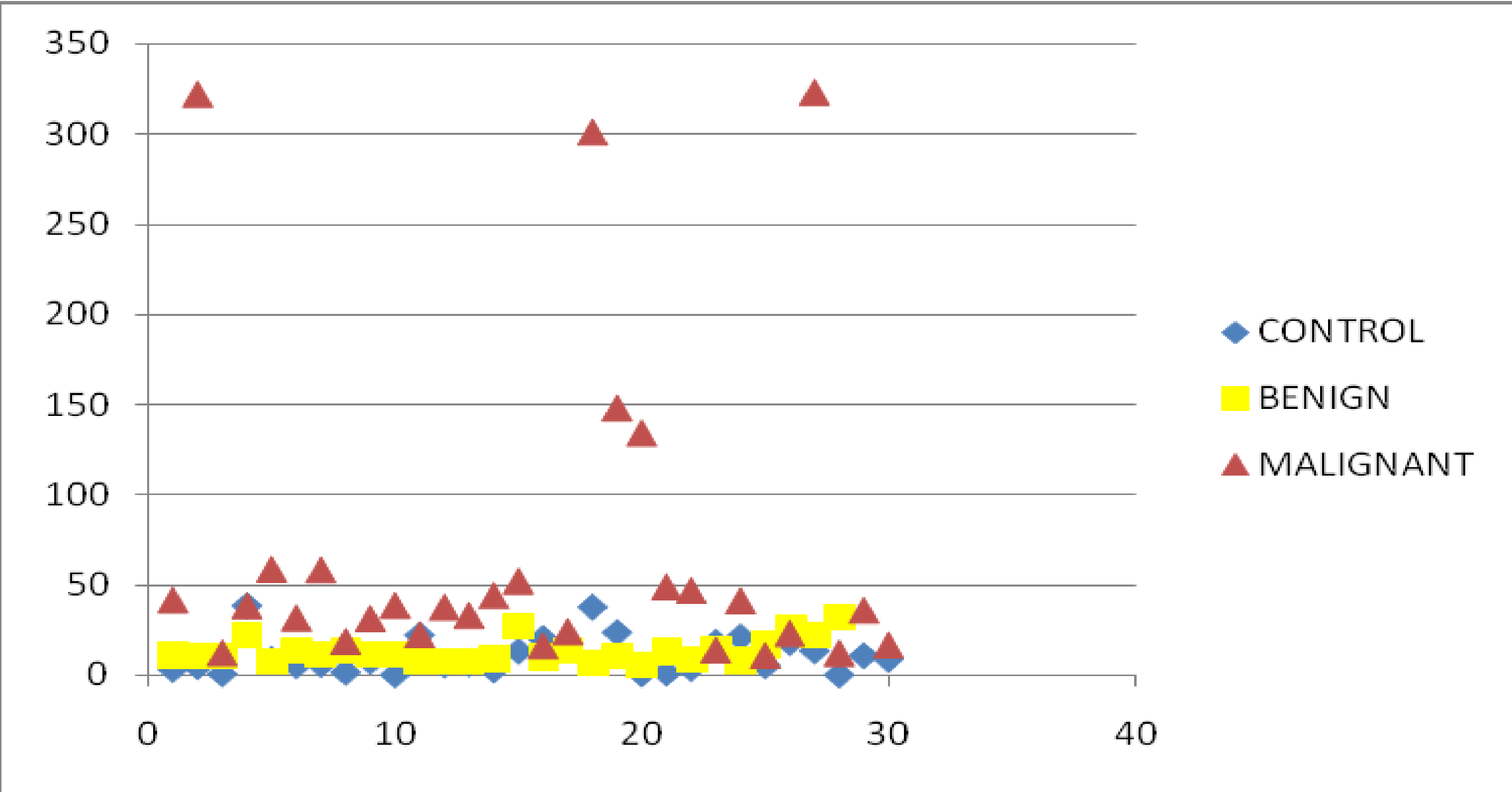


Table-1: Shows the comparison of age, family h/o breast cancer, nulliparity, first childbirth >30 years and endogenous exposure to estrogen > 30 years among the controls and cases.

S No	Study group	Age in years	Family h/o Breast Cancer %	Nulliparity %	First Childbirth > 30yrs%	Endogenous estrogen Exposure > 30 years %
1	Controls (n=30)	48.1± 9.7	0%	6.7%	3.3%	66.7%
2	Benign (n=28)	48.1± 7.8	3.6%	7.1%	0%	64.3%
3	Malignant (n=30)	51.03± 9.5	3.3%	10%	3.3%	66.7%
p-value		0.39	0.59	0.58	0.93	0.90
Level of significance		Not significant	Not significant	Not significant	Not significant	Not significant

Table-1: Shows the comparison of age, family h/o breast cancer, nulliparity, first childbirth >30 years and endogenous exposure to estrogen > 30 years among the controls and cases.

S No	Study group	Age in years	Family h/o Cancer %	Nulliparity %	First Childbirth > 30 %	Endogenous estrogen Exposure > 30 years %
1	Controls (n=30)	48.1± 9.7	0%	6.7%	3.3%	66.7%
2	Benign (n=28)	48.1± 7.8	3.6%	7.1%	0%	64.3%
3	Malignant (n=30)	51.03± 9.5	3.3%	10%	3.3%	66.7%
p-value		0.39	0.59	0.58	0.93	0.90
Level of significance		Not significant	Not significant	Not significant	Not significant	Not significant

Table-2 Shows the comparison of TSH among the controls and cases.

	N	Mean mIU/L	Std. Deviation	oneway ANOVA	Multiple comparison using Bonferroni t-test
Control	30	2.59	0.89	F=4.82 P=0.01	Control Vs Cancer
Benign	28	2.76	0.68		
Cancer	30	3.17	0.62		

Fig: 6 - Comparison of TSH among the controls and cases.

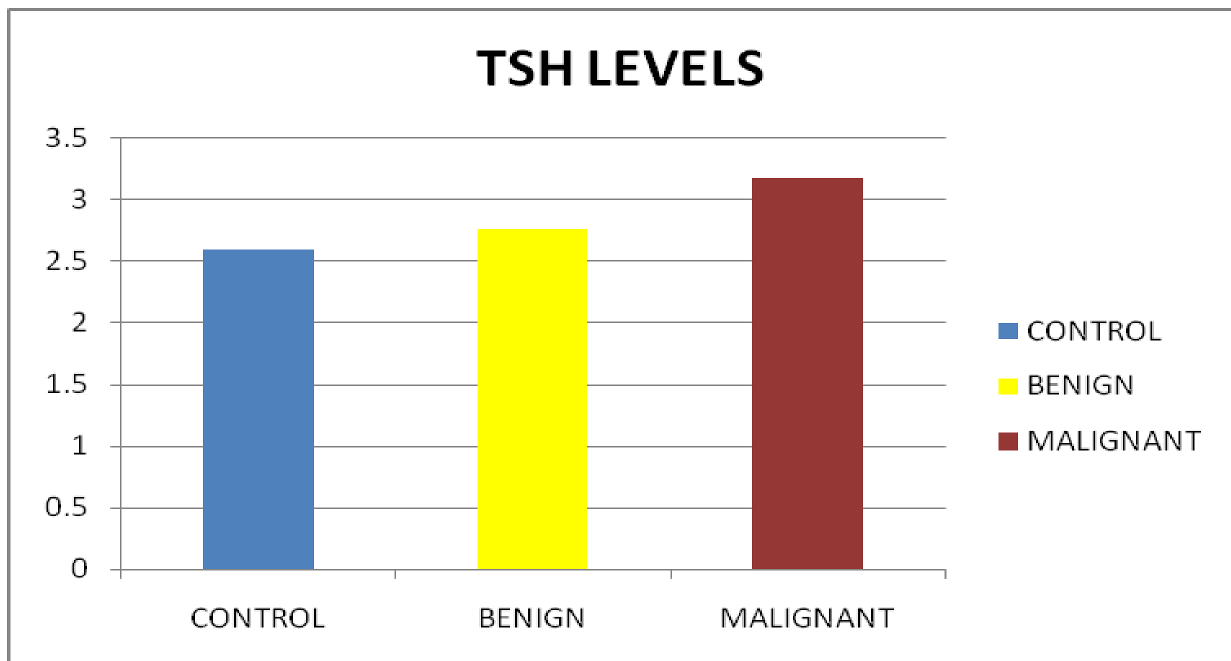


Table-3 Shows the comparison of free T_4 among the controls and cases.

	N	Mean ng/dL	Std. Deviation	oneway ANOVA	Multiple comparison using Bonferroni t-test
Control	30	1.61	0.46	F=21.85 P=0.001***	Control Vs Benign Cancer Vs Control, Benign
Benign	28	1.43	0.33		
Cancer	30	1.00	0.28		

* P<0.05 significant   **P<0.01 highly significant   ***P<0.001 very high significant

Fig: 8:Comparison of free T4 among the controls and cases

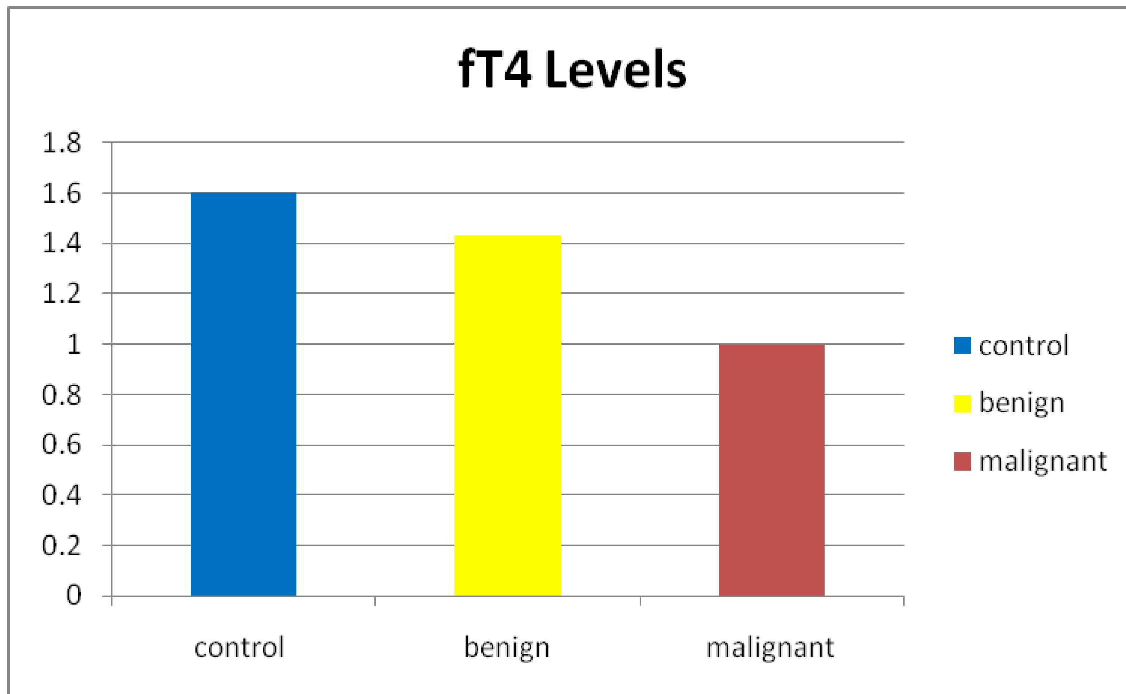


Table-4 shows the comparison of freeT3 among the controls and cases.

	N	Mean pg/mL	Std. Deviation	oneway ANOVA	Multiple comparison using Bonferroni t-test
Control	30	2.89	0.85	F=9.82 P=0.001***	Control Vs Benign
Benign	28	2.66	0.70		Cancer Vs Control, Benign
Cancer	30	2.05	0.73		

* P<0.05 significant   **P<0.01 highly significant   ***P<0.001 very high significant

Fig:10: shows the comparison of freeT3 among the controls and cases.

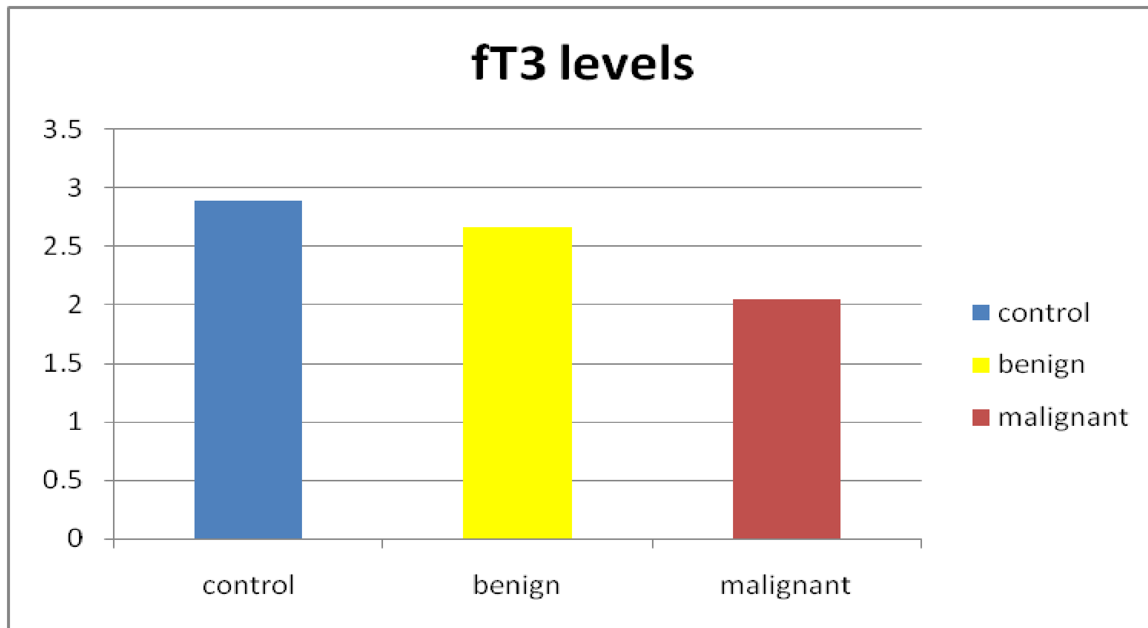


Table-5 shows the comparison of antiTPO among the controls and cases.

	N	Mean AU/mL	Std. Deviation	oneway ANOVA	Multiple comparison using Bonferroni t-test
Control	30	11.11	10.21	F=11.07 P=0.001***	Control Vs Benign Cancer Vs Control, Benign
Benign	28	12.98	6.84		
Cancer	30	67.77	89.41		

* P<0.05 significant **P<0.01 highly significant ***P<0.001 very high significant

Fig: 12: shows the comparison of antiTPO among the controls and cases

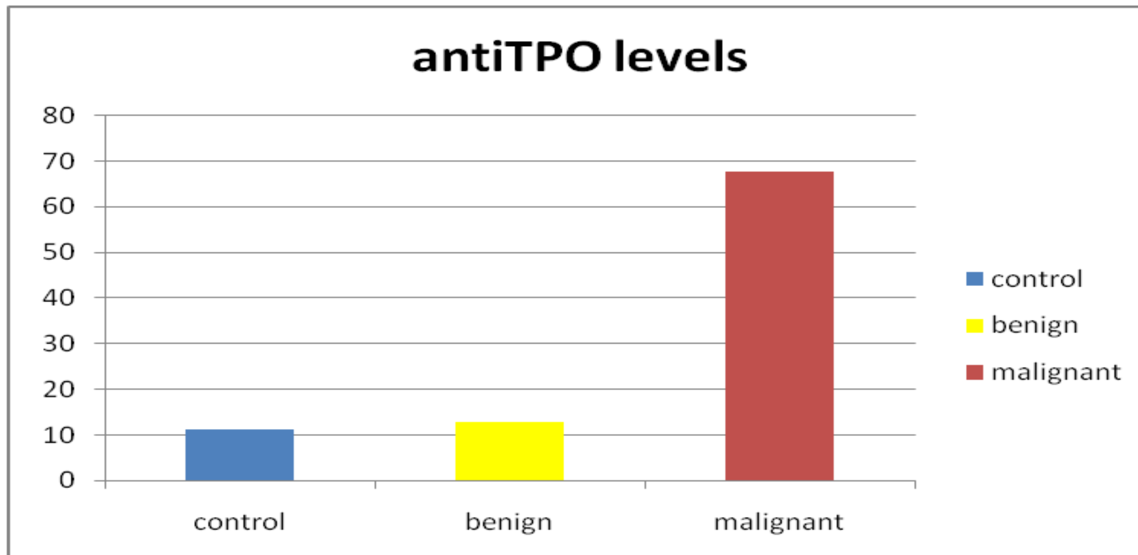


Table 6: Shows frequency of cancer patients by their staging and their respective anti-TPO values.

Stage of Breast cancer	Frequency N =30	Anti-TPO antibody level AU/mL		Oneway ANOVA
		Mean	SD	
II	9 (30%)	35.13	16.06	F=5.16 P=0.001***
III	20 (66.7%)	70.80	93.37	
IV	1 (3.3%)	301.00	.	

* P<0.05 significant **P<0.01 highly significant ***P<0.001 very high significant

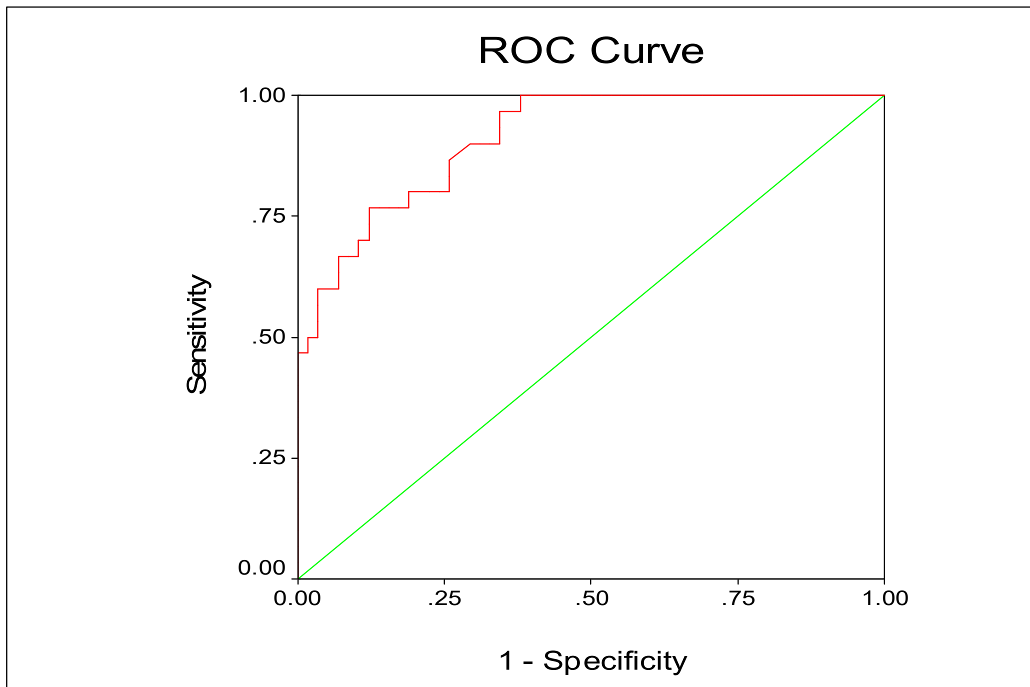
Table 7: Shows the Odds ratio calculation with anti-TPO cut-off value of 20.350AU/mL

	Breast cancer	controls	total
antiTPO > 20.35AU/mL	23	6	29
antiTPO < 20. 35AU/mL	7	24	31
Total	30	30	60

Odds ratio = 13.14

95%confidence interval of 3.8 to 45.0

Figure 14: Receiver operator characteristic curve of anti-TPO values



17.050	.800	.241
17.900	.800	.224
18.400	.800	.190
19.400	.767	.190
20.350	**.767**	**.172**
20.750	.767	.155
21.650	.767	.138
22.250	.767	.121
22.650	.733	.121
23.350	.700	.121

The test result variable(s): TPO ab has at least one tie between the positive actual state group and the negative actual state group.

a The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

TNM Stage Groupings

Stage 0	Tis	N0	M0
Stage I	T1(T1 mic)	N0	M0
Stage IIA	T0	N1	M0
	T1(T1 mic)	N1	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1(T1 mic)	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

Relative Risk Estimates for the Gail Model	
Variable	Relative Risk
Age at menarche (years)	
>14	1.00
12–13	1.10
<12	1.21
Number of biopsies/history of benign breast disease, age <50 y	
0	1.00
1	1.70
2	2.88
Number of biopsies/history of benign breast disease, age >50 y	
0	1.02
1	1.27
2	1.62
Age at first live birth (years)	
<20 years	
Number of first-degree relatives with history of breast cancer	
0	1.00
1	2.61
2	6.80
20–24 years	
Number of first-degree relatives with history of breast cancer	
0	1.24
1	2.68
2	5.78
25–29 years	
Number of first-degree relatives with history of breast cancer	
0	1.55
1	2.76
2	4.91
≥30 years	
Number of first-degree relatives with history of breast cancer	
0	1.93
1	2.83
2	4.17