STUDY OF THYROID AUTOANTIBODIES IN THYROTOXICOSIS PATIENTS BEFORE AND DURING MEDICAL TREATMENT USING THE INDIRECT IMMUNOFLUORESCENCE TECHNIQUE

THESIS

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Presented by

Ghada Mohammad Sadek Sabbour

Under the Supervision of

Prof. Dr. Aida Abdel-Azim
Professor of Clinical Pathology

Dr. Laila El-Shawarby
Assistant Professor of Clinical Pathology

Dr. Soheir Gamal El-Din
Assistant Professor of Internal Medicine

FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY
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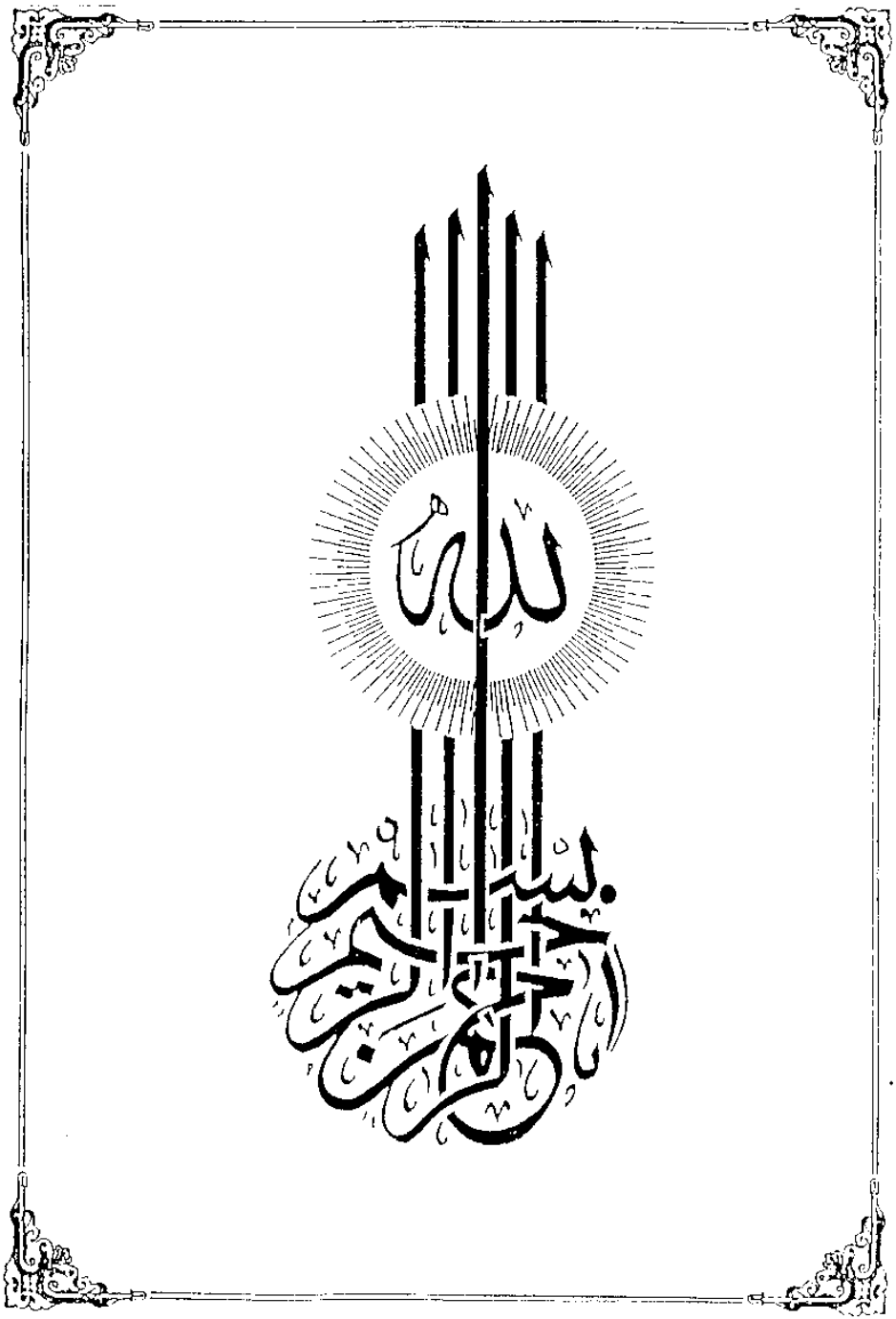

ABBREVIATIONS

AITD	autoimmune thyroid disease
DIT	di-iodotyrosine
X	Ci Squared
FITC	fluorescein isothiocynate
IL	interleukin
K cells	killer cells
LMIF	lymphocyte migration inhibition factor
M-Ab	microsomal antibody
M-Ag	microsomal antigen
MIF	migration inhibition factor
MIT	monoiodothyrosine
NK cells	natural killer cells
P	probability
CA_2	second colloid antigen
Tg	thyroblobulin
Tg-Ab	thyroglobulin antibody
TPO	thyroid peroxidase
TSAb	thyroid stimulating antibody
TSH	thyroid stimulating hormone (thyrotropin)
TRAb	thyrotropin receptor antibody
TRF	thyrotropin-releasing factor
T_4	thyroxine
T.G.N.	toxic nodular goitre
T_3	triiodothyronine

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INTRODUCTION

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AIM OF THE WORK

Background

Thyroid antibodies are frequently found in sera of patients with Graves' disease. Studies have shown that drug therapy is followed by a progressive reduction of TSH displacing immunoglobulin. However, authors have reported conflicting results regarding thyroglobulin and microsomal antibodies during antithyroid drug therapy. Some of this conflict might be ascribed to the method used in the laboratory detection.

Aim of the Work

Comparison of two methods of detection of two of the thyroid antibodies; microsomal and thyroglobulin, in hyperthyroid patients, before and after drug control of hyperthyroidism.

The more commonly used method, passive haemagglutination was compared with the indirect immunofluorescence method in patients with hyperthyroidism before and after treatment; the same thyroid antibodies were tested for in the sera of normal controls.

REVIEW OF LITERATURE

REVIEW OF LITERAUTRE

Embryology

The thyroid gland is identifiable in embryos of about 20 somites, as a median thickening of endoderm in the floor of the pharynx between the first and the second pharyngeal pouches and immediately dorsal to the aortic sac. (Davis 1923). The area is later evaginated to form a median diverticulum which appears in the later half of the fourth week in the furrow immediately caudal to tuberculum impar. It grows caudally as a tubular duct which bifurcates and subseqently divides into a series of double cellular plates from which the isthmus and the lateral lobes of the thyroid gland are developed. The primary thyroid follicles differentiate by reorganization and proliferation of the cells of these plates. Secondary follicles subsequently arise by budding and subdivision (Norris 1916).

The connection of the median diverticulum with the pharynx is termed the thyroglossal duct. The foramen caecum marks the site of its connection with the epithelial floor of the mouth. The distal part of the duct usually differentiates to a variable extent to form the pyramidal lobe of the thyroid (Boyd 1964).

The parafollicular C-cells that secrete calcitonin arise from mesodermal contribution to endodermal thyroid premordium (Gray 1973).

At the end of the 3rd month of foetal development, follicles containing colloid become visible and it is probable that the gland begins to release thyroid hormone at this time (Harrison 1981).

Anatomy of the thyroid gland:

The thyroid gland is a brownish-red, highly vascular organ, situated anteriorly in the lower part of the neck at the level of the fifth, sixth and seventh cervical vertebrae and the first thoracic vertebra. It consists of right and left lobes connected across the median plane by a narrow region called the isthmus. It weighs about 20-25 gm. The pyramidal lobe, which is often present, is a conical process of the thyroid tissue which arises from the upper border of the isthmus.

Histology and Structure of the Thyroid Gland:

The thyroid gland is invested by a thin capsule of connective tissue and it is divided into masses of irregular form and size by extensions of this connective tissue.

The gland is made up of two types of secretory cells, firstly, those of thyroid follicles (follicular cells) secreting the hormones tri-iodothyronine and thyroxine, and secondly, in smaller numbers, the parafollicular or C cells which secrete thyrocalcitonin. The follicles are closed hollow, spheroidal groups of epithelial cells of varying sizes, 0.02-0.9 mm across, and just visible to the naked eye on the surface of the gland as minute lobules. They contain a viscid, semi-fluid material (colloid), and are separated from each other by a highly vascular connective tissue. The colloid stains

pink readily with eosin. The follicles are arranged in groups or gland units. Each follicle is lined with a single layer of cubical epithelium. A basement membrane can be seen only with the electron microscope.

The shape of the follicular cells varies with the state of activity of the gland, being flattened if the follicles are filled with colloid material and the gland is resting, becoming columnar when the gland is active and the follicles contain little colloid. The epithelial cells have a variable number of microvilli at their free surfaces and show typical junctional complexes at their apices. Numerous mitochondria, abundant granular endoplasmic reticulum, a relatively small Golgi apparatus and numerous vesicles and granules are present in the cytoplasm. Some vesicles are believed to contain colloid, others resemble lysosomes and are believed to contain various enzymes, including peroxidases. There is a reduction in the quantity of granular endoplasmic reticulum after hypophysectomy, whilst administration of thyroid stimulating hormone leads to an increase in the number of colloid droplets in the apical part of the cell, and in the number and size of the microvilli. The epithelial cells lining the follicles selectively absorb iodide ions from the blood in the rich capillary plexuses surrounding the follicles. By the use of the radioactive isotope of iodine it has been shown that ingested iodine is rapidly taken up by the thyroid and is concentrated to a level up to fifty times that of the plasma content. Autoradiographic studies on histological sections of the thyroid of the thyroid glands showed that amino acids including

tyrosine are used to synthesize large protein molecules in the rough endoplasmic reticulum of the basal and perinuclear cytoplasm. To these molecules, carbohydrate groups are added in the cistence of the endoplasmic reticulum and in the saccules of the Golgi apparatus, so forming a complicated molecule of thyroglobulin - a glycoprotein. Membrane-bound vesicles transport this glycoprotein to the apical surface of the cell, where it is released into the colloid. Iodide added to the tyrosyl radicals of this molecule at or near the cell surface adjacent to the colloid, the latter being formed of the resulting iodothyroglobulin. As the colloid is continually being added to, so it is continually being taken up again by the follicular cell surface in a manner similar to phagocytosis; and, by the action of lysosomal enzymes, the iodothyroglobulin is broken down intracellularly to release the hormones tri-iodothyronine and tetra-iodothyronine which pass through the base of the cell into the circulation. Other products of degradation are re-used by the follicular cell to synthesize more iodothyroglobulin.

Physiology

The function of the thyroid gland is to synthesize, store and secrete the hormones thyroxine (T4) and triiodothyronine (T3). Iodide is absorbed by the gastrointestinal tract and actively trapped by the acinar cells of the thyroid gland. It is then oxidized and combined with tyrosine in thyroglobulin to form monoiodotyrosine (MIT) and di-iodotyrosine (DIT). These are coupled

to form the active hormones T4 and T3, which initially are stored in the colloid of the gland. Following hydrolysis of the thyroglobulin, T4 and T3 are secreted into the plasma, becoming almost instantaneously bound to plasma proteins. T3 is also produced by extrathyroidal conversion of T4 to T3.

The function of the thyroid gland is regulated by a feedback mechanism which involves the hypothalamus and the pituitary. Thyrotropin-releasing factor (TRF), a tripeptide amide, is formed in the hypothalamus and stimulates the release of thyrotropin (TSH), a glycoprotein from the pituitary, and this stimulates thyroid hormone production.

IMMUNOPATHOLOGICAL ASPECTS OF GRAVES' DISEASE

Hyperthyroidism

Hyperthyroidism (thyrotoxicosis) is not a single disease entity, rather, it is a syndrome with diverse aetiologies, each with its own pathogenesis, pathophysiology, optimum therapy. In terms of frequency, diffuse toxic goitre (Graves' Disease) is the most prevalent form of hyperthyroidism in all age groups, followed by toxic nodular goitre, and toxic adenoma ("hot" nodule).

Thyrotoxicosis is one of the most common endocrine disorders. Its highest incidence is in women between the ages of 20 and 40 years. (Marcus et al, 1976). When hyperthyroidism is associated by ophthalmopathy and diffuse enlargement of the thyroid gland, the condition is called Graves' disease. Less commonly, Graves' disease may be accompanied by skin lesions (pretibial myxoedema) or changes in the fingers (acropathy). (Scott, 1975). However, this term (Graves' disease) is commonly used to mean all forms of hyperthyroidism. Instead of a diffuse goitre, there may be toxic nodular goitre, or all the metabolic features of thyrotoxicosis may occasionally be present without visible or palpable thyroid enlargement. The latter form is quite common in the elderly patient, who may even lack some of the hypermetabolic signs (apathetic Graves' disease) but may present with a refractory cardiac illness. Also, a poorly understood syndrome of marked eye signs, often without hypermetabolism, may precede, accompany, or follow treatment of thyrotoxicosis, and has been termed

hyperexophthalmic Graves' disease, exophthalmic ophthalmoplegia, and malignant (progressive) exophthalmos infiltrative ophthalmopathy). A rare cause of hyperthyroidism is stuma ovarii or hydatiform mole, although asymptomatic elevation of T4 may be seen in chronic tumours, presumably due to production of ectopic TSH. (Marcus et al, 1976).

Aetiology

A genetic or consitutional predisposition is strongly suggested by a familial incidence, particularly in the mother or the sisters of the proband, of Graves' disease. Since Graves' disease is now recognized as the probable consequence of thyroid antibodies, it is likely that the condition is related to an inherited instability of immunological tolerance. Although difficult to obtain convincing scientific support, there is a clinical suspicion that in predisposed subjects Graves' disease may develop as the result of an emotional disturbance. (Scott, 1975).

Pathology of Graves' Disease

The thyroid gland is uniformly enlarged with a marked increase in vascularity. Colloid is scanty and the acini vary in size. The epithelial cells are cubical or columnar and project into the vesicles as plicated folds. Infiltration of the gland by foci of lymphocytes, plasma cells and areas of fibrosis indicating focal