

Serum Enzyme Activities in Human Thyroid Diseases

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ABSTRACT:

BACKGROUND:

The study enrolled a total of 76 patients with thyroid gland diseases, hyperthyroidism and hypothyroidism. The activities of the enzyme amylase, gamma glutamyltransferase and alkaline phosphatase were evaluated in these diseases state and were compared with normal healthy thyroid gland. The specimens were obtained from different hospitals in Baghdad and from routine clinical works.

OBJECTIVE:

Diagnosis of patients with thyroid gland diseases hyper and hypothyroidism by evaluation of enzymes activity of amylase, GGT and ALP.

METHOD:

Two groups of patients were used: The first consists of 38 patients with hyperthyroidism, the second consists of also 38 are patients with hypothyroidism. Twenty five of each group was investigated for amylase, GGT, ALP and were compared with third group of 25 normal healthy thyroid gland. The remaining 13 out of 38 patients of each group were tested for enzymes, amylase, GGT, ALP, GOT, GPT and evaluate the hormones, they were compared with third group of 10 normal healthy thyroid gland.

RESULTS:

There was a highly significant increase and decrease in the activity of amylase, GGT and ALP in hyper and hypothyroidism patients ($P < 0.001$) when compared with third group normal.

CONCLUSION:

Diagnosis of patients with thyroid gland diseases can be confirmed by enzymes investigation in blood serum and hormonal assay.

KEYWORDS: hyperthyroidism, hypothyroidism, amylase, gamma glutamyl transferase, alkaline phosphatase(alp) and thyroid gland

INTRODUCTION:

The thyroid gland is one of the largest endocrine glands in the body. In healthy people it produces thyroid hormones (TH) which are important at a cellular level, affecting the growth development and rate of function of many other systems in the body⁽¹⁾. It has functions as a stimulus to metabolism and is critical to normal function of the cell. These hormones also have direct effect on most organs including heart, beats faster and harder under influences of thyroid hormone. The thyroid hormone control how quickly the body burns energy, makes proteins, and how sensitive the body should be to other hormones. As well many functions of the liver, this is the primary organ responsible for the maintenance of the metabolic process^(2,3). Hyperthyroidism is the

medical term to describe the signs and symptom associated for over active tissue within the thyroid gland, resulting in over production and thus an excess of circulating free thyroid hormone (triiodothyronine T3, thyroxine T4) or both^(3,4). Functional thyroid tissue producing an excess of thyroid hormone occurs in a number of clinical condition Thyrotoxicosis is a common clinical syndrome consequent to excess circulating thyroid hormone, can be caused by a number of different disorders such as Graves's disease, toxic multinodular goiter or toxic thyroid adenoma, thyroiditis and iodide which induced hyperthyroidism⁽⁵⁾. Hypothyroidism results from undersecretion of thyroid hormone from the thyroid gland. The most common cause of primary hypothyroidism is chronic auto-immune thyroiditis (Hashimoto's disease)⁽⁶⁾. The other

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causes are surgical removal of thyroid gland, thyroid gland ablation with radioactive iodine, external irradiation, a biosynthetic defect in iodine organification, replacement of the thyroid gland by tumor. Secondary causes of hypothyroidism include pituitary hypothalamic disease^(7,8). Human α -amylase (1,4- α -glucan glucanohydrolase, EC 3.2.1.1) found in all types of organs and tissue, it is highest concentration in salivary glands and pancreas⁽⁹⁾. Blood serum amylase may be measured for purpose of medical diagnosis. Higher concentration may reflect one of several medical conditions including acute inflammation of pancreas, macroamylasemia, peptic ulcer and mumps^(10,11). Normally, only low level of amylase is found in the blood or urine, but when the pancreas or salivary glands become damaged or blocked amylase is usually released into the blood or urine⁽¹⁰⁾. Amylase has also been detected in the normal thyroid gland and in thyroid adenomas. Salivary- type amylase is generally found, but there have been reports claiming that the other type is also detectable⁽¹²⁾. However, it is not clear whether the enzyme detected by biochemical studies was actually produced in the thyroid gland or just accumulated there⁽¹²⁾ and the amylase isoenzyme were expressed in thyroid cancer⁽¹³⁾.

Alkaline phosphatase, ALP [EC 3.1.3.1] is found in all tissue of the body, serum, cell membrane, liver, bile duct, placenta, chromosome and intestinal epithelium⁽¹⁴⁾. Gamma glutamyltransferase, GGT [EC 2.3.2.3], is the enzyme responsible for extracellular catabolism of glutathione (GSH-Gamma Glutamyl-Cysteiny- Glycine), the main thiol intracellular antioxidant agent⁽¹⁵⁾ and the larger function of enzyme is located in the cell membrane and may act to transport amino acid and peptide into the cell across the cell membrane in the form of gamma glutamyl peptidase. The enzyme is present on the surface of most cells, in serum and also in various body compartment of human body tissue⁽¹⁵⁾. The aim of the present study is to investigate the activity of serum amylase, alkaline phosphatase and gamma glutamyltransferase in patients with thyroid diseases, hyperthyroidism and hypothyroidism. Moreover serum transaminase activities (GOT and GPT) have been evaluated in group of patients in addition to amylase, GGT and ALP. All patients were investigated for thyroid hormones (T3, T4) and TSH which is a marker for thyroid diseases. There is no reference about information for investigation of serum enzyme activities in healthy and patients

PATIENTS AND METHOD:

The study enrolled a total of 76 patients with thyroid gland diseases. The first group of 38 patients comprised of 16 male of age range 25-65 years and 22 females of age range 21-63 years and were suffering from hyperthyroidism. The second group G2 of 38 patients comprised of 14 males of age range 24-66 years and 24 females of age range 23-62 years and were suffering from hypothyroidism. Twenty five patients of each were investigated by measuring the activities of three enzymes namely amylase, GGT and ALP. These were compared with a third group of 25 normal healthy individuals which comprised of 12 males with age range 23-62 years and 13 females with age range 20-64 years. Thirteen patients of each group were investigated for the activities of 5 enzymes amylase, Gamma glutamyl transferase (GGT), Alkaline phosphatase (ALP), Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT) and for investigation of thyroid hormones Triiodothyronin (T3), Thyroxine (T4) and thyroid stimulating hormone (TSH). They were compared with a third group of 10 normal healthy individual. All patients with thyroid gland diseases, hyperthyroidism and hypothyroidism were investigated for thyroid hormones which are a marker for thyroid diseases. So the diagnosis of hyperthyroidism and hypothyroidism can be confirmed by routine laboratory test by measuring the amount of thyroid hormones Triiodothyronine (T3), Thyroxin (T4) and thyroid stimulating hormone (TSH) in blood serum. The samples of all groups were collected from routine clinical work from different hospitals and laboratories in Baghdad. Collection of whole blood samples was done by vein puncture from patients with hyperthyroidism and hypothyroidism. Five milliliter of venous blood was obtained from each subject. The samples were centrifuged at 10000 rpm for 10 minute and sera were separated and put in plain tubes and refrigerated until time of analysis after 45-72 hrs.

Methods of assay Assay of Amylase

The activity of amylase was determined by kit method, quantitative determination of amylase activity in human serum by amylase CNPG3 using kit (Biolabo SA, Reagents, 02160, mazy, France). The measurement was done by using spectrophotometer at 405 nm⁽¹⁶⁾.

Assay of Alkaline phosphatase (ALP)

ALP was estimated by kit colorimetric determination of alkaline phosphatase (manufactured by Biomerieux 61511, France)⁽¹⁷⁾

ENZYME ACTIVITIES THYROID DISEASES

Assay of Gamma glutamyl .transferase (GGT)

Serum GGT activity was determined by kinetic method using special kit (Biolabo Reagents-France). The assay method is based on transport of gamma glutamyl group from gamma glutamyl P-nitroaniline to glycylglycine by GGT enzyme leaving the yellow product of P-nitroaniline. The analytical method was performed at 30C by measuring the absorbance change with a spectrophotometer. The rate of formation of P-nitroaniline is directly proportional to GGT activity in the specimens and it is measured at 405 nm⁽¹⁸⁾.

Assay of GOT and GPT

They were measured by kit method (Reitman-France colorimetric method, linear chemical, S.L, Spain)⁽¹⁹⁾.

Assay of Hormones of ThyroidI.

Measurement of Total Triiodothyronine TT3: The quantitative determination of TT3 concentration in human serum or plasma by a microplate enzyme immunoassay. Serum TT3 was determined by competitive radioimmunoassay technique using RIA kits supplied by monobind Inc. AccuBind ELISA microwells USA⁽²⁰⁾.

II. Measurement of Total Thyroxine TT4: The quantitative determination of TT4 concentration in human serum or plasma by microplate enzyme immunoassay. Serum TT4 was determine by competitive radioimmunoassay technique using RIA kits supplied by, Monobind Inc Accubind ELISA Microewlls USA⁽²⁰⁾.

III. Measurement of Thyroid Stimulating. Hormone (TSH): The quantitative determination of TSH concentration in human serum or plasma by microplate immunoenzymetric assay. Serum TSH was determined by an enzymatic immunoassay technique using EIA kits supplied by, Monobind Inc Accubind ELISA Microewlls USA⁽²¹⁾.

Statistical Analysis

Statistical Analysis included mean and standard deviation (M±SD), coefficient of variation (CV), unpaired students t test and P<0.001 was considered a statistical significant difference⁽²²⁾.

RESULTS:

Table (1) shows serum enzyme activities of Amylase, GGT and ALP in patients with hyperthyroidism and hypothyroidism compared with the control normal individuals. A significant increase in the enzyme activities of hyperthyroidism with the normal healthy group (P<0.001). Table (2) demonstrates the mean values of serum enzyme activities of Amylase, GGT, ALP, GOT and GPT in patients with thyroid diseases. There was significant increase in Amylase, GGT, ALP but decrease in GOT and GPT in comparison with normal. Table (3) represented mean value of serum thyroid hormones in patients with hyperthyroidism and hypothyroidism compared to the normal healthy. In hyperthyroidism there was significant increase in T3, T4 and TSH (P<0.001). In hypothyroidism a decrease in T3, T4 and increase in TSH (P<0.001).

Table 1: M±SD values of serum enzyme activities of Amylase, GGT&ALP in patients with thyroid gland diseases compared to the control.

Parameter for both sexes	Amylase U/L	GGT U/L	ALP U/L
Normal N=25	67.04±5.46 CV=8.14%	27.08±5.21 CV=19.24%	36.5±3.96 CV=10.96%
Hyperthyroidism N=25	117.24±6.46 CV=5.51% t*=29.68 P<0.001	138.2±5.67 CV=4.10% t*=72.155 P<0.001	158.44±5.26 CV=3.32% t*=92.85 P<0.001
Hypothyroidism N=25	41.44±4.24 CV=10.24% t*=18.52 P<0.001	18.44±4.0 CV=21.69% t*=5.44 P<0.001	21.32±3.76 CV=17.64% t*=4.65 P<0.001

t* = comparison between normal and patients of thyroid diseases.

ENZYME ACTIVITIES THYROID DISEASES

Table 2: M±SD serum values of different enzyme activities in patients with thyroid diseases compared to the control.

Parameter	Amylase U/L	GGT U/L	ALP U/L	GOT U/L	GPT U/L
Normal N=10	65.9±4.5 CV=6.82%	25.8±1.5 CV=5.81%	38.0±3.0 CV=7.89%	32.2±2.8 CV=8.69%	22.5±2.4 CV=10.66%
Hyperthyroidism N=13	121.3±5.2 CV=4.28% t*=26.82 P<0.001	145.2±4.8 CV=3.3% t*=75.5 P<0.001	161.0±4.8 CV=2.98% t*=70.89 P<0.001	25.7±1.92 CV=7.47% t*=6.612 P<0.001	13.1±2.1 CV=16% t*=10 P<0.001
Hypothyroidism N=13	38.5±2.9 CV=7.53% t**=17.74 P<0.001	16.0±2.1 CV=13.12% t**=12.48 P<0.001	19.2±2.1 CV=10.93% t**=17.7 P<0.001	24.0±2.0 CV=8.33% t**=8.206 P<0.001	18.0±2.9 CV=16.11% t**=3.96 P<0.001

t* = comparison of normal with Hyperthyroidism groups.

t** = comparison of normal with Hypothyroidism groups.

Table 3: Serum thyroid hormones in healthy and patients with diseases of thyroid gland.

Parameter	S.T3 (ng/ml)	S.T4 (mg/dl)	S.TSH MIU/ml
Normal N=10	1.43 ± 0.25 CV=17.48%	9.06 ± 0.48 CV=5.29%	2.8 ± 0.17 CV= 6.07%
Hyperthyroidism N=13	8.0 ± 0.85 CV=10.62% t*=23.56 P<0.001	18.95 ± 0.96 CV=5.06% t*=29.73 P<0.001	0.12 ± 0.025 CV= 20.83% t*= 56.4 P<0.001
Hypothyroidism N=13	0.75 ± 0.06 CV=8% t**=9.5 P<0.001	3.06 ± 0.35 CV= 11.43% t**=34.74 P<0.001	12.0 ± 0.7 CV= 5.83% t**=40.45 P<0.001

t* = comparison of normal with Hyperthyroidism groups.

t** = comparison of normal with Hypothyroidism groups.

DISCUSSION:

Thyroid diseases are usually present as a spectrum of clinical and metabolic features of varying severity. Although primary diseases of the thyroid gland are the most common, secondary disorder are due to hypothalamic pituitary insufficiency can also give rise dysfunctional state⁽²³⁾. Many other factors, both exogenous and endogenous may affect the thyroid function⁽²⁴⁾. These include the pathway of the thyroid hormone, biosynthesis, secretion, transport in the circulation and metabolism which offer numerous targets for drug interaction⁽²⁵⁾. Results in tables (1) and (2) represent the mean value of serum enzyme activities of amylase, GGT and ALP with two

pathological state hyperthyroidism and hypothyroidism, there was significantly a remarkable increase in the activities of the three enzymes (P<0.001) with hyperthyroidism, while there was a significantly reduced activities with hypothyroidism (P<0.001) in comparison with normal healthy thyroid glands. The possible explanation could be that goiterous tissue produce high enzymatic activity than in healthy individuals and that may be released in blood stream and cause rising of enzymes activities in serum during presence of goiter and damage of thyroid gland tissue. Patients with thyroid diseases may present with a thyroid swelling or

goiter and characterized by presence of thyroid stimulating antibodies in the blood (2). Table (2) illustrates the serum enzyme transaminase activities GOT and GPT were reduced in hyperthyroidism and hypothyroidism ($P < 0.001$). Goiter or enlargement of the thyroid can occur in patient with hyperthyroidism. Both hyperthyroidism and hypothyroidism are commonly the result of autoimmune disease (26) and caused by a variety of thyroid disorder (25). Insufficient of the antioxidant defense may lead to, as has been reported in various thyroid disease oxygen free radical mediated degenerative processes (2).

The causes of hyperthyroidism due to inflammation of thyroid gland called thyroiditis, can lead to the release of excess amount of thyroid hormone that are normally stored in the gland and can be triggered by different disorders, as Hashimoto's and sub acute thyroiditis (1,3).

Results in table (3) show significantly higher concentration of thyroxine and triiodothyronine ($P < 0.001$) and low thyrotropic stimulating hormone ($P < 0.001$) in hyperthyroidism. A low TSH indicate that the pituitary gland is being inhibited by increased level of T3 and T4 in the blood and is therefore a reliable marker of hyperthyroidism so the diagnosis of hyperthyroidism is confirmed by blood test that show a decreased thyroid stimulating hormone TSH level and elevated T3 and T4. High level of TSH indicate that the thyroid is not producing sufficient levels of thyroid hormone as T4 and a smaller amount of T3. A major cause of hypothyroidism is iodine deficiency affected individuals usually have a goiter as a result of increased secretion of TSH (6). The pain in patient with hyperthyroidism and caused goiter could be due to hemorrhagic into a cyst or thyroiditis or due to rapid increase in size of goiter (26). In excess of thyroid hormone, it over stimulate metabolism and exacerbates the effect of the sympathetic nervous system causing speeding up of various body system and symptoms and increase the enzyme activities and releasing into blood stream in patients.

Amylase has also been detected in the normal thyroid gland and in thyroid adenoma (12), amylase isoenzyme expressed in thyroid cancer. It is not clear whether the enzyme detected by biochemical studies was actually produced in the thyroid gland (12). In excess of thyroid hormone it over stimulates causing speeding up of various body

system including fast heart beat and symptoms of nervous system, tremor and anxiety symptoms, digestive system hypermotility and weight loss (3).

CONCLUSION:

diagnosis of patients with hyperthyroidism and hypothyroidism can be confirmed by enzymes investigation in blood serum the enzyme amylase, GGT and ALP. This need further research since there is little information on this subject. High blood level of thyroid hormone can occur due to inflammation of thyroid gland which leads to secretion of excess thyroid hormone.

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ENZYME ACTIVITIES THYROID DISEASES

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