

Assessment of Serum Enzymes Level in Patients with Thyroid Alteration Attending Manipal Teaching Hospital, Pokhara

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Abstract

Thyroid hormones exert their effect on all tissue and modulate the rate of metabolic activity. Alterations in thyroid function can affect the various organ system of body and perturb measures like AST, ALT, GGT, ALP, CPK and LDH. Both hypothyroidism and hyperthyroidism have potentially fatal systemic manifestations. The main aim of study was to determine the relationship between thyroid alteration and serum enzymes level. The study included 110 subjects visiting Endocrinology Unit of Department of Biochemistry, MTH, Pokhara, with the request of TFT. Thyroid profile and serum enzymes were analyzed using standard kits. Each thirty hyperthyroid and hypothyroid cases show slight elevation of AST, ALT and GGT. However, the values were higher in hyperthyroid subjects. ALP, CPK and LDH were markedly elevated both in hyperthyroidism and hypothyroidism as compared to controls. In conclusion, conducted study demonstrated that there is a positive association between increased serum AST, ALT, GGT, CPK, ALP and LDH in hyperthyroidism and hypothyroidism.

Keywords: thyroid disorders, TSH, FT3, FT4, AST

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INTRODUCTION

The thyroid is a small butterfly shaped endocrine gland, located in the lower part of the neck, in front of the windpipe which secretes thyroid hormones. The main hormones released by the thyroid are T3 and T4; deliver energy to cells of the body [1, 2]. Thyroid hormone synthesis and secretion is regulated by a negative feedback system that involves the hypothalamus, pituitary, and the thyroid gland [2, 4].

Thyroid hormones control the metabolism—the process by which oxygen and calories are converted to energy for use by the cells and organs. When the thyroid works normally, it produces and secretes the amount of T4 and T3 necessary to keep various body functions moving at their proper pace [2].

The thyroid frequently is a common target of disease or dysfunction [2]. Thyroid disorders are commonly separated into two major categories, hyperthyroidism (caused by an

overactive thyroid gland) and hypothyroidism (due to a poorly functioning thyroid gland), depending on whether serum thyroid hormone levels (T4 and T3) are increased or decreased, respectively. Both hypothyroidism and hyperthyroidism have potentially fatal systemic manifestations [15].

The symptoms of hyperthyroidism are weight loss, rapid or irregular heartbeat, anxiety, irritability, trouble sleeping, trembling in the hands and fingers, increased sweating, increased sensitivity to heat, muscle weakness, etc. The symptoms of hypothyroidism are weight gain, increased sensitivity to cold, muscle weakness, joint or muscle pain, depression, fatigue, pale dry skin, a puffy face, a hoarse voice, etc. [13].

Subclinical hypothyroidism occurs when TSH levels are elevated but T3 and T4 levels are normal [15]. Subclinical hyperthyroidism is characterized by a low or undetectable concentration of serum TSH with FT3 and FT4 levels within laboratory reference ranges

[14]. Euthyroidism refers to the state of normal functioning of thyroid gland [3]. As thyroid hormones are essential for normal organ growth, development, function and regulate the basal metabolic rate of all cells, its alteration can affects the entire metabolism [3]. Most affected organs include liver and heart. So, it alters the liver enzymes like ALP, AST, ALT, GGT and cardiac enzymes like CPK, LDH and AST [1, 5, 6].

Therefore, accurate diagnosis of thyroid abnormalities is critical for clinicians as well as medical laboratories worldwide for appropriate management. Laboratory measurements of T3, T4 and TSH are crucial in helping clinicians to diagnose thyroid abnormalities [15].

According to the World Health Organization, iodine deficiency is the world's most prevalent – yet easily preventable – cause of brain damage. It affects more than 740 million people worldwide – 13% of the world's population [2]. As many as an additional 30% of the population worldwide is at risk of iodine deficiency-related problems. Besides iodine deficiency, there are number of risk factors for thyroid disease. These include genetics and heredity, personal or family, history of endocrine or autoimmune disease, infection, exposure to goitrogenic foods, cigarette smoking, pregnancy, certain drugs, particular chemical exposures, radiation exposure, and many other factors. It is estimated that more than 200 million people at minimum worldwide have thyroid disease [2].

From the study done in Nepal, it was found that the prevalence of thyroid dysfunction was 25%. Females have more thyroid dysfunction than males. Hypothyroidism (8%) and subclinical hypothyroidism (8%) have higher prevalence compared to subclinical hyperthyroidism (6%) and hyperthyroidism (3%). Higher prevalence of thyroid dysfunction was observed in subjects with age above 30 years [7]. Based on the comparison with contemporary studies it is found that thyrotoxicosis has a significant effect on liver that is reflected in increased level of liver specific enzymes i.e., AST, ALT and ALP [1, 8, 9]. In particular, there seems a significant positive relationship between serum TSH, ALT and GGT activities throughout the

normal and high TSH ranges, and a similar inverse relationship between FT4 and serum liver enzyme activity concentrations [3, 6]. In another study, serum CPK level shows an inverse relation with serum T3, T4 levels [5, 10–12].

Thyroid dysfunction is one of the major public health problems in Nepal. Laboratory tests facilitate early diagnosis before clinical features are obvious, increased sensitivity carries the price of decreased diagnostic specificity. Laboratory tests coupled with supportive clinical findings are frequently used to diagnose thyroid dysfunction [15].

MATERIALS AND METHODS

METHODS

The study was conducted in the Department of Biochemistry laboratory of Manipal Teaching hospital, Phulbari, Pokhara from July to December 2011. Fifty apparently healthy subjects were selected randomly from healthy volunteers and students from School of health and Allied sciences and each thirty hypothyroid and hyperthyroid cases were selected from the patients visiting MTH. After selection of the participants and signing of the consent form, they were interviewed by the principal investigators by asking the questions included in the questionnaire. Health screening of the participants was done using survey questionnaires.

Study Type

An analytical cross-sectional study was conducted for 180 days from July to December 2011.

Study Design

The study population included native resident of Pokhara Valley and surrounding areas and those who have migrated from other parts of Nepal. Participants included in this study were of following religions: Hindu, Buddhist and Christian; and from different ethnic groups. A total of 110 individuals participated in the present study conducted from July to December 2011. Participants were from Pokhara valley and from following surrounding areas: Lekhnath, Kaskikot and Bharatpokhari VDC. The age range of participants was 20–60 yrs. The objective of this project was to determine the relationship

between serum enzymes level and thyroid among the apparently healthy and diseased people of western development region. 50 blood samples were collected from staff of MTH and students from School of health and Allied sciences as control. Abnormal 60 samples (30 for hyperthyroidism and 30 for hypothyroidism) were collected from the patients visiting MTH. Individuals with an active infection or a recent infection including liver disease, bone and muscle disease, cardiac, pancreatic, hepatobiliary, diabetes, hypertension, malignancy, oral contraceptive pills (OCP), pregnancy, alcoholics, and drug abusers were excluded. All the blood samples were labeled with code number and various other information including age, sex, location etc. were also recorded. The samples were stored in deep freezer until processing.

Inclusion Criteria

The individual within 20–60 age groups and without any chronic condition other than thyroid are included in this study.

Exclusion Criteria

Individuals with an active infection or a recent infection including liver disease, bone and muscle disease, cardiac, pancreatic, hepatobiliary, diabetes, hypertension, malignancy, oral contraceptive pills (OCP), pregnancy, alcoholics, and drug abusers were excluded.

Sample Collection and Laboratory Analysis

5 ml of venous blood was collected from the selected patients in a plain test tube in Sample Collection Department of MTH, Pokhara. Blood collected in plain tube was allowed to clot at room temperature and then centrifuged at 1,500 rpm for 5 min. Serum so obtained was used to determine the thyroid hormones (TSH, FT3, FT4) and serum enzymes (ALT, AST, CPK, ALP, GGT, LDH). The final report was approved by a senior faculty member of the Biochemistry Department.

Measurement of TSH

It was determined by ELISA method.

Test Principle

The TSH ELISA makes use of highly specific monoclonal anti-TSH antibody coated on the surface of the microtiter wells. In the first

incubation step, specimens, calibrators or controls and enzyme conjugates are mixed to form the sandwich complex which is bound to the surface of the wells by the interaction with immobilized antibody. At the end of the incubation, excess enzyme conjugate is washed out. Substrate reagent added and resulting color, which turns into yellow after stopping the reaction with stop solution, is measured photometrically. The intensity of color is directly proportional to the TSH concentration in the sample.

Measurement of FT3

It was determined by ELISA method.

Test Principle

ELISA is based on the principle of competitive binding between FT3 in a test specimen and T3-peroxidase conjugate for a limited number of binding sites on the anti-T3 (sheep) coated well. Thus, the amount of T3- peroxidase conjugate bound to the well is inversely proportional to the concentration of FT3 in the specimen. After incubation of specimen and T3-peroxidase conjugate unbound enzyme conjugate is removed in the equilibrium state by washing. TMB/substrate solution is added and a blue color develops. The intensity of this color, which changes to yellow after stopping reaction, is inversely proportional to the amount of FT3 in the specimen.

Measurement of FT4

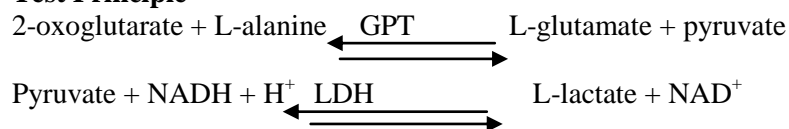
It was determined by ELISA method.

Test Principle

ELISA is based on the principle of competitive binding between FT4 in a test specimen and T4-peroxidase conjugate for a limited number of binding sites on the anti-T4 (sheep) coated well. Thus, the amount of T4- peroxidase conjugate bound to the well is inversely proportional to the concentration of FT4 in the specimen. After incubation of specimen and T4-peroxidase conjugate unbound enzyme conjugate is removed in the equilibrium state by washing. TMB/substrate solution is added and a blue color develops. The intensity of this color, which changes to yellow after stopping reaction, is inversely proportional to the amount of FT4 in the specimen.

Measurement of ALT

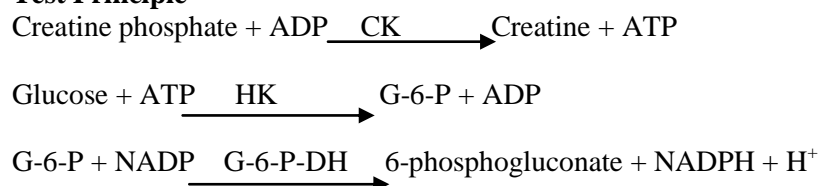
ALT was determined by Kinetic method.

Test Principle**Measurement of AST**

AST was determined by Kinetic method.

Test Principle**Measurement of CPK-Nac**

CPK-Nac was determined by Kinetic method.

Test Principle**Measurement of ALP**

ALP was determined by Kinetic method.

Test Principle**Measurement of GGT**

GGT was determined by Kinetic method.

Test Principle**Measurement of LDH**

LDH was determined by Kinetic method.

Test Principle**Statistical Analysis**

All the statistical analysis were performed using SPSS version 17.0 and Microsoft excel 2007. The significant differences between two groups will be compared using Mann-Whitney U-test. Pearson's correlation coefficient will be determined between hypothyroidism,

hyperthyroidism and control. Data were presented as mean \pm SD. Probability values were calculated throughout, and $p < 0.05$ was considered statistically significant. The results were expressed in the forms of bar diagrams, and tables, etc.

RESULTS

Patients visiting Endocrinology Unit of Department of Biochemistry, MTH, Pokhara, with the request of TFT were recruited for the study. Six variables were measured for hypothyroidism, hyperthyroidism and controls, i.e., serum enzymes level ALT, AST, CPK, GGT, ALK and LDH. Thyroid profile FT3, FT4 and TSH were measured to categorize hypothyroidism, hyperthyroidism and controls. The data were analyzed to compare the mean values between hypothyroidism, hyperthyroidism and controls and to find out correlation between thyroid profile and serum enzymes in the hypothyroidism, hyperthyroidism and controls. Overall the cases and controls were in the age range of 20–60 years. About 110 patients were included. Out of 110 subjects, there were 38 (34.54%) males and 72 females (65.45%) with mean age 33.13 ± 8.45 and 35.50 ± 10.71 , respectively. In cases, and controls the normal range of the plasma ALT level is up to 40 U/L

in males and 35 U/L in females, AST level up to 40 U/L, CPK level from 24 to 195 U/L, GGT level from 9 to 50 U/L, ALK level is between 100 and 290 U/L, while LDH level is from 70 to 240 I/U in both genders. The mean levels of all the six enzymes were significantly greater in both hypothyroidism and hyperthyroidism than in controls ($p < 0.005$).

The normal range for thyroid profile was taken from the Department of Biochemistry, MTH. Among the 50 controls the mean FT3, FT4, and TSH were 2.60 ± 0.62 , 1.33 ± 0.27 , and 2.67 ± 1.07 , respectively. In hypothyroid cases, the mean FT3 (1.29 ± 0.56) and FT4 (0.62 ± 0.26) were lower in comparison to controls whereas, mean TSH (27.97 ± 10.21) was markedly high. In hyperthyroid cases the mean FT3 (5.55 ± 1.83), FT4 (2.78 ± 0.61) were higher compared to controls and hypothyroids. The mean TSH in hyperthyroid was 0.16 ± 0.07 .

Table 1: Thyroid Profile of the Hypothyroidism, Hyperthyroidism and Control.

Study group	Normal range	Control (n=50)	Hypothyroid (n=30)	Hyperthyroid (n=30)
FT3 (pg/ml) Mean \pm SD range	1.4-4.2	2.60 ± 0.62 (1.60-4.00)	1.29 ± 0.56 (0.39-3.30)	5.55 ± 1.83 (4.10-11.80)
FT4 (ng/ml) Mean \pm SD range	0.8-2.0	1.33 ± 0.27 (0.80-1.90)	0.62 ± 0.26 (0.10-1.50)	2.78 ± 0.61 (2.10-4.90)
TSH (IU/ml) Mean \pm SD range	0.4-6.2	2.67 ± 1.07 (0.70-5.00)	27.97 ± 10.21 (9.00-43.20)	0.16 ± 0.07 (0.10-0.30)

Table 2: Serum Enzymes Profile of the Hypothyroidism, Hyperthyroidism and Control.

Study Group	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	CPK (U/L)	LDH (I/U)	P value
Control (n=50) Mean \pm SD range	29.10 ± 4.26 (19.00-36.00)	24.88 ± 5.34 (15.00-35.00)	27.84 ± 6.24 (16.00-53.00)	178.84 ± 47.73 (100.00-290.00)	68.82 ± 34.34 (26.00-167.00)	153.28 ± 36.51 (85.00-223.00)	<0.005*
Hypothyroid (n=30) Mean \pm SD range	50.70 ± 8.48 (27.00-69.00)	46.93 ± 9.64 (32.00-81.00)	62.26 ± 29.55 (26.00-150.00)	314.00 ± 144.46 (95.00-600.00)	232.76 ± 102.03 (17.00-423.00)	541.06 ± 598.53 (184.00-3654.00)	<0.005*
Hyperthyroid (n=30) Mean \pm SD range	67.80 ± 19.39 (31.00-111.00)	64.33 ± 31.87 (27.00-202.00)	82.96 ± 33.15 (30.00-198.00)	918.43 ± 279.23 (512.00-1550.00)	218.76 ± 60.32 (32.00-350.00)	658.20 ± 458.45 (323.00-2852.00)	<0.005*

*Correlation is significant at the 0.005 level.

In control group, the mean values for AST, ALT, GGT, ALP, CPK and LDH were 29.10 ± 4.26 , 24.88 ± 5.34 , 27.84 ± 6.24 , 178.84 ± 47.73 , 68.82 ± 34.34 and 153.28 ± 36.51 , respectively. In hypothyroid cases AST, ALT and GGT were only slightly elevated when compared with controls. But

ALP, CPK and LDH were significantly increased. AST, ALT and GGT were slightly raised in hyperthyroid subjects whereas ALP, CPK and LDH show a marked elevation. The values of AST, ALT and GGT were found higher than those found in hypothyroids.

Table 3: Correlation Between Serum Enzymes and Thyroid Hormone Profile of the Control.

SE/TP	AST	ALT	GGT	ALP	CPK	LDH
	Correlation γ	Correlation γ	Correlation γ	Correlation γ	Correlation γ	Correlation γ
FT3	0.078	0.006	-0.091	0.110	0.211	0.075
FT4	-0.056	-0.024	0.123	-0.174	-0.111	0.211
TSH	0.186	-0.127	-0.384	0.118	0.114	-0.168

The above table shows correlation and significance between serum enzymes and thyroid hormones profile in the control.

Table 4: Correlation Between Serum Enzymes and Thyroid Hormone Profile of the Hyperthyroidism.

SE/TP	AST	ALT	GGT	ALP	CPK	LDH
	Correlation γ	Correlation γ	Correlation γ	Correlation γ	Correlation γ	Correlation γ
FT3	0.388	0.130	-0.096	-0.104	-0.479	-0.134
FT4	0.175	-0.011	0.058	-0.262	-0.517	-0.021
TSH	0.185	0.203	0.305	0.278	0.284	0.272

The above table shows correlation and significance between serum enzymes and thyroid hormones profile in the hyperthyroid cases.

Table 5: Correlation Between Serum Enzymes and Thyroid Hormone Profile of the Hypothyroidism.

SE/TP	AST	ALT	GGT	ALP	CPK	LDH
	Correlation γ	Correlation γ	Correlation γ	Correlation γ	Correlation γ	Correlation γ
FT3	-0.386	-0.320	0.298	0.154	0.072	-0.036
FT4	-0.159	-0.212	0.325	-0.095	0.086	0.100
TSH	-0.009	-0.026	0.212	-0.145	0.063	-0.106

The above table shows correlation and significance between serum enzymes and thyroid hormones profile in the hypothyroid cases.

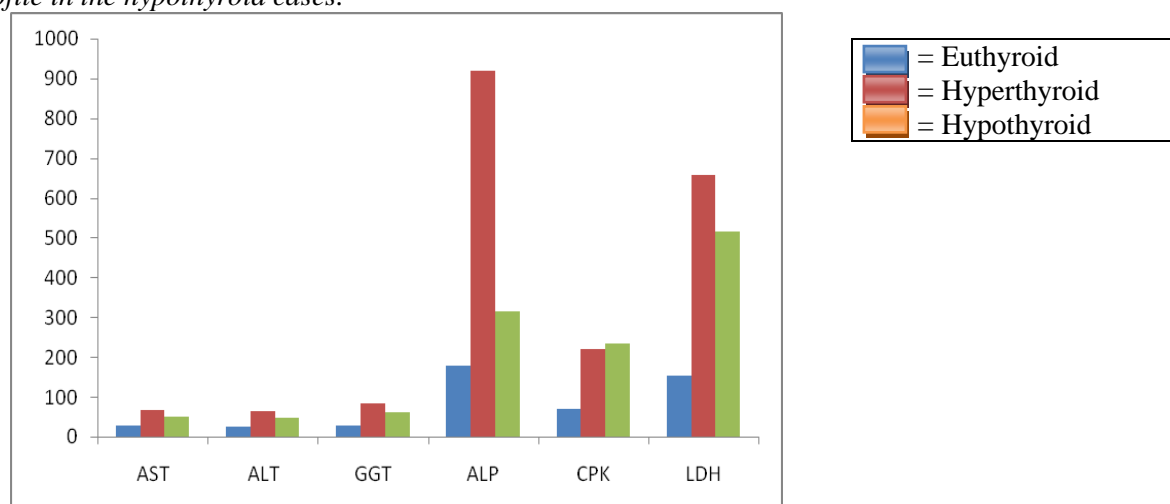


Fig. 1: Graphical Representation of Control and Cases with Serum Enzymes.

DISCUSSION

Present study of 110 subjects was conducted to assess serum enzymes level in thyroid alteration is probably the first epidemiological study carried out in Western Development Region, Nepal.

Serum enzymes profile have been proved valuable as diagnostic and prognostic guideline both in clinical practice and occupational medicine; reflecting the status, size, structure and functions of different body organs affected by age, sex, environmental factors, various diseases and drugs.

In the study, it was found that the prevalence of thyroid dysfunction was 25%. Females have more thyroid dysfunction than males. In majority of the cases diagnosis is usually straightforward on clinical grounds. However, various diagnostic tests are performed for confirmation of the disease, i.e., Serum FT3, FT4 and TSH levels. It is well known that various organs biochemical abnormalities have been shown in patients with thyroid alteration [28, 29].

In the present study, 50 blood samples were collected from staff of MTH and students from School of health and Allied sciences as control. Abnormal 60 samples (30 for hyperthyroidism and 30 for hypothyroidism) were collected from the patients visiting MTH and examined for their serum levels of enzymes ALT, AST, CPK, ALK, GGT and LDH. The serum reports of thyroid hormones FT3, FT4 and TSH were already available with the cases (hypothyroidism and hyperthyroidism), while these tests were performed for the controls.

Since, no previous data were available from the area under study, the observed serum enzymes profile was compared with normal standard and correlated with thyroid hormones profile in cases and controls. Results of the study from the MTH did not differ significantly from other studies and have been found in conformity with previous works carried out by various scientists. Therefore, results of the study from MTH shows an unexplained relationship between serum thyroid hormones and serum enzymes level in thyroid alteration. Malik and Hodgson

reviewed the relationship between thyroid gland and liver in hyperthyroidism [14] mentioned that thyroid hormones T3 and T4 are essential for the growth, development and function of all organs of the body. They regulate BMR of all cells of the body including the hepatocytes and thereby modulate all the organ function. The liver, muscle and kidney in turn metabolizes thyroid hormones and regulates their systemic endocrine effects. Therefore, thyroid dysfunction may disturb liver, muscle, other organs function and vice versa. It highlights a close relationship between thyroid and various organs in health and disease. The clinical features of liver injury caused by thyrotoxicosis are relatively common and can be conveniently divided into hepatic and cholestatic types. In hepatic injury an increase in levels of AST and ALT were reported in 27% and 37% of the patients, respectively. Although majority of them showed no other clinical or biochemical features of liver impairment. The mechanism of injury appears to be relative hypoxia in periventricular regions of the liver, due to an increase in hepatic oxygen demand without an appropriate increase in blood flow.

Therefore, results of the present study along with the earlier reports are suggestive of the fact that more the serum thyroid hormones level is elevated, higher is the serum enzymes (ALT, AST, CPK, GGT, ALP and LDH) level. Thus, showing a positive relationship between FT3, FT4 and ALT, AST, CPK, ALK, GGT and LDH levels, this is in accordance with the values reported in the previous studies. The relationship between thyroid hormones and serum enzymes levels have been well documented, though its importance as other organs dysfunction is still controversial. Findings of the present study are consistent with the previous work regarding elevated plasma serum enzymes levels in thyroid alteration. But contrary to the expectations, the correlation between thyroid and serum enzymes profile was found to be non significant which is in agreement with previous studies. Regardless of the reasons, significant alteration in serum enzymes in hypothyroidism and hyperthyroidism was not seen in enough individuals to make us feel

comfortable in using it as a reliable tool for diagnosis and prognosis of the disease.

In hypothyroid cases, the serum enzymes AST, ALT and GGT were only slightly increased whose mean values were less than those found in hyperthyroidism. Other serum enzymes CPK, ALP and LDH showed a marked elevation. Thus, study clearly revealed a positive association between increased serum AST, ALT, GGT, CPK, ALP and LDH in hyperthyroidism and hypothyroidism.

CONCLUSION

The present study ascertained that thyroid disorder causes significant effect on metabolism of various cells of the body that was reflected by increased level of serum enzymes to a varying extent. Thyroid dysfunction shows a strong female preponderance in Pokhara valley. This was an analytical cross-sectional study carried out in 110 subjects visiting Endocrinology Unit of Department of Biochemistry, MTH, Pokhara, with the request of TFT. Author's study Group was divided into euthyroid, hyperthyroid and hypothyroid. Since, there have been no such studies on the determination of serum enzymes level in this study area before, authors found it likely that the population in this respect represents that of Nepal. From the study, it is clear that thyroid hormones have significant effect on various organ systems of the body. During thyroid alteration, serum enzymes levels were also fluctuated. In hyperthyroid cases, the serum enzymes AST, ALT, GGT and CPK were slightly elevated with mean values 67.80 ± 19.39 , 64.33 ± 31.87 , 82.96 ± 33.15 and 218.76 ± 60.32 , respectively whereas, ALP and LDH shows significant elevation when compared to the controls.

In hypothyroid cases, the serum enzymes AST, ALT and GGT were only slightly increased whose mean values were less than those found in hyperthyroidism. Other serum enzymes CPK, ALP and LDH showed a marked elevation. Thus, study clearly revealed a positive association between increased serum AST, ALT, GGT, CPK, ALP and LDH in hyperthyroidism and hypothyroidism. Based on the results, it can be concluded that this association could lead us to newer avenues to investigate the pathophysiology and

management of patients with mild to moderate abnormalities of relative organs or systems.

Therefore, it necessitates the measurement of thyroid hormones in patients with abnormal serum enzymes level without any significant cause. However, further studies are required to be carried out in large sample size to confirm our findings.

LIMITATIONS

- Author's study was a hospital based cross-sectional study involving small number of patients visiting a single hospital in Pokhara. Therefore, the observation might not be true representative of the thyroid disease patients in Western Region.
- TSH, FT3 and FT4 were only determined to classify thyroid disorders excluding others.
- AST, ALT, ALP, GGT LDH, CPK were only determined excluding other serum enzymes.
- Sample size was only for 110 patients.
- Due to the limitation of time, authors couldn't include other organs marker to determine their extent of dysfunction.
- Though the most recent specific and sensitive method for the estimation of thyroid function is chemiluminescence method, authors had used microtiter well ELISA in this research.
- However, electricity, space, water supply, semi-automated analyzer and common glassware will be provided by the Biochemistry Department of Manipal Teaching Hospital.

FUTURE PERSPECTIVES

- Government should initiate and promote a routine screening program for thyroid alteration and its effect on other organs.
- Future studies are also needed to evaluate the general population and to trace the subjects under risk for development of multi organ dysfunction due to thyroid alteration.

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