Original Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ASSESSMENT OF THYROID PARAMETERS IN ALCOHOLIC LIVER DISEASE

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ABSTRACT

Normal level of thyroid hormone is important for normal hepatic function as it maintains the metabolism of bilirubin by playing a role in the enzymatic activity of glucuronyl transferase and by regulating the level of ligandin. The liver in turn glucuronidates and sulphates the thyroid hormone, excretes into bile and regulates their systemic endocrine effects. Therefore, hepatic dysfunction is commonly observed in patients with thyroid disease. Mean levels of Gamma-glutamyl transferase are increased in alcoholic cirrhosis with alcohol abuse of < 10 years duration. In this study thyroid function tests and liver function tests were performed on the 200 subjects, of which 100 subj ects were patients with Alcoholic Liver Disease, 100 were healthy controls and it was found that the serum tri-iodothyronine and free tri-iodothyronine levels were decreased and levels of Thyroid stimulating hormone were increased in patients with Alcoholic Liver Disease as compared to controls.

KEYWORDS: **ALD, Thyroid hormones, Deiodinase, Gamma-glutamyl transferase.**

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INTRODUCTION

Alcoholic liver disease is a term that encompasses the liver manifestations of alcohol overconsumption, including fatty liver, alcoholic hepatitis, and chronic hepatitis with liver fibrosis or cirrhosis.[1] Of all chronic heavy drinkers, only 15–20% develop hepatitis or cirrhosis, which can occur concomitantly or in succession.[2] 80% of alcohol passes through the liver to be detoxified. Additionally, the liver has tremendous capacity to regenerate and even when 75% of hepatocytes are dead, it continues to function as normal.[3] Alcoholism causes development of large fatty globules the liver and can begin to occur after a few days of heavy drinking. Alcohol is metabolized by alcohol dehydrogenase (ADH) into acetaldehyde, then further metabolized by aldehyde dehydrogenase (ALDH) into acetic acid, which is finally oxidized into carbon dioxide (CO_2) and water (H_2O).[4] Gamma-glutamyl transferase (GGT) is an enzyme that transfers gamma-glutamyl functional groups. It is found in many tissues, the most notable one being the liver. GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione. Isolated elevation or disproportionate elevation compared to other liver enzymes (such as ALP or ALT) can indicate alcohol abuse or alcoholic liver disease.[5] Alcohol might increase GGT production by inducing hepatic microsomal production, or it might cause the leakage of GGT from hepatocytes.GGT is a sensitive and highly specific test of liver cell injury in suspected alcoholics and is superior to transaminase determination. GGT remains the best of simple lab screening test and depending on population studied the sensitivity is in the order of 50% and specificity about 85%.[6] There are three homologous iodothyronine deiodinases which catalyses these reactions.[7] Type I deiodinase is located in liver, kidney, and thyroid. Liver has an important role in thyroid hormone transport and metabolism. These hormones are required for the normal growth, development and function of nearly all tissues, with major effects on oxygen consumption and metabolic rate.[8,9] Thyroid hormone synthesis and secretion is regulated by a negative feedback system that involves the hypothalamus, pituitary, and the thyroid gland. The free, unbound component of thyroid hormone within plasma is in equilibrium with the protein bound hormone and accounts for it's biological activities.[10] Thyroid hormones(TH) are known to stimulate basal metabolic rate for over a century.[11] Subsequent studies showed that THs induced energy expenditure in response to increased caloric intake.[12] The active form of TH, 3,3′5-triiodo-l-thyronine (T_3), is a critical regulator of cellular and tissue metabolism throughout the body. It controls gene expression in target tissues by binding to its cognate nuclear receptors (TRα and TRβ), which are ligand-inducible transcription factors.[13] In the presence of T_3, TH receptors (TRs) bind to TH response elements in the promoters of target genes and form coactivator complexes containing histone acetyltransferase activity to activate transcription.[14] Our main objective is to evaluate the thyroid hormone levels and liver function parameters in patients with liver disease .

MATERIALS AND METHODS

The present study consists of 200 subjects, 100 subjects were patients with alcoholic liver disease 100 were healthy controls.The study was conducted at Goa Medical College and Hospital , Bambolim-Goa during the period of 2013-2014. Ethical clearance was o btained from the institution's ethical committee. 7-8ml of fasting samples were collected in plain bulbs by venepuncture, under aseptic conditions of the 200 subjects. Serum was seperated by centrifuging blood samples at 3000 rpm in clinical centrifuge for 10 minutes. Serum was used to perform thyroid function tests and liver function tests on the above mentioned 200 subjects. All tests were performed on Ci2000 autoanalyser. ARCHITECT Total T_3(tri-iodothyronine) assay is a two step immunoassay to determine the presence of Total T_3 in human serum and plasma using CMIA (Chemiluminescent Microparticle ImmunoAssay) technology with flexible assay protocols , referred to as chemiflex. The sample and anti T_3 coated paramagnetic microparticles are combined. T_3 present in the sample binds to the anti-T_3 coated microparticles. After washing, T_3 acridinium-labeled conjugate is added .Pre-trigger and trigger solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as relative light units (RLUs). Reagent contains microparticles 1 or 4 bottles (6.6ml/27.0ml) anti-T_3 coated particles in MES buffer with sheep IgG Stabilizers and 1 or 4 bottles (5.9ml/26.3ml)T_3 acridinium-labeled conjugate in citrate buffer with NaCl and triton X-100 stabilizers. Antimicrobial agent is used as preservative. Calibrator range is 0.0-8.0ng/mg. Expected value of TT_3 is 0.58-1.59 ng/mL.[15] The sample and anti T_4 coated paramagnetic microparticles are combined. T_4 present in the sample binds to the anti-T_4 coated microparticles. After washing, T_4 acridinium-labeled conjugate is added .Pre-trigger and trigger solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as RLUs. Reagent contains microparticles 1 or 4 bottles of anti-T_4 coated particles in TRIS buffer. Calibrator range is 0.0-24.0 µg/dL. Expected value of TT_4 is 4.87-11.72 µg/dL. [16] The ARCHITECT TSH assay is a two step immunoassay to determine the presence of thyroid stimulating hormone (TSH) in human serum and plasma using CMIA technology with flexible assay protocols.The sample and anti-β TSH antibody coated paramagnetic microparticles and TSH Assay Diluent are combined. TSH present in the sample binds to the anti-TSH coated microparticles. After washing, anti-α TSH acridinium labeled conjugate is added. [16] Pre-trigger and trigger solutions are then added to the reaction mixture, the resulting chemilumiescent reaction is measured as RLUs. Reagent contains microparticles 1 or 4 bottles (6.6ml/27.0ml) anti- β TSH coated particles in TRIS buffer with protein (bovine) Stabilizers. Minimum concentration is 60 ng/mL. Calibrator range is 0.0000-100.0000 µIU/mL and expected value is 0.35- 4.94 µIU/mL. The ARCHITECT Free T_3 assay is a two step immunoassay to determine the presence of free (unbound) T_3 in human serum and plasma using CMIA technology. The sample and anti T_3 coated paramagnetic microparticles are combined. Free T_3

present in the sample binds to the anti-T_3 coated microparticles. After washing, T_3 acridinium-labeled conjugate is added. Pre-trigger and trigger solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as RLUs. Reagent contains microparticles 1 or 4 bottles (6.6ml/27.0ml) anti-T_3coated particles in MES buffer with sheep IgG Stabilizers. Calibrator Range is 0.0-30.0 pg/ml. Expected value is 1.71-3.71 pg/mL.[17] The sample and anti T_4 coated paramagnetic microparticles are combined. Free T_4 (unbound) present in the sample binds to the anti-T_4 coated microparticles. After washing, T_3 acridinium-labeled conjugate is added. Pre-trigger and trigger solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as RLUs. Reagent contains microparticles 1 or 4 anti-T_4 coated particles in TRIS buffer. Calibrator range is 0.0-6.0pg/mg and expected value is 0.70-1.48ng/dL.[18] Quality control procedures for TT_3, TT_4, TSH, FT_3, FT_4 requires a single sample of all control levels tested once every 24 hours each day of use. The ARCHITECT TT_3, TT_4, TSH, FT_3, FT_4 utilizes a 4 parameter logistic curve fit data reduction method to generate a calibration curve. Total Bilirubin-Total (conjugated and unconjugated) Bilirubin couples with diazoreagent in the presence of surfactant to form azobilirubin. The increase is absorbance at 548nm due to azobilirubin is directly proportional to the total Bilirubin concentration. Expected values are 0.2 to 1.2 mg/dL in adult serum. Direct (conjugated fractions) bilirubin couples with a diazonium salt in the presence of sulfamic acid to form the coloured compound azobilirubin. The increase in absorbance at 548 nm due to azobilirubin is propotional to the direct bilirubin concentration. Expected values are 0.0 to 0.5 mg/dL in adult serum.[19] Aspartate Aminotransferase (AST) present in the sample catalyzes the transfer of the amino group from *L*-aspartate to α-ketoglutarate, forming oxaloacetate and *L*-glutamate. Oxaloacetate in the presence of NADH and malate dehydrogenase (MDH) is reduced to *L*-malate. In this reaction, NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD. Expected values are 5 to 34U/L in adult serum.[20] Alanine Aminotransfererase (ALT) present in the sample catalyzes the transfer of the amino group from *L*-alanine to α-ketoglutarate, forming pyruvate and *L*-glutamate. Pyruvate in the presence of NADH and lactate dehydrogenase (LD) is reduced to *L*-lactate. In this reaction, NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD. Expected values are 0-55U/L in adult serum.[20] Alkaline Phosphatase (ALP) present in the sample catalyzes the hydrolysis of colourless *p*-nitrophenyl phosphate (*p*-NPP) to give *p*-nitrophenyl and inorganic phosphate. The rate of increase in absorbance at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Expected values are 40-150 U/L in adult serum.[19,20] Total protein polypeptides containing at least two peptide bonds react with biuret reagent. In alkaline solution, cupric ion forms a coordination complex with protein nitrogen with very little difference between albumin and globulin on a protein-nitrogen basis. Expected values are 6.4 to 8.3 g/dL.in adult serum. The Albumin BCG procedure is based on the binding of bromcresol green specifically with albumin to produce a coloured complex. The absorbance of the complex at 628 nm is directly propotional to the albumin concentration in the sample.Expected values are 3.5 to 5.2 gm/dl in adult serum.[20] Gamma Glutamyl Transferase(GGT) catalyzes the transfer of amino group between L γ Glutamyl-3-Carboxy-4 nitroanilide and Glycylglycine to form L γ Glutamylglycylglycine and 5-amino-2-nitrobenzoate. The rate of formation of 5-amino-2-nitrobenzoate is measured as an increase in absorbance which is proportional to the GGT activity in the sample. Expected values are 10-50 U/L in (Males) and 7-35 U/L (Females). Quality Control was maintained for all the above parameters.[21]

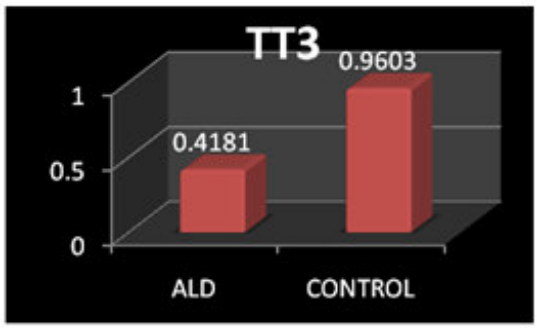
STATISTICAL ANALYSIS

The results of this study were analysed using windows statistical package for social science (SPSS) software, version 14. All the continuous variables were expressed as mean±SD. Pearson correlation was performed to find the correlation between study group and the control group. Student T test was performed and P value < 0.05 was considered statistically significant.

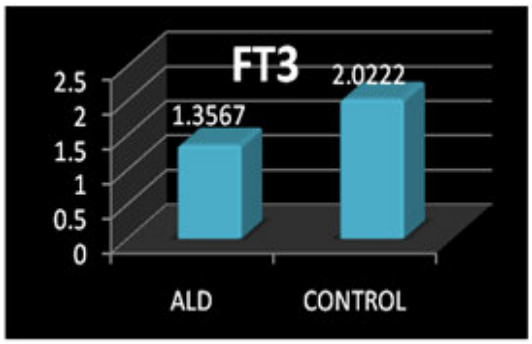
RESULTS

Table 1
Mean serum level of TT_3, FT_3 and TSH in diseased and control groups

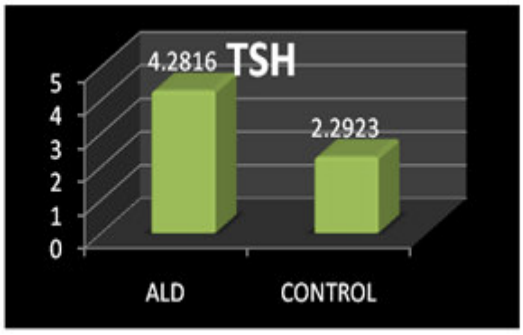
Parameters	Group	N	Mean	Std. Deviation
TT_3	DISEASED	100	.4181	0.08230
	CONTROL	100	.9603	0.27543
FT_3	DISEASED	100	1.3567	0.41663
	CONTROL	100	2.0222	0.62613
TSH	DISEASED	100	4.2816	2.21290
	CONTROL	100	2.2923	1.33532



Graph 1
Diagramatic representation of mean serum T_3 levels in patients with ALD (0.41± 0.082) and Controls(0.96±0.27). p value <0.001.



Graph 2
Diagramatic representation of mean serum FT_3 levels in patients with ALD (1.35±0.41) and Controls (2.29±0.62). p value <0.001.



Graph 3
Diagramatic representation of mean serum TSH levels in patients with ALD(4.28± 2.21) and Controls(2.29±1.33). p value <0.001.

The present study consists of 200 subjects, 100 subjects were patients with alcoholic liver disease 100 were healthy controls.In this study, patients with alcoholic liver disease were evaluated for thyroid function tests and it was found that the serum T_3 and FT_3 levels were decreased and levels of TSH were increased as compared to controls. The mean serum T_3 level in patients with ALD was 0.41± 0.082 and in control group was 0.96±0.27.This difference was statistically significant (p value <0.001).The mean serum FT_3 level in patients with ALD was 1.35±0.41 and in control group was 2.29±0.62. The mean serum TSH level in patients with ALD was 4.28± 2.21 and in control group was 2.29±1.33.The This difference was statistically significant(p value<0.001).

DISCUSSION

Thyroid hormones are essential for normal organ growth, development, function and regulation of the basal metabolic rate of all cells and therefore, its alteration can affect the entire metabolism. 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. [22,23,24] These biochemical changes, usually mild, are also reversible with adequate thyroid replacement therapy.[25] The findings of our study is in corroboration with findings of the study by Yadav A. et al.[26] and Pandey R. et al.[27] Malik and Hodgson et al who mentioned that thyroid hormones T_3 and T_4 regulate BMR of hepatocytes and modulate all the organ functions. The liver, muscle and kidney in turn metabolizes thyroid hormones and

regulates their systemic endocrine effects. Therefore, thyroid dysfunction may disturb liver, muscle and other organ functions and vice versa.[28]

Thyroid hormones regulate the basal metabolic rate of all cells including hepatocytes. The liver in turn metabolizes the thyroid hormones and regulates their systemic endocrine effects.[28]Thyroid hormones are glucuronidated and sulphated within the liver and subsequently excreted into bile; in addition, these hormones maintain the metabolism of bilirubin by playing a role in the enzymatic activity of glucuronyltransferase and by regulating the level of ligandin, a major organic anion-binding protein.[29] In fact, there are several clinical and laboratory associations between thyroid and liver diseases namely- (i) Liver damage secondary to the systemic effect of thyroid hormone excess or direct toxic effects and subclinical physiological effects of thyroid hormone on liver functions.(ii) Some patients with chronic liver diseases may have thyroiditis, hyperthyroidism or hypothyroidism through autoimmune mechanisms. (iii) Alterations of thyroid hormone metabolism or tests secondary to liver disease, and(iv) Liver or thyroid disorders related to the therapy of thyroid or liver disease. [30,31,32] Ethanol intake was associated with impaired hepatic 5'-deiodination. Among patients with alcohol-induced liver cirrhosis, low T_3 and T_4, elevated rT_3, and normal TSH values have been observed.[33]In patients with alcoholic liver disease Israel et al[34] reported an inverse correlation between serum T_3 concentrations and the severity of liver dysfunction as well as a progressive T_3 increase suggesting that T_3 concentrations in patients with alcoholic liver disease may be considered as helpful prognostic indicator. The low total and free T_3levels may be regarded as an adaptive hypothyroid state that serves to reduce the basal metabolic rate within hepatocytes and preserve liver function and total body protein stores. Indeed, a recent study in cirrhotic patients showed that the onset of hypothyroidism from intrinsic thyroid disease of various aetiologies during cirrhosis resulted in biochemical improvement in liver function.[35]

CONCLUSION

Hepatic dysfunction is commonly observed in patients with thyroid disease. The mean T_3 and FT_3 levels were decreased in patients with alcoholic liver disease has compared to controls probably because type I deiodinase which is the major enzyme in liver carries out 5' deiodination of T_4 to formT_3. Type II 5' deiodinase is important for providing the T_3 required to stimulate the pituitary to synthesize and secrete TSH.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. O'Shea RS, Dasarathy S, McCullough AJ. Alcoholic liver disease:AASLD Practice Guidelines. Hepatology. 2010 January; 51 (1): 307–28.
2. Menon KV, Gores GJ, Shah VH. Pathogenesis, diagnosis, and treatment of alcoholic liver disease. Mayo Clin. Proc. 2001 October; 76 (10): 1021–9.
3. Longstreth, George F.; Zieve, David. Alcoholic Liver Disease. MedLinePlus: Trusted Health Information for You. Bethesda, MD: US National Library of Medicine & National Institutes of Health. 2009 October.
4. Courtay C, Oster T, Michelet F, Visvikis A, Diederich M, Wellman M, Siest G. Gamma-glutamyltransferase: nucleotide sequence of the human pancreatic cDNA. Evidence for a ubiquitous gamma-glutamyltransferase polypeptide in human tissues. Biochemical Pharmacology.1992 Jun; 43 (12): 2527–2533.
5. Lamy J, Baglin MC, Ferrant JP, Weill J. Determination de la gamma-glutamyl transpeptidase senque des ethyliques a la suite du sevrage. Clin Chim Acta. 1974; 56: 169.
6. Barouki R, Chobert MN, Finidori J, Aggerbeck M, Nalpas B, Hanoune J. Ethanol effects in a rat hepatoma cell line: induction of gamma-glutamyltransferase. Hepatology 1983; 3 (3): 323–9.
7. Larsen PR, Berry MJ. Nutritional and hormonal regulation of thyroid hormone deiodinases. Annu Rev Nutr.1995;15: 323-52.
8. Visser TJ. Pathways of thyroid hormone metabolism. Acta Med Austr. 1996;23: 10-11.
9. Malik R, Hodgson H. The relationship between the thyroid gland and the liver. Q J Med. 2002;95: 559-69.
10. Shupnik MA, Ridgway EC, Chin WW. Molecular biology of thyrotropin. Endocr Rev. 1989;10: 459-75.
11. Thompson WO, Thompson PK, Brailey AG, Cohen AC. The calorigenetic action of thyroxine at different levels of basal metabolism in myxedema. J Clin Invest. 1929;7(3):437–463.
12. Landowne M. Specific dynamic action of carbohydrate and of protein in human hypothyroidism after total ablation of the normal thyroid gland. J Clin Invest. 1935;14(5):595–603.
13. Yen PM. Physiological and molecular basis of thyroid hormone action. Physiol Rev. 2001;81(3):1097–1142.
14. Ekins RP. Methods for measurement Of Free Thyroid Hormones. Amsterdam:Excerpta Medica Foundation. 1979;72-92.
15. Robbins J, Rall JE.The Iodine-containing Hormones.In:Hormones in blood. 3rd ed.London : Academic Press; 1979.1:632-667.
16. DeGroot LJ, Larsen PR, Refetoff S, Stanbury JB. Transport of Thyroid Hormone and Cell Uptake In The Thyroid and Its Diseases. 1984;62-66.

17. Kaplan MM, Larsen PR, Crantz FR, Deau VJ, Rossing TH. Prevelence of Abnormal Thyroid Function Test Results in Patients with Acute Medical Illnesses. Am J Med. 1982;72:9-16.
18. Visser TJ. Role of sulphation in thyroid hormone metabolism. Chem Biol Interact. 1994;92:293-303.
19. Larsen PR,Thyroidal triiodothyronine and thyroxine in Grave's disease:correlation with presurgical treatment, thyroid status, and iodine content. J Clin Endocrinol Metab. 1975;41:1098-104.
20. Burtis CA, Ashwood ER. Teitz Textbook Of Clinical Chemistry and molecular diagnostics.2nd ed. Philadelphia: Saunders;1994.
21. Theodorsen L, Stromme JH. Gamma- Glutamyl-3-Carboxy-4 nitroanilide the substrate of choice for routine determinations of Gamma- Glutamyl transferase activity in serum. Clin Chim Acta 1976; 72:205.
22. Targher G., Montagnana M., Salvagno G. et al. Association between serum TSH, free T4 and serum liver enzyme activities in a large cohort of unselected outpatients. Clin Endocrinol .2008; 68(3):481–484.
23. Khan T.M., Malik S, Diju I.U. Correlation between plasma thyroid hormones and liver enzymes level in thyrotoxic cases and controls in Hazara division. J Ayub Med Coll Abbottabad. 2010; 22(2):176–179.
24. Prakash A., Lal A.K., Negi K.S. Serum Creatine Kinase activity in thyroid disorders. J Med Edu Research. 2007; 9(1):25–26.
25. Couzigou P, Latapie J.L, Fleury B. et al. Evolution of serum gammaglutamyl transpeptidase activity in treated hyperthyroid and hypothyroid patients. Gastroenterol Clin Biol. 1985; 8(5): 458–463.
26. Yadav A., Arora S., Saini V., Arora MK., Singh R., Bhattacharjee J. Influence of thyroid hormones on biochemical parameters of liver function: a case-control study in North Indian population. Internet Journal of Medical Update. 2013;8(1):4-8
27. Pandey R., Jaiswal S., Sah JP., Bastola K., Dulal S. Assessment of Serum Enzymes Level in Patients with Thyroid Alteration Attending Manipal Teaching Hospital, Pokhara. A Journal of Life Sciences. 2013; 3(1):1-9.
28. Malik R, Hodgson H. The relationship between the thyroid gland and the liver. Q J Med . 2002; 95:559-69.
29. Fagiuoli S, Van Thiel DH. The liver in endocrine disorders. The Liver in Systemic Disease. 1993; 285-287.
30. Babb R.R. Associations between diseases of the thyroid and the liver. Am.J. Gastroenterol. 1984; 79:421-3.
31. Fong T.L, Mchutchison J.G., Reynolds T.B. Hyperthyroidism and hepatic dysfunction. Clin. Gastroenterol. 1992; 14: 240- 4.
32. Huang M.J., Li K.L., Wei J. S., Wu S.S., Fank D., Liaw Y.F. Sequential liver and bone biochemical changes in hyperthyroidism. Am. 3 Gastroenterol. 1994; 89:1071-6.
33. Camacho PM, Dwarkanathan AA. Sick euthyroid syndrome. What to do when thyroid function tests are abnormal in critically ill patients. Postgraduate med 1999;105:215-19.
34. Isreal Walfish PG, Orrego H, Blake J, et al.Thyroid hormones in alcoholic liver disease, effect of treatment with 6-propylthiouracil. Journal of gastroenterology 1979;76:116-22.
35. Van Thiel DH, Udani M, Schade RR, et al. Prognostic value of thyroid hormone levels in patients evaluated for liver transplantation. Hepatology 1985;5:862-6.