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### Research Article

# STUDY OF THYROID HORMONE LEVELS AND PITUITARY THYROID AXIS IN CHRONIC RENAL FAILURE

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## ABSTRACT

The present study suggests that in chronic renal failure there is increase TSH levels and decrease in T<sub>3</sub>, T<sub>4</sub> levels. The decrease T<sub>3</sub>, T<sub>4</sub> levels is not only due to urinary loss of thyroid binding proteins in CRF but also due to the presence of circulating inhibitors, which impairs binding of T<sub>4</sub> to thyroxine binding globulin and decrease in the peripheral synthesis of T<sub>3</sub> from T<sub>4</sub>.

**Objectives:** To estimate thyroid hormone levels i.e. T<sub>3</sub>, T<sub>4</sub> and TSH and to study the pituitary thyroid axis in patients of chronic renal failure.

**Materials and Methods:** Cross sectional study was conducted consisting of 30 patients of chronic renal failure and 30 healthy age matched controls. Fasting venous blood samples were collected and serum levels of T<sub>3</sub>, T<sub>4</sub> and TSH were analysed by using CLIA method in both the groups.

**Results:** T<sub>3</sub>, T<sub>4</sub> decreases and TSH increases significantly in cases compare to the controls.

**Conclusion:** Mean of T<sub>3</sub>, T<sub>4</sub> decreases TSH increases significantly in cases compare to controls. There are 10% of patients of CRF i.e cases are hypothyroid compare to 0% in controls. In CRF TSH increases as T<sub>3</sub>, T<sub>4</sub> decreases which suggests the maintenance of pituitary thyroid axis.

**Keywords:** Chronic Renal Failure, Hypothyroidism, Hyperthyroidism, Thyroid Stimulating Hormone (TSH).

## INTRODUCTION

Chronic renal failure (CRF) refers to an irreversible deterioration in renal function which classically develops over a period of years. Initially it manifests only as a biochemical abnormality eventually loss of excretory, metabolic & endocrine functions of the kidney. This leads to the development of the clinical symptoms & signs, which are referred to as uremia. When death is likely without renal replacement therapy it is called as End stage renal failure. There has been a dramatic increase in the incidence of ESRD as well as a shift in the relative incidence of etiologies of CRF during the past two decades. Whereas glomerulonephritis was the leading cause of CRF in the past, diabetic and hypertensive nephropathy are now much more frequent underlying etiologies. Thyroid hormones in the blood are almost entirely bound to plasma proteins. They are bound to a globulin named thyroid binding globulin (TBG), thyroxine binding prealbumin and albumin. Affinity of thyroxine is maximum for thyroid binding globulin and hence TBG is the major

determinant of binding. Affinity for T<sub>3</sub> and T<sub>4</sub> are slightly different because T<sub>3</sub> is not bound significantly by thyroxine binding prealbumin and binds to TBG less firmly than T<sub>4</sub>. The levels of free T<sub>3</sub> are 8 to 10 times more than T<sub>4</sub>. As only the free hormone is available to tissues, metabolic state correlates closely with the concentration of free thyroxine.

Patients with CRF often have signs & symptoms suggestive of thyroid dysfunctions. Various Studies of thyroid functions in uremic patients have been carried out which have shown conflicting results. Hyperthyroidism, hypothyroidism & euthyroid state have all been reported by various Workers<sup>1,2</sup>. Serum Tri-iodothyronine (T<sub>3</sub>) level were consistently found to be low, serum total & free thyroxine (T<sub>4</sub>) concentration have been reported as low, normal or high. Serum thyroid stimulating hormone (TSH) levels were found to be normal in most of the patients of CRF even in those whose CRF is complicated by low T<sub>3</sub> concentration<sup>3</sup>.

Serum hormonal concentration may be altered by changes in the binding capacity of serum proteins. In CRF there is massive proteinuria mainly albuminuria. Globulin levels are

not much altered. Hypothyroidism in CRF is mainly due to decreased level of albumin & thyroid binding pre-albumin<sup>4</sup>. In CRF Circulating thyroid binding inhibitors are increased, which inhibits the binding of thyroid hormones to carrier proteins, It may be additional cause for hypothyroidism<sup>5</sup>. Because of these variability in previous studies, A definite change in the thyroid hormone levels in CRF is yet to be determined. So study of thyroid hormone levels in CRF is taken.

## MATERIALS AND METHODS

- Source of Data:** The present study was undertaken in Al-Ameen medical college and hospital both in patients & outpatients and patients attending to dialysis unit.
- Inclusion Criteria:** The study subjects are divided in to 2 groups as cases & controls.
  - Cases:-** 30 Male patients aged between 40-70 years of having history of chronic kidney disease with serum creatinine > 5.5 mg/dl and urea > 55mg/dl and dipstick test positive for protein with symptoms of chronic renal failure.
  - Controls:-** 30 Healthy men aged between 40 -70 years
- Exclusion Criteria:** Patients with diabetic nephropathy, patients on treatment with estrogen, corticosteroids, sulphonylurea, phenobarbitones &  $\beta$ -blocker, Female & children's are excluded from the study.
- Data Collection:** All the subjects i.e. both cases & controls were subjected to medical examination as per a fixed proforma.
- Biochemical Tests:** Morning sample blood was drawn after 12 hrs fasting. The samples of blood were allowed to stand to clot. Serum was separated by centrifugation, and analyzed by the following methods.

### Estimation of $T_3$ , $T_4$ & TSH by Chemiluminescence immunoassay (CLIA)

#### a. Estimation of $T_3$ & $T_4$

The principle & procedure for estimation of  $T_3$  &  $T_4$  are similar

**Principle:** (Competition principle). The  $T_3$ ,  $T_4$  assay employs a competitive test principle with polyclonal antibodies specially directed against  $T_3$ ,  $T_4$ . Endogenous  $T_3$ ,  $T_4$  released by the action of 8 anilino-1- naphthalene sulphonic Acid (ANS), competes with the added biotinylated  $T_3$ ,  $T_4$  derivate for the binding sites on the antibodies labeled with the ruthenium complex.

**Procedure:** Total duration of assay 18min.

1<sup>st</sup> incubation: 30  $\mu$ l for  $T_3$  & 15  $\mu$ l for  $T_4$  of sample and a  $T_3$  or  $T_4$  specific antibody labeled with a ruthenium complex; bound  $T_3$  or  $T_4$  released from the binding proteins in the sample by ANS.

2<sup>nd</sup> incubation: After addition of streptavidin coated microparticles and biotinylated  $T_3$  or  $T_4$  the still free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin & streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured into the

surface of the electrode. Unbound substances are then removed with procell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by photomultiplier.

Results are detected via calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

#### Reagents:

- Streptavidin-coated microparticles contains 0.72mg/ml in preservative,
- Anti  $T_3$ -Ab & Anti  $T_4$ -Ab (separate for both) contains polyclonal Anti- $T_3$  & Anti  $T_4$  antibody (sheep) labeled with ruthenium complex 75 ng/ml for  $T_3$  & 100ng/ml for  $T_4$ ; ANS 0.8 mg/ml for  $T_3$  & 1 mg/ml for  $T_4$ ; phosphate buffer 100 m mol/l,  $p^H$  7-4; preservative for both.
- $T_3$  or  $T_4$  – biotin: Contains biotinylated  $T_3$  3 ng/ml & biotinylated  $T_4$  20ng/ml, phosphate buffer 100 m.mol/l,  $p^H$  7-4; preservative.

#### b. Estimation of TSH

**Principle:** (Sandwich principle) The TSH assay employs monoclonal antibody specifically directed against human TSH. The antibodies labeled with ruthenium complex consist of chimeric construct from human & mouse specific components. As a result, interfering effects due to HAMA (human anti-mouse antibodies) are largely eliminated.

**Procedure:** Total duration, 18min

1<sup>st</sup> incubation: 50  $\mu$ l of sample, a biotinylated monoclonal TSH specific antibody and a monoclonal TSH specific antibody labeled with a ruthenium complex react to form a sandwich complex.

2<sup>nd</sup> incubation: After addition of streptavidin coated microparticles, the complex becomes bound to the solid phase via interactions of biotin & streptavidin.

The reaction mixture is aspirated in to the measuring cell where the microparticles are magnetically captured on to the surface of the electrode. Unbound substances are then removed with pro-cell Application of voltage to the electrode then induce chemiluminescent emission which is measured by photomultiplier.

Results are determined via calibration curve which is instrument specifically generated by 2 point calibration and a master curve provided via the reagent barcode

#### Reagents:

Streptavidin -coated microparticles

Contains -0.7 mg/ml in preservative.

Anti-TSHAb-biotin

Contains-Biotinylated monoclonal ant-TSH- antibody (mouse) 2.0 mg/l; phosphate buffer 100 m.mol/l  $p^H$  7.2 in preservative.

Anti-TSH-Ab

Contains-monoclonal anti-TSH antibody (mouse/human) labeled with ruthenium complex 1-2 mg/l; phosphate buffer 100 m.mol/l  $p^H$  7.2 in preservative.

## RESULTS

Study design: a case control study with 30 controls and 30 cases is undertaken to study the thyroid hormone levels and pituitary thyroid axis in patients with chronic renal failure.

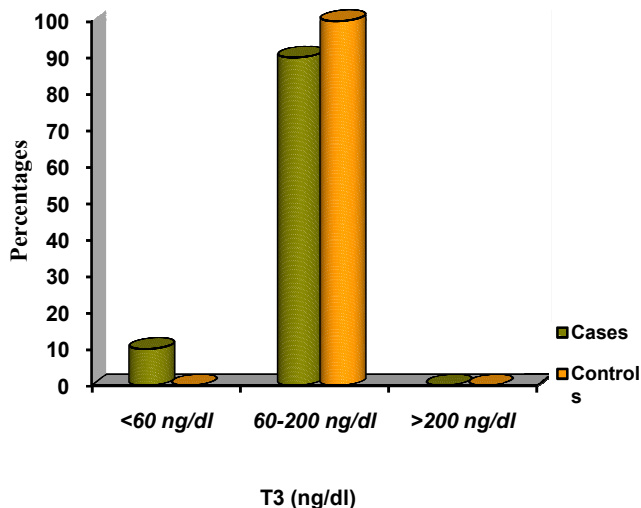
**Table 1: Comparison of study parameters in cases and controls (mean  $\pm$ SD)**

Study variables	Cases	Controls	P value
Blood urea (mg/dl)	96.23 $\pm$ 12.24	28.47 $\pm$ 8.40	<0.001**
S. creatinine (mg/dl)	5.83 $\pm$ 0.69	1.09 $\pm$ 0.17	<0.001**
T <sub>3</sub>	81.67 $\pm$ 15.07	111.96 $\pm$ 10.17	<0.001**
T <sub>4</sub>	5.80 $\pm$ 0.50	8.36 $\pm$ 0.46	<0.001**
TSH	4.81 $\pm$ 0.38	3.02 $\pm$ 0.79	<0.001**

**Comparison of study parameters in cases and controls**

Comparison of study parameters in cases and controls is shown in table 1. Mean of blood urea among cases is 96.23 $\pm$ 12.24 and in controls is 28.47 $\pm$ 8.40 p value is <0.001 which is statistically significant.

Mean of Sr. creatinine in cases is 5.83 $\pm$ 0.69 and in controls is 1.09 $\pm$ 0.17 (mg/dl). p value is <0.001 which is statistically significant. Mean of T<sub>3</sub> among all 30 cases is 81.67 $\pm$ 15.07 and in 30 controls is 111.96 $\pm$ 10.17 (ng/dl). it means T<sub>3</sub> is decreases in cases compares to controls. P value is <0.001 which is statistically significant. Mean of T<sub>4</sub> also decreases in cases compare to controls. P value is <0.001 which is statistically significant. Mean of TSH in cases increases compare to controls. P value is <0.001 which is statistically significant.

**Graph 1: Comparison of T<sub>3</sub> in cases and controls****Table 2: Comparison of T<sub>3</sub> in cases and controls**

T <sub>3</sub> (ng/dl)	Cases	Controls
<60 ng/dl	3 (10.0%)	0
60-200 ng/dl	27(90.0%)	30(100.0%)
>200 ng/dl	0	0
Total	30(100.0%)	30(100.0%)

**Comparison of T<sub>3</sub> in cases and controls**

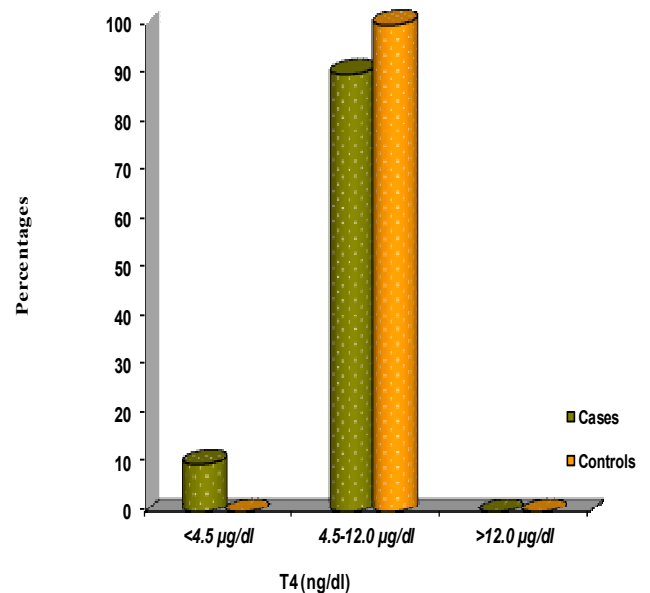
Comparison of T<sub>3</sub> in cases and controls are shown in table 2 and graphically represented in graph 1.

The normal range of T<sub>3</sub> is 60-200 ng/dl 3 patients among 30

cases having T<sub>3</sub> levels less than 60 ng/dl. All 30 controls and remaining 27 patients of cases group fall in normal range. No subjects among cases and control group fall in above 200 ng/dl category.

**Table 3: Comparison of T<sub>4</sub> in cases and controls**

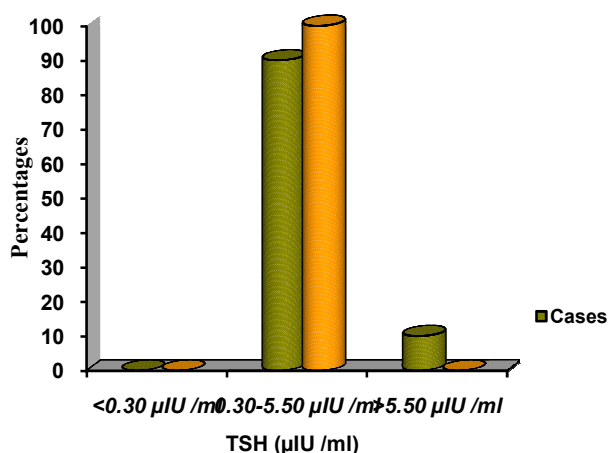
T <sub>4</sub> ( $\mu$ g/dl)	Cases	Controls
<4.5 $\mu$ g/dl	3 (10.0%)	0
4.5-12.0 $\mu$ g/dl	27(90.0%)	30(100.0%)
>12.0 $\mu$ g/dl	0	0
Total	30(100.0%)	30(100.0%)

**Graph 2: Comparison of T<sub>4</sub> in cases and controls****Comparison of T<sub>4</sub> in cases and controls**

Comparison of T<sub>4</sub> in cases and controls are shown in table 3 and graphically represented in graph 2. The normal range of T<sub>4</sub> is 4.5-12  $\mu$ g/dl 3 patients among 30 cases having T<sub>4</sub> levels less than 4.5  $\mu$ g/dl. All 30 controls and remaining 27 patients of casaes group fall in normal range. No subjects among cases and control group fall in above 12  $\mu$ g/dl category.

**Table – 4: Comparison of TSH in cases and controls**

TSH ( $\mu$ IU/ml)	Cases	Controls
<0.30 $\mu$ IU/ml	0	0
0.30-5.50 $\mu$ IU/ml	27(90.0%)	30(100.0%)
>5.50 $\mu$ IU/ml	3(10.0%)	0
Total	30(100.0%)	30(100.0%)



Graph 3: Comparison of TSH in cases and controls

### Comparison of TSH in cases and controls

Comparison of TSH in cases and controls are shown in table 4 and graphically represented in graph 3. The normal range of TSH is 0.03-5.5 µIU/ml 3 patients among 30 cases having TSH levels more than 5.5 µIU/ml. All 30 controls and remaining 27 patients of cases group fall in normal range. No subjects among cases and control group fall in below 0.3 µIU/ml category.

## DISCUSSIONS

A study of thyroid dysfunctions in chronic renal failure is done with 30 cases and 30 controls. Cases and control subjects are selected according to inclusion and exclusion criteria which are mentioned earlier. The cases and controls include different age groups. Among cases 6 patients are in age group 44-49 yrs, 9 patients are in age group 50-54 yrs, 8 patients are in age group 55-59 yrs and 7 patients are  $\geq 60$  yrs. Control subjects are selected almost equal age distribution for comparison of study parameters. Only male subjects are selected as cases and controls for the study.

Mean of blood urea levels in cases are  $96.23 \pm 12.24$  mg/dl and in controls are  $28.47 \pm 8.40$  mg/dl. The mean of serum creatinine in cases are  $5.83 \pm 0.69$  mg/dl and in controls are  $1.07 \pm 0.17$  mg/dl. The mean of blood urea and serum creatinine is high when compared to the controls. All patients in cases are positive for frank proteinuria by urine dip stick test.

The mean of  $T_3$  in all 30 cases is decreased when compared to controls even though most of them are within the normal range. In this study the findings are comparable with the previous study<sup>6-11</sup> showing decreased levels of  $T_3$  in uremic and hemodialysis patients. This reduction in  $T_3$  concentration has been linked to the decrease in the peripheral synthesis of  $T_3$  from  $T_4$ <sup>12-14</sup>. The mean of  $T_4$  in all 30 cases is decreased when compared to the controls, even though majority of them are within normal range. The findings are comparable with previous study<sup>15-18</sup>. The different studies mentioned various reasons to decreased levels of  $T_4$ . The decreased levels of  $T_4$

may be secondary to the protein loss, which occur in CRF. Serum albumin and thyroid binding pre-albumin decreases<sup>18</sup>. Decrease in  $T_4$  is also attributed to the presence of circulating inhibitors, which impairs binding of  $T_4$  to thyroxine binding globulin<sup>19</sup>.

Mean TSH in cases is  $4.81 \pm 0.38$  µIU/ml which is high compare to controls of having  $3.02 \pm 0.79$  µIU/ml. even though majority of cases TSH still remains within the normal range. The findings of previous studies are variable. Patients with low  $T_3$ ,  $T_4$  and free  $T_4$  showed high TSH suggesting maintenance of pituitary thyroid axis<sup>20</sup>. Studies conducted by G. Avasthi-et.al,<sup>21</sup> Joseph et.al,<sup>22</sup> shows increased TSH in those patients who had low  $T_3$ ,  $T_4$  &  $FT_4$  suggesting maintenance of pituitary thyroid axis. which is similar to this study. In some studies plasma TSH levels are not increased in spite of low  $T_3$  &  $T_4$  levels. It is not due to dysfunction in hypothalamo-pituitary axis but because truly hypothyroid renal failure patients can mount a high TSH response<sup>19,21</sup>. But in some studies shows that normal TSH response is due to blunted TSH response to TRH. Suggesting probability of pituitary dysfunction as well<sup>23,24</sup>. Blunted TSH after TRH administration was also reported by Ramirez et.al,<sup>25</sup>, Alvarez-de-et.al,<sup>26</sup> Czernichow et.al,<sup>27</sup>.

In this study 3 patients (i.e 10% of cases) among 30 cases have  $T_3$ ,  $T_4$  levels below normal range and TSH above the normal range. These 3 patients are hypothyroid, compared to none among control groups. The remaining 27 patients that is 90% of cases are euthyroid. There is no hyperthyroid in both cases and controls. In this study, findings are comparable with previous studies. Prevalence of hypothyroidism in patients with terminal renal failure is 5%, in comparison with that in hospitalized patients with normal renal function<sup>28</sup>.

## CONCLUSION

Serum thyroid hormone levels in chronic renal failure patients have been evaluated with age matched controls. Mean of  $T_3$ ,  $T_4$  decreases TSH increases significantly in cases compare to controls. There is 10% of patients of CRF i.e cases are hypothyroid compare to 0% in controls. There is no hyperthyroidism both in cases & controls. TSH increases as  $T_3$ ,  $T_4$  decreases which suggests the maintenance of pituitary thyroid axis. The present study is consistent with previous studies shows increased TSH in those patients who had low  $T_3$ ,  $T_4$  &  $FT_4$  suggesting maintenance of pituitary thyroid axis.

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