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AN EVALUATION OF THYROID HORMONE STATUS AND OXIDATIVE STRESS IN UNDIALYZED CHRONIC RENAL FAILURE PATIENTS

PON AJIL SINGH, ZACHARIAH BOBBY*, N. SELVARAJ AND R. VINAYAGAMOORTHI

Department of Biochemistry,
Jawaharlal Institute of Postgraduate Medical Education and Research,
Pondicherry – 605 006

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Abstract : Chronic renal failure (CRF) patients on prolonged dialysis have been found to have significant alteration in their thyroid status, but little is known about the same in undialyzed CRF patients. Oxidative stress has been implicated as the key player in altering the levels of thyroid hormone in euthyroid sick syndrome. This study was performed to evaluate the levels of oxidative stress and thyroid status in undialyzed CRF patients. A case control study was performed on 20 undialyzed CRF patients and 20 control subjects. There was a significant decrease in the levels of T_3, T_4, total protein and albumin levels in CRF patients when compared to control. There was a significant increase in the level of malondialdehyde and total antioxidant status in CRF patients when compared with control subjects. The present study confirms oxidative stress along with altered thyroid status in CRF patients.

Key words : chronic renal failure, lipid peroxidation, total antioxidant status, euthyroid sick syndrome, thyroid profile

INTRODUCTION

Chronic renal failure (CRF) is a state of irreversible deceleration in renal function. When only less than 10% of renal function remains, it is termed as end stage renal failure. This permanent loss of renal function culminates in signs and symptoms termed uremia. Unlike acute renal failure, from which recovery is frequent, CRF is not reversible and may lead to a vicious cycle with progressive loss of remaining nephrons (1).

A high percentage of chronic renal failure patients who are on hemodialysis therapy develop goiter and thyroid dysfunction (2, 3). However, the literature describing *in vitro* thyroid function tests in non-dialyzed chronic renal failure patients is somewhat controversial. There is agreement that plasma thyroid binding globulin concentrations are usually normal (4, 5), but both normal (6) and low normal serum total T_4 levels have been reported (7). Serum free T_4 concentrations have been reported as normal

*Corresponding Author : E-mail : zacbobby@yahoo.com; Tel.: +91-413-2273078, Fax : +91-413-2372067

(8) as well as low (9). Normal serum T_3 levels have been reported (10), but others have found T_3 concentrations to be subnormal in varying proportions of patients studied (11, 12).

Knowledge of alterations of thyroid hormone metabolism in euthyroid end stage renal disease (ESRD) patients is required to accurately diagnose and treat concurrent hypothyroidism and hyperthyroidism. Furthermore, thyroid diseases including goiter, hypothyroidism, thyroid nodules, and thyroid cancer may occur more frequently in ESRD patients than in general population and may be under diagnosed due to limited clinical awareness (13). Oxidative stress has been implicated as the key player in altering the levels of thyroid hormone in euthyroid sick syndrome (14–18).

We along with others have previously reported an increased oxidative stress in non-dialyzed chronic renal failure patients (9–23). Oxidative stress has been implicated in many pathological processes of euthyroid sick syndrome (4–18). Oxidative damage to unsaturated lipids (lipid peroxidation) is a well established general mechanism for oxidant mediated cellular injury (24, 25). Additionally, free radicals have been shown to alter the activity of some membrane bound tissue enzymes (26). Data from in vitro study indicates that free radicals contribute to reduced 5'-monodeiodination of iodothyronines in euthyroid sick syndrome (15, 16). For the above mentioned reasons, we conducted a study of thyroid function in patients on chronic renal failure who where not on dialysis therapy.

The aim of the present study was (i) to study the changes in thyroid hormone and oxidative stress status in undialyzed chronic renal failure (CRF) patients, and (ii) to evaluate if changes in thyroid hormone profile have any association with oxidative stress.

MATERIAL AND METHODS

Twenty Chronic renal failure (CRF) patients (12 men and 8 women) with mean age of 43 ± 6 years were selected for this study. Twenty age matched healthy volunteers (10 men and 10 women) were taken as control. The blood sample collected from these subjects was ceritrifuged and the serum was used for the estimation of total antioxidant assay, malondialdehyde, urea, creatinine, protein, albumin, T_3, T_4 and TSH.

Malondialdehyde was measured using the established thiobarbituric acid (TBARS) method (27). This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and malondialdehyde, a colorless product of lipid peroxidation, measured at 532 nm. The MDA values were calculated using the extinction coefficient of MDA-thiobarbituric acid complex (1.56×10^5 l $\times$ mol^{-1} $\times$ cm^{-1}) at 532 nm and expressed as nmol/ml.

The total antioxidant activity was measured by the ferric reducing/antioxidant power (FRAP) assay (28). The working FRAP reagent consisted of 300 mmol/1 of acetate buffer (pH 3.6), 10 mmol/1 2,4,6,-tri-pyridyl-s-triazine (TPTZ) in 40 mmol/1 HC1 and 20 mmol/1 $FeCl_3.6H_2O$ in he ratio of 10:1:1. Seven hundred fifty microliters of working FRAP reagent was mixed with 25 µl of serum or standard in a test tube. After

exactly 10 minutes at room temperature, the absorbance at 593 nm was read against reagent blank. The change in absorbance was directly related to the "total reducing power" of the electron-donating antioxidants present in the reaction mixture.

The thyroid status of all subjects was estimated by radioimmunoassay (BARC, Mumbai, India). Serum concentrations of urea, creatinine, total protein and albumin were estimated by using commercial kits adapted to 550 Express plus autoanalyser (Ciba Corning Diagnostics, Oberlin, Ohio, Canada).

Statistical analysis

All variables are shown as the mean ± SD. The data between control and test groups was compared using unpaired student's *t* test. Correlation was determined by Pearson's correlation coefficient. The level of significance used was P value less than 0.05.

RESULTS

The data for the chronic renal failure (CRF) patients and healthy subjects are shown in Table I. There was no significant difference between the two groups with respect to age and serum TSH levels. Serum creatinine and urea levels were significantly increased in CRF patients compared to control subjects. Serum T_3, T_4, total protein and albumin levels of CRF patients were significantly decreased compared to control subjects. There was a significant increase in the level of malondialdehyde and total antioxidant status as measured by FRAP assay. No significant correlation was observed between either total antioxidant status or lipid peroxides with the thyroid profile in CRF patients.

TABLE I: Mean and standard deviation of serum biochemical parameters in controls (n = 20) and chronic renal failure (n = 20).

	Controls	*CRF*
Age (in years)	45.50±6.39	43.70±6.04
Urea (mg/dl)	31.60±5.40	94.80±62.91*
Creatinine (mg/dl)	0.67±0.10	3.58±2.61*
T_3 (µg/dl)	123.00±25.36	70.40±27.06*
T_4 (µg/dl)	7.99±1.02	5.08±1.20*
TSH (µIU/ml)	4.23±2.35	3.65±3.77
Total Protein (g/dl)	6.15±0.43	5.40±0.96*
Albumin (g/dl)	4.02±0.28	3.11±0.57*
MDA (nmol/ml)	1.59±0.28	3.02±0.53*
Antioxidant power (FRAP) (µmol/l)	979.8±128.92	1598.3±315.57*

*P<0.05.

DISCUSSION

Abnormalities of thyroid function in nonthyroidal sick syndrome have been classified as 1) low T_3 syndrome, 2) low T_3 – low T_4 syndrome, 3) high T_4 syndrome, and 4) other abnormalities (29).

Serum T_3 concentration was less than the normal range in 12 of the 20 patients with chronic renal failure (60%). The mean serum T_3 concentration of 70.4 ± 27.06 ng/dl in patients with chronic renal failure group was significantly (P<0.001) lower than that in control subjects (123 ± 25.36 ng/dl). These results confirm earlier observations of several authors (3, 30) that in about one third to one half of cases of chronic renal failure serum T_3 are below the normal range.

In nonthyroidal illness, reduced T_3 levels are due to decreased peripheral conversion of T_4 to T_3, while thyroid gland production of T_3 is normal and T_3 clearance rates are normal or decreased, as in other nonthyroidal illness (30). T_4 is a prohormone requiring 5'-monodeiodination to produce the most active thyroid hormone T_3. Selenium functions as a cofactor of 2 functionally distinct enzymes: glutathione peroxidase and 5'-deiodinase (31). Reduced levels of selenium have been reported in patients with chronic renal failure (32). 5'deiodination of T_4 occurs in practically all tissues of the body and the reaction is catalyzed by the family of enzymes known as the iodothyronine deiodinases. The liver, kidney and muscle supply more than 80% of plasma T_3 (33). Impaired conversion of T_4 to T_3 may be related to malnutrition and humoral factors including cytokines that are generally associated with CRF (34). The works of Hung et al (15) and Brzezinska-Slebodzinska & Pietras (14) showed that free radicals may influence 5'-monodeiodenase, and indirectly reduce plasma T_3 level.

The initiation of lipid peroxidation has often been considered the proximal cause of cell damage due to free radicals (24) Increased amounts of malondialdehyde have been found in patients with renal failure (22, 23). Our results also indicate an increased lipid peroxidation in chronic renal failure patients. In our study, we found an increase in FRAP values as measured as total antioxidant capacity. Previous studies have also reported an increased total antioxidant capacity in chronic renal failure and have attributed this increase to the increase in uric acid level (35). We did not find any significant correlation between thyroid profile and either total antioxidant capacity or malondialdehyde. This lack of correlation between oxidative stress parameters and T_3 levels indicate that the alteration in T_3 levels in CRF is multifactorial and other factors like malnutrition and plasma protein levels may also have a predominant role.

Serum T_4 concentration was diminished below the normal range in 15 patients (75%) with chronic renal failure in the present study. The mean differed significantly ($P<0.001$) for chronic renal failure (5.08 ± 1.20 µg/dl) and for control subjects (7.99 ± 1.02 µg/dl). Low total T_4 values in chronic renal failure patients may be primarily related to impaired T_4 binding to serum carrier proteins. It has been reported that many inhibitors of T_4 binding to serum carrier proteins are present in CRF patients and thus contributing to the decreased levels of T_4 in CRF (15). The decreased total T_3 levels can also be attributed to the increase in excretion of bound and free T_4 in urine of chronic renal failure as reported in other previous study (36).

Serum mean TSH concentrations were within the normal range in chronic renal failure and did not differ from that found in the controls. Reduced serum TSH levels have not been reported to date in euthyroid chronic renal failure patients.

In conclusion T_3 and T_4 levels were significantly reduced in CRF patients when compared with healthy controls. TSH levels were similar in both the groups.

Further study with a larger cohort of chronic renal failure patients, taking into account all the confounding factors involved in alteration of thyroid status could help in unraveling the possible nexus between oxidative stress and euthyroid state in undialyzed chronic renal failure patients.

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