

Original Research Article

An Observational Cross Sectional Study to Evaluate the Effect of Thyroid Hypo Function on Serum Creatinine and Serum Uric Acid Levels to Assess the Renal Status in an Urban Hypothyroid Female Population of Reproductive Age Group in Eastern India

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ABSTRACT

Background: Thyroid disorders are the most common worldwide and also in India. It is well documented that renal status such as elevated serum creatinine and serum uric acid levels are associated with hypothyroid state of the body.

Aims: To investigate the effect of hypothyroid state on Serum Creatinine and Serum Uric acid levels in an urban female hypothyroid population of reproductive age group in Eastern India.

Materials & methods: This present observational cross sectional study was conducted on 200 hypothyroid subjects in Burdwan Medical College for a period of 12 months after taking Institutional Ethical Clearance. 100 controls were taken for the study. Serum TSH, FT4, Creatinine and Uric acid levels were estimated. The subjects were age matched. The computer software SPSS, version 16.0 was used for analyzing data.

Results: 200 hypothyroid subjects were enrolled for the study. 48 hypothyroid patients were with increased Serum creatinine level (24%) and 43 with increased serum uric acid level (21.5%) Significant difference was observed between control and hypothyroid subjects for mean TSH ($P < 0.0001$), mean FT4 ($P < 0.0001$), mean Creatinine ($P < 0.0001$) and mean Uric acid ($P < 0.0001$). No significant difference of age was found between two groups. Serum creatinine level was positively correlated with TSH ($R = 0.662$, $P < 0.00001$) and negatively correlated with Sr. FT4 ($R = -0.273$, $P = 0.000092$). Also we found a positive correlation between serum uric acid and TSH ($R = 0.669$, $P < 0.00001$), a negative correlation between serum uric acid and FT4 ($R = -0.276$, $P = 0.000076$).

Conclusion: It is observed from our present study that serum creatinine and serum uric acid levels are significantly higher in Hypothyroid females in reproductive age group compared to control subjects. Therefore, patients with increased serum creatinine and serum uric acid levels should be investigated for hypothyroidism and it will help to avoid unnecessary treatment and concern related to such biochemical abnormalities with undetermined thyroid status of the subjects.

Keywords: Hypothyroidism, Indian female population, FT4, TSH, Creatinine, Uric acid

INTRODUCTION

Thyroid gland produces and secretes T3 (Triiodothyronine) and T4 (Thyroxine)

and these thyroid hormones are regulated by negative feedback involving hypothalamus, pituitary and thyroid glands. ^[1,2] Thyroid

hormones help to maintain metabolic homeostasis in the adult and are required for growth and development of the kidney. Moreover, kidney helps in the metabolism and elimination of thyroid hormones. [3]

Thyroid hormones increase the renal blood flow and GFR by their prerenal and intrinsic renal effect. Thyroid and renal disorder might coexist with common etiologies. [4]

Significant changes such as impairment of concentrating and diluting capacity of distal tubule, a decline in urate excretion, decrease in renal blood flow and glomerular filtration rate (GFR) and decrease in sodium reabsorption by proximal tubule may occur in long standing hypothyroidism. [2]

It has been shown by various studies that hypothyroidism is associated with increased serum creatinine and uric acid levels. [5-27] Reduction of glomerular filtration rate following hemodynamic changes and also hypothyroid myopathy may contribute to increased serum creatinine levels. [20] Hyperuricemia in hypothyroidism occurs due to decreased renal plasma flow and impairment of glomerular filtration. [11,20] Also purine metabolism can be affected by thyroid hormone disturbances causing hyperuricemia and subsequent gout. [11] A study by Giordano et al. observed 33.3% prevalence of hyperuricemia in hypothyroid patients. [28]

It has been observed that surgical hypothyroidism or drug induced hypothyroidism in experimental animal decrease in GFR. Minor degree of hypothyroidism can cause adverse effects on various tissues. [29]

The present study was conducted to evaluate the effect of hypothyroid state on Serum Creatinine and Serum Uric acid levels in an urban female hypothyroid population of reproductive age group in Eastern India. Hypothyroid state is more common in females as compared to males, so they were chosen as the study population. Thyroid disorders are very common

endocrine disorders in India but the data on its prevalence specifically in young women is lacking. So, the female population of reproductive age group was taken for our present cross sectional study.

MATERIALS AND METHODS

This observational cross sectional study was conducted on 200 newly diagnosed hypothyroid female subjects in Burdwan Medical College for a period of 12 months after taking approval from institutional ethics committee and informed consent from the participating subjects. 100 controls (normal thyroid status) were taken for the present study. The formula used to calculate the size of the required sample was $n = (z)^2 p(1-p)/d^2$, $n =$ sample size, $z = z$ statistic for a level of confidence (95% level of confidence used, so z value is 1.96), $p =$ expected prevalence of proportion, $d =$ desired precision taken as 6% and previous studies were taken into consideration.

Inclusion criteria:

200 newly diagnosed hypothyroid female subjects of reproductive age group attending in the Department of Biochemistry, Burdwan Medical College and 100 control subjects were enrolled for the study.

Exclusion criteria:

- Pregnancy
- Renal disorder
- Hepatic disorder
- Bone disorder
- Gout
- Malignancies
- Diabetes, hypertension or any other systemic illness that may affect the renal function
- Patients on drug for treatment of thyroid disorders or any other medications that might affect renal function

Parameters studied:

- Age
- TSH
- FT4
- Serum Creatinine
- Serum Uric acid

Methods:

Approval from the Institutional ethics committee was taken before conduction of the study and Informed consent was taken from the participants. Subjects were recruited by random sampling using an online randomizer. Detailed history was taken from each subject as per case record format. Participants were also screened based on the inclusion and exclusion criteria.

Fasting Blood samples (5 ml) were drawn from subjects by sterile needle and syringes and sent to biochemical laboratory in sterile vials for analysis.

Estimation of Serum TSH was done by Quantitative determination of TSH concentration by Microplate immunoassay using Monobind Inc. USA manufactured TSH Accu Bind ELISA Kit (Normal value:0.39-6.16 micro IU/ml).

Estimation of Serum FT4 level was done by Quantitative determination of FT4 concentration by Microplate Enzyme Immuno assay using Monobind Inc. USA manufactured FreeT4 AccuBind ELISA Kit (Normal value:0.8-2.0 ng/dl)

Serum Creatinine was analyzed by modified Jaffe's method (Normal value:0.6-1.1 mg/dl) and Serum Uric acid was analyzed by Uricase enzymatic method (Normal value:2.6-6.0 mg /dl) on fully automated analyzer(EM 360,Germany) using kits supplied by Erba Mannheim, Germany.

Biochemical methods:

Estimation of TSH:

Measurement of serum TSH is generally regarded as the most sensitive indicator for the diagnosis of Hypothyroidism.

Test principle: (Method-Immunoassay)

The immobilization occurs at the surface of microplate well between the interaction of streptavidin coated on the well and biotinylated monoclonal anti-TSH antibody. By mixing the monoclonal

biotinylated antibody, the enzyme labelled antibody and a serum containing native antigen, reaction occurs between native antigen and the antibodies to form a soluble sandwich complex. Then the complex is deposited to the well. After equilibrium is achieved, the antibody bound fraction is separated from unbound antigen by aspiration or decantation. The enzymatic activity of the antibody bound fraction which is directly proportional to the native free antigen concentration is measured by adding substrate. By utilizing calibrators of known antigen concentrations, a dose response curve can be generated from which the antigen concentration of an unknown sample can be ascertained

Kit content:

1. Streptavidin Coated Microplates-96 wells coated with streptavidin and packaged in an aluminium bag with a drying agent.
2. TSH Enzyme Reagent- 13 ml/vial containing enzyme labelled polyclonal antibody, biotinylated monoclonal IgG in buffer, dye and preservative.
3. Thyrotropin Calibrators-seven vials(0.5ml/vial) of references for TSH Antigen at levels of 0,0.5,2.5,5.0,10,20 and 40 μ IU/ml
4. Substrate A(7ml/vial) -one bottle containing tetramethyl benzidine (TMB) in buffer.
5. Substrate B(7ml/vial)-one bottle containing hydrogen peroxide (H₂O₂) in buffer.
6. Stop Solution (8ml/vial)- one bottle containing a strong acid(1 N HCL)
7. Wash Solution Concentrate (20 ml)-one vial containing a surfactant in buffered saline. A preservative has been added.

Calculation of results:

- (1) Calculation of the mean absorbance value of calibrator and samples was done at 450 nm.
- (2) A point to point curve was plotted by plotting the absorbance of each calibrator on the vertical Y-axis against concentration of each calibrator on the horizontal or X-axis.

(3) Using the absorbance value for each sample the corresponding concentration of TSH was determined in microIU/ml. from the standard curve.

Estimation of FT4: -

Thyroxine circulates in blood almost bound to carrier proteins. Thyroxine binding globulin (TBG) is the main carrier protein. Only the free (unbound) fraction of thyroxine is biologically active. Concentrations of the carrier proteins are altered in different clinical conditions. So the free thyroxine (FT4) concentration remains constant. The measurement of FT4 concentration correlates better than total thyroxine level.

Test principle: (Competitive Enzyme Immunoassay-EIA)

In competitive EIA, a competitive reaction results between the native free antigen and enzyme-antigen conjugate for limited number of insolubilized binding sites on antibody coated on the micro well. After the equilibrium is attained the antibody-bound fraction is separated from unbound antigen by aspiration or decantation. The enzymatic activity of the antibody bound fraction which is inversely proportional to the native free antigen concentration is measured by adding substrate. By utilizing calibrators of known antigen concentrations, a dose response curve can be generated from which the antigen concentration of an unknown sample can be found out.

Kit contents: -

1. FT4 Antibody coated microplate(96 wells)-one96 well microplate coated with anti-thyroxine serum.
2. Enzyme reagent (13ml/vial)-one vial of thyroxine-horseradish peroxidase(HRP) conjugate in a protein stabilized matrix.
3. FT4 calibrators (0.5 ml/vial)- six vials of human serum based reference calibrators for free thyroxine.
4. Substrate A (7 ml/vial)-one bottle containing tetramethylbenzidine (TMB) in acetate buffer.

5. Substrate B (7 ml/vial)-one bottle containing hydrogen peroxide (H₂O₂) in acetate buffer.

6. Wash solution concentrate (20 ml)-one vial containing a surfactant in buffered saline.

7. Stop solution (8 ml/vial)-one bottle containing a strong acid (1 N HCL).

Calculation: -

1. Calculation of absorbance value was done at 450 nm.
2. A point to point curve was plotted by plotting the absorbance of each calibrator on Y axis against concentration of each calibrator on X axis.
3. Using the absorbance value for each sample the corresponding concentration of FT4 was determined in ng/dl. from the standard curve.

Hypothyroidism was defined as an elevated TSH (>6.16micro IU/ml) with a decreased (<0.8ng/dl) or normal serum FT4 level (range:0.8-2.0 ng/dl) as per kit values.

Estimation of serum Creatinine:

Principle: Creatinine reacts with alkaline picrate to produce a reddish colour complex (creatinine picrate)

Reagents composition:

Reagent 1-Sodium hydroxide (240 mmol/L)

Reagent 2- Picric acid (26 mmol/L)

Hypercreatininemia was considered above 1.1 mg/dl as per kit value.

Estimation of serum Uric acid:

Principle: It is based on trinder reaction Uric acid is oxidized to allantoin by uricase with production of H₂O₂.The peroxidase reacts with 4- aminoantipyrine(4-AAP) and TOOS in presence of peroxidase to yield a quinoneimine dye.

Reagents:

Pipes buffer, TOOS, Uricase, Peroxidase,4-Aminoantipyrine

Hyperuricemia was considered above 6.0 mg /dl as per kit value.

Statistical analysis:

The computer software “Statistical Package for the Social Sciences (SPSS) version 16

(SPSS Inc. Released 2007.SPSS for Windows, Version 16.0. Chicago, SPSS Inc.)” was used to analyze the data, P<0.05 was considered as significant and P<0.01 was considered as highly significant.

RESULTS

Newly diagnosed 200 hypothyroid female subjects and 100 controls were taken for our present study. 48 subjects were having increased serum Creatinine level and 43 subjects were with increased Serum Uric acid level out of 200. There was no significant difference in age between the two groups (Age in years: 30.82±6.55 vs. 31.54 ±6.68; P value: 0.376). Prevalence of increased serum Creatinine was 24% and serum Uric acid was 21.5% among all hypothyroid subjects.

Significant difference was noticed between control and hypothyroid subjects for mean TSH (P<0.0001), mean FT4 (P<0.0001), mean serum Creatinine (P<0.0001) and mean serum Uric acid (P<0.0001). No significant difference was observed for mean age between control and hypothyroid subjects (Table1, Figure 1).

Serum Creatine was positively correlated with serum TSH (R=0.662, P<0.00001) and also serum Uric acid was positively correlated with serum TSH (r=0.669, P<0.00001 Figure 2).

Serum Creatinine level was negatively correlated with Sr. FT4 (r= - 0.273, P=0.000092) and also Serum Uric

acid level was negatively correlated with Sr. FT4 (r= - 0.276,P=0.000076) (Figure 3).

Table 1: Shows Age, TSH, FT4 , serum Creatinine and serum Uric acid values of control and hypothyroid subjects

Parameter	Control (Mean±SD)	Hypothyroidism (Mean±SD)	P value
Age (yrs)	30.82 ± 6.55	31.54 ± 6.68	0.376
TSH(micro IU/ml)	1.90 ± 0.91	18.93± 9.50	<0.0001**
FT4(ng/dl)	1.32 ± 0.24	0.78± 0.36	<0.0001**
Serum Creatinine (mg/dl)	0.7± 0.08	1.01±0.27	<0.0001**
Serum Uric acid (mg/dl)	4.12±0.64	5.48±1.07	<0.0001**

P<0.05*significant, <0.01** highly significant

Table 1: shows that the difference of TSH, FT4 and serum Creatinine and serum Uric acid were highly significant.

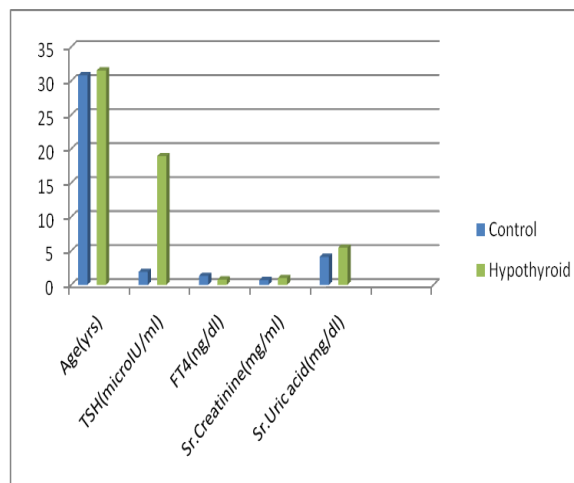


Figure 1: shows the difference of Age, TSH, FT4 and serum Creatinine and serum Uric acid values between control and hypothyroid subjects

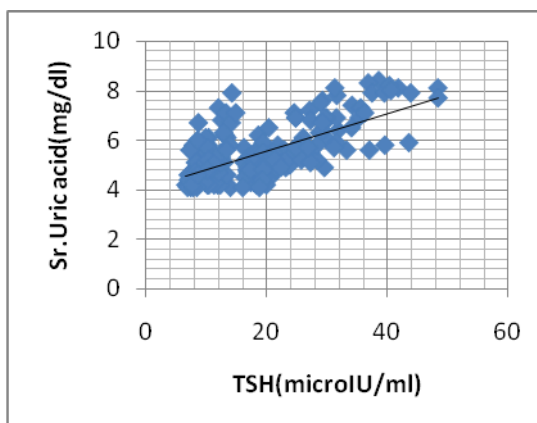
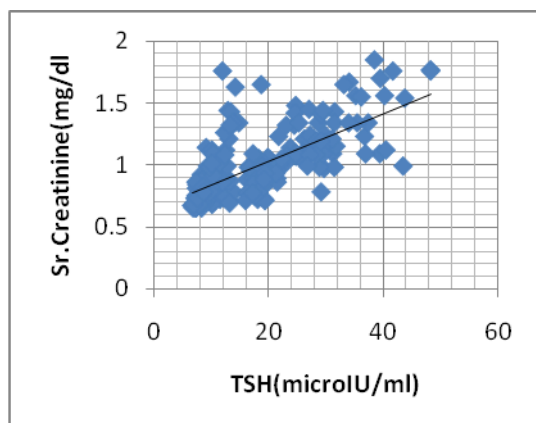


Figure2: Shows serum Creatine was positively correlated with serum TSH (R=0.662, P<0.00001) and also serum Uric acid was positively correlated with serum TSH (R=0.669, P<0.00001).

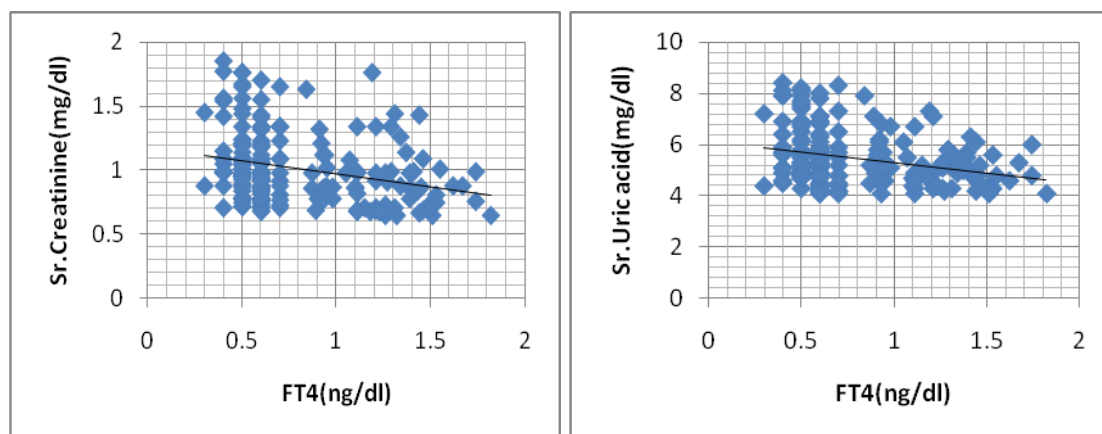


Figure 3: Shows Serum Creatinine level was negatively correlated with Sr. FT4 ($r = -0.273, P = 0.000092$) and also Serum Uric acid level was negatively correlated with Sr. FT4 ($r = -0.276, P = 0.000076$).

DISCUSSIONS

Hypothyroidism is divided into primary hypothyroidism and central (secondary) hypothyroidism. Primary hypothyroidism (95%) is the most common form of hypothyroidism, mainly caused by either iodine deficiency or by autoimmune thyroiditis. [30]

Functional renal mass is the kidney to body mass ratio and is decreased in hypothyroid subjects. [31] In hypothyroidism, reduced cardiac output and increased vascular resistance diminish renal blood flow and GFR. [30] Also, decreased responsiveness to β adrenergic stimuli causing decreased renin release, diminished angiotensin II and alteration in RAAS (renin-angiotensin-aldosterone) activity decrease in GFR. [31] It has been documented that in 55% case of hypothyroidism GFR is reversibly reduced by 40%. [32]

The present study was conducted to evaluate the effect of hypothyroid state on Serum Creatinine and Serum Uric acid levels in an urban female hypothyroid population of reproductive age group in Eastern India. We had taken 200 hypothyroid female subjects and 100 controls for our present study. Significant difference was noticed between control and hypothyroid subjects for mean TSH ($P < 0.0001$), mean FT4 ($P < 0.0001$), mean Creatinine ($P < 0.0001$) and mean Uric acid ($P < 0.0001$). No significant difference of age was observed between two groups

Thyroid hormones are involved in the development and function of kidney. Also kidney function can influence the metabolism and concentration of thyroid hormones. [30]

Jia et al. in their study found that TSH showed no correlations with serum uric acid ($r = -0.01, P = 0.648$) and serum creatinine ($r = -0.02, P = 0.284$), FT4 showed negative correlations with serum uric acid ($r = -0.978, P = 0.001$) and serum creatinine ($r = -0.599, P = 5.012$). [5] Saini V et al. observed that TSH showed significant positive correlation with serum creatinine and uric acid level and FT4 had a negative correlation with serum uric acid in overt hypothyroidism. [33]

Studies conducted by Erickson et al [34] and Dariyerliet et al. [35] noticed hyperuricemia in hypothyroid subjects. Kreisman SH et al. [36] observed in their study that serum creatinine levels in hypothyroid cases were significantly higher in compared to controls. Also other studies showed increased serum uric acid and creatinine levels in hypothyroidism. [8,13,37-44]

In the present study we observed the prevalence of increased Serum Creatinine was 24% and increased Uric acid was 21.5%. Serum creatinine level was positively correlated with TSH ($r = 0.662, P < 0.00001$) and negatively correlated with Sr. FT4 ($r = -0.273, P = 0.000092$). Also we found a positive correlation between serum uric acid and TSH ($r = 0.669, P < 0.00001$) and

a negative correlation between serum uric acid and FT4($r = -0.276, P = 0.000076$).

CONCLUSION

It is observed from our present study that serum creatinine and serum uric acid levels are significantly higher in hypothyroid females in reproductive age group compared to control subjects. Therefore, patients with increased serum creatinine and serum uric acid levels should be investigated for hypothyroidism and it will help to avoid unnecessary treatment and concern related to such biochemical abnormalities with undetermined thyroid status of the subjects. Further study is needed to evaluate the mechanism of different biochemical parameters related to thyroid dysfunction.

Conflict of interest: The authors declared no conflict of interest

Source of funding: Nil

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