Original Article

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The Effect of Hyperthyroidism on the Level of Urinary Neutrophil Gelatinase Associated Lipocalin and Markers of Kidney Function

Biochemistry Section

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ABSTRACT

Introduction: Thyroid hormones affect kidneys in various ways ranging from their development to their functions. Serum and urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) are emerging as the most promising biomarkers for early determination of Acute Kidney Injury (AKI) and Chronic Kidney Disease (CKD).

Aim: To evaluate the effects of hyperthyroidism (subclinical and overt) on the markers of kidney function.

Materials and Methods: In this cross-sectional study, 300 subjects were recruited from May 2015 to June 2018 and divided in three categories of euthyroid, subclinical and overt hyperthyroid subjects. All the subjects were analysed for Thyroid function (Total T_3, T_4, Thyroid Stimulating Hormone (TSH)) and Kidney Function (Serum Urea, Serum Creatinine, Urinary NGAL, estimated Glomerular Filtration Rate (eGFR)).

Results: Statistical analysis revealed a significant decrease in serum urea and serum creatinine in both subclinical (17.32±3.51 mg/dL and 0.58±0.13 mg/dL, respectively) and overt hyperthyroid (13.52±2.78 mg/dL and 0.29±0.10 mg/dL, respectively) patients as compared to euthyroids (19.99±5.38 mg/dL and 0.87±0.25 mg/dL, respectively). Urinary NGAL and eGFR were significantly increased in both subclinical (30.24±8.63 ng/mL and 119.57±11.31 mL/min, respectively) and overt hyperthyroid (44.05±13.02 ng/mL and 155.12±19.75 mL/min, respectively) patients as compared to euthyroids (21.94±18.06 ng/mL and 97.97±25.92 mL/min, respectively). A negative correlation of creatinine and positive correlation of urinary NGAL and eGFR with T_3 and T_4 was observed in both subclinical and overt hyperthyroidism ($p<0.05$).

Conclusion: The study shows that there are negative effects of hyperthyroidism on kidney functions.

Keywords: Creatinine, Glomerular filtration rate, Lipocalin 2, Serum urea, Thyroid dysfunction

INTRODUCTION

The CKD is emerging as a prevalent and serious threat. A recent study estimated the prevalence of CKD to be 17.2% out of which 7% were with only stage one [1]. Thyroid and kidneys share a special relation. Any dysfunction of thyroid can change Renal Blood Flow (RBF), GFR, electrolyte homeostasis, tubular function and kidney structure. Also, kidney helps in the metabolism, degradation and excretion of thyroid hormone and its metabolites. Hyperthyroidism results in increased RBF and GFR [2] by increase in positive chronotropic [3] and inotropic effects [4] as well as reduction in systemic vascular resistance [5]. Tri-iodothyronine increase, results in the increased tubular mass, renal mass and tubular re-absorptive capacity [6].

Raised level of Urinary-*N*-acetyl-β-D-glucosaminidase (NAG) in hyperthyroidism shows disruption of glomerular basement membrane due to hyper filtration, hypertrophy and hyperplasia [7]. It ought to be emphasised and taken care of by the primary care physicians to screen for early kidney damage in cases of hyperthyroidism. Serum creatinine and urea are the conventional markers to assess kidney function but both of them can be affected by various other factors.

NGAL is also known as human neutrophil lipocalin, lipocalin-2, siderocalin, 24p3, or Lcn2. NGAL is a petite protein which has 178 amino acids in its structure and belongs to the family of lipocalins. These proteins are specialised in capturing and transferring small hydrophobic molecules. NGAL, similar to the other members of lipocalins, is able to attach to some ligands, including the siderophores. NGAL reacts with iron-binding siderophores that gives it, characteristic bright red colour and modulates most of its biological effects. Numerous tissues including lungs, trachea, stomach, colon and kidneys exude NGAL at low levels. In case of any kidney damage NGAL is rapidly released from renal tubular cells which lead to an increase in the level of serum and urinary NGAL [8,9].

Kidneys seem to be the chief source of NGAL, but quite a few studies [10,11] have demonstrated that acute renal injury results in an augmented expression of NGAL mRNA in distant organs, such as liver and lungs, causative to the increased levels. All these reasons can further raise urinary levels of NGAL as a result of insufficient reabsorption of the filtered NGAL molecule. Being a minuscule protein molecule, NGAL is freely filtered by the glomerulus, and most of it is reabsorbed in the proximal tubules by efficient megalin-dependent endocytosis. Any NGAL excretion through urine is possible only when there is an associated proximal renal tubular injury that precludes NGAL reabsorption and/or increased de novo NGAL synthesis. Serum and urinary NGAL are emerging as the most promising biomarkers for early determination of AKI. Various studies have recognised the role of NGAL in CKD and showed serum and urinary NGAL levels are the potential markers of kidney dysfunction and severity in CKD [12-14].

So the present study aimed to evaluate the levels of traditional biochemical markers of kidney function and urinary NGAL in patients of hyperthyroidism.

MATERIALS AND METHODS

This was a cross-sectional study, conducted in the Department of Biochemistry, Santosh Medical College and Hospital, Ghaziabad, Delhi-NCR, India from May 2015 to June 2018. Sample size was estimated according to the prevalence [15] of subclinical and overt hyperthyroidism. A total 300 subjects were recruited from the medicine OPD and divided in 3 groups: (i) Euthyroid (100 subjects); (ii) Subclinical Hyperthyroid (100 subjects); (iii) Overt Hyperthyroid (100 subjects).

The approval vide letter no. SU/2015/793(1) was taken from local Ethics Committee. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, revised in 2013.

Inclusion criteria: Subjects with no history of any thyroid disorder, diagnosed and confirmed with complete clinical and biochemical investigations.

Exclusion criteria: Subjects with Diabetes mellitus, Hypertension, Coronary Heart Diseases, other endocrine disorders, alcohol abusers, pregnant women and patients already on Thyroid treatment or CKD treatment.

A single date was fixed for the participants to report to the hospital, with 12 hours overnight fasting. Blood samples were collected by venipuncture. The samples were centrifuged for 15 minutes at 2500 rpm and aliquots of serum were used to analyse different parameters.

Following Biochemical parameters were analysed:

- Serum Triiodothyronine [16] and Thyroxine concentration [17]- estimated using competitive ELISA technique.
- Serum TSH concentration [18]- estimated using sandwich ELISA technique using the reagent kit by Monobind, Lake forest, USA.
- Serum urea- analysed by enzymatic Urease Glutamate Dehydrogenase Method [19] using the reagent kit by Erba diagnostics Mannheim Germany.
- Serum Creatinine- analysed by modified Jaffe's method [20] using the reagent kit by Autospan liquid gold creatinine (Aarkay healthcare, India).
- Urinary NGAL- analysed by Sandwich ELISA Technique [21] using the reagent kit by Bioporto Diagnostics, Denmark.
- eGFR- calculated by Cock-croft Gault Formula [22].

STATISTICAL ANALYSIS

The difference between groups was compared by Student's t-test. Pearson's correlation coefficient was used to assess the correlation between different parameters for thyroid and kidney function. Microsoft Excel and available online calculators (Mean and Standard Deviation: Microsoft Excel, Student's t-test: https://tinyurl.com/s2bs4a3, Pearson's Correlation Coefficient: https://tinyurl.com/yyqup3nd) were used.

RESULTS

Most of the participants were females (64.66%). All three groups were age and sex matched. Mean age of euthyroid group was 34.38±11.61 years and that of subclinical and overt hyperthyroid group was 34.28±11.58 years and 36.79±13.97 years, respectively.

There was a statistically significant decrease in the level of TSH in case of subclinical hyperthyroidism (0.24±0.09 µIU/mL) and overt hyperthyroidism (0.16±0.05 µIU/mL) as compared to euthyroid subjects (2.55±1.14 µIU/mL). However, there was an increase in the level of T_3 in subclinical hyperthyroidism (1.09±0.26 ng/mL) and overt hyperthyroidism (2.63±0.59 ng/mL) as compared to euthyroid subjects (1.02±0.12 ng/mL) [Table/Fig-1]. The level of T_4 was also increased significantly in subclinical hyperthyroidism (9.80±1.52 µg/dL) and overt hyperthyroidism (15.09±2.15 µg/dL) as compared to euthyroid subjects (8.04±1.9 µg/dL).

There was a statistically significant decrease in the level of serum urea in subclinical hyperthyroid and overt hyperthyroid cases as compared to controls. The level of serum creatinine was also significantly decreased in subclinical hyperthyroid subjects however the decrease in overt hyperthyroidism was highly significant in comparison of Euthyroids. Same kind of pattern was observed in eGFR. eGFR was also statistically significantly increased in both subclinical and overt hyperthyroid subjects. Level of urinary NGAL was also statistically significantly increased in subclinical and overt hyperthyroid subjects [Table/Fig-2].

All the biomarkers of kidney function were compared with the parameters of thyroid function for statistical correlation through Pearson Correlation Coefficient.

The serum creatinine was inversely correlated to the increase in T_3 in both subclinical and overt hyperthyroidism. The eGFR was also increased in both the situations and was statistically correlated to the hike in T_3. The urinary NGAL was increased in subclinical and overt hyperthyroidism and showed a strong positive correlation with T_3. Same kind of results was observed with thyroxine also. The serum creatinine was negatively correlated to the increase in T_4 in both subclinical and overt hyperthyroidism. The eGFR was also increased in both the situations and was statistically correlated to the hike in T_3. The urinary NGAL was increased in subclinical and overt hyperthyroidism and showed a strong positive correlation with T_3 [Table/Fig-3,4].

Parameters	Euthyroid (1)	Subclinical hyperthyroid (2)	Two sample t-test with p-value (1) vs. (2)	Overt hyperthyroid (3)	Two sample t-test with p-value (1) vs. (3)
T_3 (ng/mL)	1.02±0.12	1.09±0.26	(p>0.05)	2.63±0.59	**(p<0.0001)**
T_4 (µg/dL)	8.04±1.9	9.80±1.52	**(p<0.0001)**	15.09±2.15	**(p<0.0001)**
TSH (µIU/mL)	2.55±1.14	0.24±0.09	**(p<0.0001)**	0.16±0.05	**(p<0.0001)**

[Table/Fig-1]: Comparision of T_3, T_4 and TSH in euthyroid, subclinical hyperthyroid and overt hyperthyroid patients.
T_3: Tri idothyronine; T_4: Thyroxine; TSH: Thyroid stimulating hormone
p<0.05, p<0.001=significant result

Parameters	Euthyroid (1)	Subclinical hyperthyroid (2)	Two sample t-test with p-value (1) vs. (2)	Overt hyperthyroid (3)	Two sample t-test with p-value (1) vs. (3)
Urea (mg/dL)	19.99±5.38	17.32±3.51	**(p<0.0001)**	13.52±2.78	**(p<0.0001)**
Creatinine (mg/dL)	0.87±0.25	0.58±0.13	**(p<0.0001)**	0.29±0.10	**(p<0.0001)**
eGFR (mL/min)	97.97±25.92	119.57±11.31	**(p<0.0001)**	155.12±19.75	**(p<0.0001)**
Urinary NGAL (ng/mL)	21.94±18.06	30.24±8.63	**(p<0.0001)**	44.05±13.02	**(p<0.0001)**

[Table/Fig-2]: Comparision of different biochemical parameters of kidney function in euthyroid, subclinical hyperthyroid and overt hyperthyroid patients.
eGFR: Estimated glomerular filtration rate; NGAL: Neutrophil gelatinase associated lipocalin
p<0.05, p<0.001=significant result

	Urea	Creatinine	Urinary NGAL	eGFR
T_3	r=-0.1928	r=-0.8226*	r=0.7926*	r=0.8464*
T_4	r=0.0917	r=-0.9566*	r=0.9233*	r=0.9656*
TSH	r=0.0917	r=-0.0521	r=0.1043	r=0.0292

[Table/Fig-3]: Correlation between the markers of thyroid and kidney function in subclinical hyperthyroidism.
*p<0.05, significant result

	Urea	Creatinine	Urinary NGAL	eGFR
T_3	r=-0.1034	r=-0.7574*	r=0.9718*	r=0.9451*
T_4	r=-0.1096	r=-0.7169*	r=0.9928*	r=0.9734*
TSH	r=0.1061	r=0.0629	r=-0.0372	r=-0.013

[Table/Fig-4]: Correlation between the markers of thyroid and kidney function in overt hyperthyroidism.
*p<0.05, significant result

DISCUSSION

The aim of present study was to evaluate the consequence of subclinical hyperthyroidism and overt hyperthyroidism on markers of kidney function and to compare them with euthyroids.

A significant decrease in the levels of serum creatinine and blood urea in both the groups' i.e., subclinical and overt hyperthyroidism was observed. When compared with euthyroids, all these changes were statistically significant. Similar results of decrease in serum creatinine have been reported [23-25]. The decrease in creatinine and urea may be due to increase in RBF and GFR and reduced muscle mass. The rise in eGFR was found in this study also. Hyperthyroidism is known to cause increased RBF and GFR [2].

Thyroid hormones results in increased cardiac output by positive chronotropic [3] and inotropic effects [4] as well as a reduction in systemic vascular resistance [5]. Indirectly, Nitric oxide production is increased by high arterial pressure related endothelial shear stress [26]. This is accompanied by an enhancement of endothelium-dependent flow-mediated vasodilation in hyperthyroidism. Collectively, these factors cause an increased vasodilatation inside the kidney and decreased vasoconstriction, contributing a net raise in RBF. Increased RBF results rise in GFR but there are some other causes also for the increase in GFR. Activation of Renin-Angiotensin-Aldosterone System (RAAS) also contributes to the rise in GFR. In hyperthyroidism, there's increased stimulation of RAAS by increased density of β-adrenergic receptors within the renal cortex [27].

Thyroid hormones induce the synthesis of the most of the components of RAAS like the plasma renin, angiotensin II, and level of serum angiotensin converting enzymes. There is also a raise in angiotensinogen synthesis and increased density of angiotensin receptors [28]. All these factors cause increased RAAS activity which in turn leads to afferent arteriolar vasodilatation and efferent arteriolar vasoconstriction resulting in increased filtration pressure. All the above alterations lead to boost in the GFR along with the role of increased RBF.

Urinary NGAL is proved to be early novel marker in AKI as well as CKD [29-32]. The present study shows that there was significant increase in urinary NGAL in both subclinical and overt hyperthyroidism. NGAL is a small protein that is easily excreted and detected in the urine. NGAL is released by damaged kidney by inborn and acquired inflammatory immune responses. Urinary excretion of NGAL is increased in hyperthyroidism resulting from glomerular basement membrane disruption and tubular damage due to hyperfiltration, hypertrophy, and hyperplasia.

The present study demonstrates that there is notable change in the level of creatinine and eGFR in hyperthyroidism and all the changes correlated significantly with the increased levels of T_3 and T_4 apparently due to increased RBF and activation of RAAS. The rise in the level of urinary NGAL can be a sign of AKI. Although to better understand the pathophysiology of hyperthyroidism over kidney function further studies are required.

Limitation(s)

The present study was an observational study so the effect estimates in the model are based on one time observation and the statistical analysis of the results. Although the results were analysed by appropriate statistical tools, if this would have included a follow-up, the effects of hyperthyroidism on kidney could be verified.

CONCLUSION(S)

It can be concluded that with the progression of hyperthyroidism, kidney function deteriorates. As the incidences of CKD increase, patients with hyperthyroidism should be routinely screened for deteriorating renal function.

Declaration: Another article by the same group of authors can be found in (https://www.ijcmr.com/uploads/7/7/4/6/77464738/ijcmr_2184_v1.pdf).

The title is **"The Levels of Cystatin C and Markers of Kidney Function in Hypothyroidism"**. The present article and published one are derived from Ph.D. thesis **"ALTERATIONS IN BIOCHEMICAL MARKERS OF KIDNEY FUNCTION AND ITS CORRELATION WITH OXIDATIVE STRESS IN THYROID DISORDERS."** which was done by Dr. Suyash Saxena. Both these studies involve different set of subjects except the euthyroid data as these studies were done at the same time.

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