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Research Article

Evaluation of Serum Calcitonin, Creatinine and Uric Acid in Ckd Complicated By Dysthyroidism

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Abstract:

This study evaluated the serum calcitonin, creatinine and uric acid levels in chronic kidney diseases (CKD) complicated by dysthyroidism. A total of 119 participants were recruited in this study, majority of who were males. This may likely be as a result of the bread winning role of men and hence are often economically empowered to seek health care. The higher proportion of males in this study could reflect a positive change in health seeking behavior of the male gender. Majority of the participants in this study were between the ages of 30 to 68 years with a median of 49 years. The high prevalence of CKD among this age group can be attributed to underlying pathologies such as hypertension, diabetes or both. Result from this study showed a significant increase ($p \leq 0.05$) in serum calcitonin,TSH, creatinine and uric acid levels of CKD subjects when compared to the control and a significant decrease ($p \leq 0.05$) was observed in serum T_3 and T_4 levels of CKD subjects were observed.T_3 value showed significant decrease when the value of T_3 gotten from stage 3,4 and 5 of CKD subjects were compared.

Introduction

Chronic kidney disease (CKD) encompasses a vast array of different pathophysiologic processes associated with abnormal kidney function and a progressive decrease in glomerular filtration rate (GFR). It is a global public health problem associated with premature mortality, decreased quality of life and a high cost of healthcare (Kefale *et al*., 2019).

Chronic kidney disease is a major public health problem and its prevalence has reached epidemic proportions in some countries. It has become a significant cause of morbidity and mortality. Impaired kidney function can affect thyroid hormone metabolism and hypothyroidism, nonthyroidal illness as well as hyperthyroidism have been reported in CKD patients. Thyroid dysfunction may worsen the morbidity in CKD patients and increase cardiovascular mortality. Low T3 has been found to be an independent predictor of cardiovascular mortality in CKD patients (Carrero et al., 2007).

Chronic kidney disease (CKD) is recognized as a major health problem affecting approximately 13% of the US population (Coresh *et al.*, 2007). Numbers of prevalent CKD patients will continue to rise, reflecting the growing elderly population and increasing numbers of patients with diabetes and hypertension. As numbers of CKD patients increase, primary care practitioners will be confronted with management of the complex medical problems unique to patients with chronic renal impairment. As well documented in the literature, the nephrologist rarely manages the medical needs of CKD patients until renal replacement therapy is required.

There is a relationship between plasma levels of T3 and various markers of inflammation, nutrition and endothelial activation in patients with CKD (Carrero *et al.*, 2006). These patients show an association between low serum values of T3 with inflammation markers (elevated levels of high sensitivity C-reactive protein, hs-CRP, interleukin 6, IL-6, and vascular adhesion molecule-1, VCAM-1) and nutrition (decrease of albumin and IGF-1) and cardiac function. The lower concentration of T3 the greater degree of inflammation, poorer nutritional status and worse cardiac function. Therefore, low T3 is associated with a survival disadvantage. The relationship between survival and T4 is less defined. A reduction in total T3, but not in free T3, concentrations was associated with an increased all-cause and cardiovascular mortality in euthyroid CKD patients (Carrero *et al.*, 2006). Total and free T3 behave as survival markers in patients with CKD both in HD (201) and in PD (Zoccali *et al.*, 2006). For these reasons, some

authors have recommended measuring T3 levels to assess the relationship between thyroid dysfunction and risk of mortality in this population. Finally, it has been recently reported that low levels of T3 before renal transplantation are associated with decreased survival of the graft (Rotondi *et al.,* 2008). Several factors, including malnutrition and intercurrent processes, may be involved in the reduction of serum T3 in uraemic patients. Fasting and disease alter iodothyronine deiodination, thus reducing peripheral production of T3. The presence of chronic protein malnutrition is associated with a reduction of binding protein synthesis and could reduce plasma total T3 concentration. TNFα and interleukin-1 inhibit the expression of type 1 5'-deiodinase, enzyme responsible for T4 to T3 conversion in peripheral tissues. This would explain how chronic inflammation and vascular damage associated to CKD interfere with the normal process of T3 synthesis from T4 (Enia *et al.,* 2007; Carrero *et al.,* 2006).

Materials and Method

Study Area:

This study was carried out at the Irrua specialist Teaching Hospital, Edo State.

Study Population:

The study population for this research are renal unit patients attending clinic at the Irrua Specialist Teaching Hospital, Benin City, Edo state, Nigeria.

Inclusion Criteria: (Test Group)

Adult male and female subjects with renal insufficiency

Exclusion Criteria:

Male and Female subjects without renal insufficiency

Control Group:

Apparently healthy male and female subjects

Sample Size:

The sample size (N) was calculated using prevalence from previous studies done on prevalence of chronic Kidney diseases among civil servants in Bayelsa ,Nigeria, which was 7.8% (Egbi *et al.,* 2014). The sample size for this study was obtained using the formula described by Daniel *et al.,* (1995).

Sample Collection:

5ml of blood sample was collected from the cubital fossa of each subject by an experienced Phlebotomist using aseptic collection procedure as described by Cheesbrough (2000), dispensed into plain sample container and allowed to clot.

Laboratory Analysis

Determination of Thyroid Stimulating Hormone Using ELISA Method (Uotila, 1981)

All reagents and clinical specimen were allowed to attain room temperature (18^0-22^0).A known volume, 50µl each of standards, specimens and controls were dispensed into appropriate microplate wells. 100µl of Enzyme Conjugate Reagent was dispensed into each wells and then mixed thoroughly for 30 seconds. The strip was then covered with a lid and then Incubate at room temperature (18-22^{o}C) for about 60minutes.The wells were washed 5 times with 300ul of working washing solution. The plate was firmly tapped against absorbent paper to remove all the residual water droplets. 100ul of TMB solution was then added into each of the wells and mixed gently for 5 seconds. It was later incubated at room temperature for 20 minutes in a dark place. The reaction was stopped by adding 100ul of stop solution to each well, mixed and then read. The optical density was read at 450nm with a microtiter well reader.

Determination of Total Thyroxine (T_4) Using ELISA Method (Wisdom, 1976)

All reagents and clinical specimen were allowed to attain room temperature (18^0-22^0).A known volume, 50µl each of standards, specimens and controls were dispensed into appropriate microplate wells. 100µl of Enzyme Conjugate Reagent was dispensed into each wells and then mixed thoroughly for 30 seconds. The strip was then covered with a lid and then Incubate at room temperature (18-22^{o}C) for about 60minutes.The wells were washed 5 times with 300ul of working washing solution. The plate was firmly tapped against absorbent paper to remove all the residual water droplets. 100ul of TMB solution was then added into each of the wells and mixed gently for 5 seconds. It was later incubated at room temperature for 20 minutes in a dark place. The reaction was stopped by adding 100ul of stop solution to each well, mixed and then read. The optical density was read at 450nm with a microtiter well reader.

Determination of total triiodothyronine (T_3) using ELISA Method (Wisdom, 1976)

In the T_3 EIA, a certain amount of anti-T_3 antibody is coated on microtiter wells. A measured amount of patient serum, and a constant of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the anti-T_3antidody is bound to the second antibody on the wells, and T3 and conjugated T_3 compete for the limited binding sites on the anti-T3 antibody. After incubation at room temperature, the wells are washed to remove unbound T_3 conjugate. Addition of TMB solution results in the development of blue colour. The colour development is stopped with the addition of 2 N HCL, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T3 in the sample.

Assay procedure for total triiodothyronine (T_3)

All reagents and clinical specimen were allowed to attain room temperature (18^0-22^0).A known volume, 50µl each of standards, specimens and controls were dispensed into appropriate microplate wells and mixed for 10 seconds. 100µl of Enzyme Conjugate Reagent was dispensed into each wells and then mixed thoroughly for 30 seconds. The strip was then covered with a lid and then Incubated at room temperature (18-22^{o}C) for about 60minutes.The wells were washed 5 times with 300ul of working washing solution. The plate was firmly tapped against absorbent paper to remove all the residual water droplets. 100ul of TMB solution was then added into each of the wells and mixed gently for 5 seconds. It was later incubated at room temperature for 20 minutes in a dark place. The reaction was stopped by adding 100ul of stop solution to

each well, mixed gently for about 15 seconds and then read. The optical density was read at 450nm with a microtiter well reader.

Calcitonin Estimation

- Wells for diluted standard, blank and sample were determined. 5 wells for standard point prepared, I well for blank. Add 50PL each of dilutions of standard, blank and samples into the appropriate wells, respectively. And then 50 microlitre of detection reagent A were added to each well immediately shake the plate gently using a microplate shaker. It was covered with a plate sealer. Solution was incubated for 1 hour at 37^0C. Detection reagent A may appear cloudy. Allow to assume room temperature and allow to mine gently until solution appear uniform.
- Solution was aspirated and washed with 350 microlitre of IX wash solution to each well using a squirt bottle, multi channel pipette manifold dispenser or auto washer, and it is allowed to slit for 2 minutes. Remaining liquid from all wells are removed by snapping the plate onto absorbent paper. This is repeated thrice after the last wash, all remaining wash buffer are removed by decanting plate is inverted and blotted using absorbent paper.
- 100 microlitre of detection reagent B is added to each well solution is incubated for 30 minutes at 37^0C after covering it with plate sealer.
- The aspiration / wash process is repeated for 5 times as conducted in step 2.
- 90 microlitre of substrate solution is added to each well, after which it is covered with a plate sealer incubation for 20 minutes at 37^0c is carried out protect against light. The liquid will turn blue by the addition of substrate solution.
- 50 microlitre of stop solution was added to each well. The liquid turned yellow by addition of stop solution liquid was thoroughly mixed by tapping the side of the plate. if colour change does not appear uniform, side of plate will be tappeds to ensure thorough mixing.
- Ensure no drop of water and finger print on the bottom of the plate and no bubble present on the surface of liquid then run microplate reader and conduct measurement at 450 nanometers immediately.

Estimation of Uric Acid (Young *et al.,* 1974**)**

- 20 microlitre of sample pipetted into sample test tube
- 20 microlitre of uric acid standard was pipetted into test tube labeled standard
- 1 Millilitre of reagent pitetted into both tubes containing both sample and standard.
- 1 Millilitre of reagent pipetted into a third test tube to at as reagent blank
- It was mixed and incubated for 5 mins at 37^0C and absorbance was read at 520 nm against a reagent blank within 30 minutes.

Assay for Creatinine by Jaffe Reaction (Taussky, 1961).

- 100 microlitre of sample pipetted into sample test tube
- 100 microlitre of creatinime standard pipetted into the test tube labeled standard.
- A third test tube derived of sample or creatinine standard is labeled Reagent blank
- 1 Millilitre of picric acid is added to respective test tubes
- 1 Millilitre of sodium hydroxide is added to the three test tubes
- It was mixed and incubated at room temperature for 10minutes, and read at 500nm.

Data Analysis

Data was collected, screened for completeness and entered into the SPSS version 20 for analysis. The mean, standard error of mean, and probability value (p-value) was gotten using chi-square, also correlation analysis will be done using the Pearson's correlation and data will be presented as tables and bar graphs. Differences will be considered statistically significant at an error probability (P) of less than or equal to 0.05 ($p \leq 0.05$) and not significant at $p \geq 0.05$.

Results

Table 1: Comparison of Serum Levels of Calcitonin, Thyroid Hormones, Creatinine and Uric Acid among Controls and CKD Subjects

Parameters	Normal Range	Control Subjects (n = 50)	CKD Subjects (n = 70)	t value	p value
Calcitonin (pg/L)	Males==19ng/L Females=14ng/L	15.4±0.22	23.9±0.40	4.29	0.001
T3 (ng/mL)	0.6 - 2.0	1.49±0.0498	0.774±0.0317	12.8	0.001
T4 (mμg/dL)	6.0 - 12	9.03±0.256	7.31±0.195	5.43	0.001
TSH (miu/mL)	0.4 - 4.2	2.31±0.218	8.25±0.525	9.2	0.001
Creatinine (mg/dL)	0.7 - 1.4	0.758±0.0376	8.19±0.585	10.8	0.001
Uric Acid (mg/dL)	3.4 - 6.5	4.98±0.190	15.4±0.935	9.42	0.001

Table 2: The serum levels of measured parameters based on stages of renal disease among study participants

Parameters	Normal Range	Stage 3	Stage 4	Stage 5	F value	p value
Calcitonin (pg/L)	=10	85.7±17.3	109±16.8	74.5±17.8	1.02	0.367
T3 (ng/ml)	0.6 - 2.0	0.773±0.0771	0.731±0.0419	0.48±0.0288	3.02	0.056
T4 (mμg/dl)	6.0 – 12	12.9±5.45	8.06±0.255	5.35±0.299	2.38	0.101
TSH (miu/ml)	0.4 - 4.2	5.63±0.620	15.2±4.72	6.91±0.644	1.56	0.217
Creatinine (mg/dl)	0.7 - 1.4	7.04±0.823	8.33±0.797	9.11±1.41	0.758	0.473
Uric Acid (mg/dl)	3.4 - 6.5	15.6±1.74	14.1±0.993	15.7±2.35	0.401	0.671

Discussion

A total of 119 participants were recruited in this study, majority of who were males. This may likely be as a result of the bread winning role of men and hence are often economically empowered to seek health care. The higher proportion of males in this study could reflect a positive change in health seeking behavior of the male gender. Majority of the participants in this study were between the ages of 30 to 68 years with a median of 49 years. The high prevalence of CKD among this age group can be attributed to underlying pathologies such as hypertension, diabetes or both (Lesley et al., 2007).

There are no reports on local prevalence of CKD. Regionally, a study done in Nigeria by Afobai et al, gave a prevalence of 12.4% in patients aged between 20 years and 74 years who had CKD with demonstrable association with modifiable risk factors such as hypertension, diabetes and obesity. Same study was done in the US, which found that the prevalence of albuminuria and decreased GFR increased from 1988-1994 to 1999-2004 and this was attributed to an increase in diabetes, hypertension and high body mass index. It has been estimated that the prevalence of CKD among adults in the United States has risen to 13% (Boyd et al., 2007). Changes in life style in our population is responsible for increase in diabetic and hypertensive cases, which could also play a role in rising CKD cases. Apart from the above named risk factors, HIV associated nephropathy also account for rising CKD cases.

CKD was classified in this study into different stages using the National Kidney Foundation guidelines (Coresh et al., 2007), with estimated GFR using the 4-varaible MDRD formula. Majority of the participants were in CKD stage 4 (45.2%), with none of these participants being in stage 0 to 2. This could be attributed to delay in seeking medical care, hence patients accessed health care when the disease has progressed to more severe stages. The clinicians use creatinine levels and only estimate GFR when the former are elevated. It is recommended that estimation of GFR should be carried out in all cases where creatinine levels have been determined. According to United States population survey data, at least 6% of the adult population has CKD at stages 1 and 2.

Subclinical hypothyroidism is defined biochemically as a state characterized by elevated TSH but normal FT4 levels. Studies have shown that subclinical hypothyroidism is more common among older adults. Laboratory tests show low Thyroid function in 40% of the general population (Salvagno et al., 2008). Increased rates of thyroid dysfunction have been reported in patients with ESRD and newer studies shown an increased rate of subclinical hypothyroidism in CKD patients not requiring chronic dialysis (Chonchol *et al.*, 2015). This study reported primary and subclinical hypothyroidism at 19.7% and 31.9 % of the participants respectively. Michel Chonchol *et al*., 2015 also found that the prevalence of hypothyroidism was common at 18% of all patients with CKD not requiring dialysis.

Both primary and subclinical hyperthyroidism was reported in same proportion (2.5%) of the participants in this study. The finding of hyperthyroidism is uncommon and manifest with same signs and symptoms as uremia. Niemczyk *et al* found multinodular goiter and grave's disease in patients with ESRD and hyperthyroidism. However, not many studies have been done on hyperthyroidism in CKD.

Sick Euthyroid syndrome (SES) is defined as biochemical changes in thyroid hormones in the absence of underlying intrinsic thyroid dysfunction. It is characterized by low T3 and fT3 with an increase in rT3 and TSH. While T4 may be low or normal. In this study, 4.2% of the participants were found to have sick euthyroid syndrome. This is primarily due to impaired peripheral tissue conversion of T4 to T3 by the deiodinase enzymes. These low T3 levels have been reported in other studies (Verger, *et al*, 1987).

Serum calcitonin has been suggested as a marker of various clinical conditions including renal failure (Ardailou *et al*, 1990). This study showed a significantly high level of serum calcitonin in male subjects as compared to their female counterpart, including control subjects. These findings are supported by earlier studies (Suzuki, H, 1998). Several other investigators have reported a significant rise in serum calcitonin levels in subjects with renal failure. Apart from calcitonin derangement, many abnormalities of endocrine function are commonly observed in renal failure especially end stage renal diseases (Morrison, *et al.*, 1995). Since the kidney play a key role in calcitonin degradation significantly (Mahoney, *et al*., 1988)

Conclusion

- Most of the study participants were euthyroid
- About 52% of participants in this study have abnormalities in thyroid function
- Primary and subclinical hyperthyroidism accounted for 5% of entire study participants, which is within rate of prevalence previously reported
- Derangement in thyroid hormone profile increased with severity of chronic kidney disease.
- The most common deranged thyroid hormone was low T3 values

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