Evaluation of Indoxyl Sulfate in Chronic Kidney Disease associated with Left Ventricular Hypertrophy

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ABSTRACT

Chronic kidney disease CKD is widely prevalent globally and Left ventricular hypertrophy (LVH) is very common among CKD patients and contributes to CV mortality which can be monitored by measuring the levels of indoxyl sulfate (IS). Study the correlation of indoxyl sulphate and the degree of left Ventricular hypertrophy in CKD patients. A case control study was done on 90 patients with Chronic kidney disease CKD, 45 of them with who were recruited the Imamian Al-Khadhemian Medical City and Baghdad Hospital, Baghdad, Iraq between January, and September 2020. The levels of indoxyl sulfate and other parameters were measured in the serum of Left ventricular hypertrophy (LVH) and compared with those with a no evidence of LVH who considered as BMI and sex matched control group. Indoxyl sulfate (IS) levels in patients with chronic kidney disease and an evidence of lift ventricular hypertrophy (LVH-CKD) group were significantly (p=0.001) higher than those of patients with chronic kidney disease without an evidence of lift ventricular hypertrophy (NLVH-CKD) and IS levels were associated significantly with the severity of LVH. The possibility to use IS as a marker for early diagnosis of LVH in patients suffered from CKD and it can be used to determine the severity of LVH in those patients.

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Table 1: General characteristics of the study groups.

|                     | LVH-CKD | NLVH-CKD | P value |
|---------------------|---------|----------|---------|
| Number of cases     | N 45    | 45       |         |
| Gender              | Male 32 | 33       | 0.814   |
|                     | Female 13 | 12       |         |
| Diabetes Mellitus (DM) | DM 24 | 17       | 0.138   |
|                     | Non-DM 21 | 28       |         |
| Hypertension (HTN)  | HTN 40 | 5        | <0.001  |
|                     | Non-HTN 15 | 30       |         |
| BP                  | Pulse pressure 66.69±8.1 | 46.44±5.96 | <0.001  |
|                     | Systolic 176.16±13.05 | 134.47±25.01 | <0.001  |
|                     | Diastolic 103.2±17.63 | 80.53±28.42 | <0.001  |
| Address             | Kerkh 15 | 16       | 0.844   |
|                     | Resafa 22 | 23       |         |
|                     | Outskirts 8 | 6        |         |
| Smoking             | Chronic 7 | 0        | 0.012   |
|                     | Current 7 | 4        |         |
|                     | ex-smoker 18 | 30       |         |
|                     | Never 13 | 11       |         |
| Causes of CKD       | DM 12 | 10       | 0.036   |
|                     | HTN 20 | 12       |         |
|                     | History 7 | 5        |         |
|                     | Other 6 | 18       |         |
| Age (year)          | -     | 37.04±11.47 | 39.56±12.18 | 0.317   |
| BMI (Kg/m^2)        | -     | 26.32±3.98 | 26.29±3.64 | 0.967   |
| CKD-duration        | -     | 28.23±25.41 | 22.59±25.68 | 0.3     |

Figure 1: Age and BMI levels in all studied groups.

LVH in patients with CKD known also as uremic cardiomyopathy as it occurs even in the absence of anemia, hypertension or other conventional risk factors (Wolf et al., 2000). Furthermore correction of some risk factors like anemia did not result in prevention of LVH (Foley et al., 2000). Indoxyl sulfate is considered as a one of the most important uremic solute that derived from bacterial action on dietary protein specifically tryptophan. In CKD patients, the diminished GFR lead to a reduction in the rate of IS excretion, that causes a gradual elevation in serum IS levels with both pre- and post-dialysis which associated with CVD. It was reported that IS affect CVS by its role in the development of atherosclerosis due to its induction effect vascular endothelial cell that lead to a dysfunction as a consequence of enhancing oxidative stress (Yang et al., 2015).

MATERIALS AND METHODS

This case control study was carried out on a 90 patients age range from 18 to 60 years, consist of patients diagnosed as CKD, all this patient has LVH compare to 45 patient have CKD only as control were enrolled serially in the study. Age, gender and body mass index (BMI) matched control subjects were taken from the same socioeconomic population of cases. All persons in control group were having CKD but not LVH. All patients subjected to the
study were notified and informed about the study goals. All biochemical tests were performed free of charges for all participants and all results obtained were provided to them. All patients were attended from the Imamian Al-Khadhemian Medical City and Baghdad Hospital. Patients and controls were with a comparable age. The practical part of the study was conducted at Department of Chemistry and Biochemistry, College of Medicine and Middle East Clinical Laboratory, Baghdad- Iraq. The study has approved by the Institutional Review Board (IRB) of the College of Medicine, University of Al-Nahrain, Baghdad, Iraq. In addition, an informed written consent for participation in the study was signed by investigated subjects according to the Helsinki principles.

Sample collection and preparation

Samples of seven milliliters of blood were collected from all participants in plain tube and left to clot for 30 min at room temperature then were centrifuged at 4000 rpm (1252 x) g for 10 min, the separated sera were divided into small aliquots and store at (-20°C) until assayed for the evaluation of Indoxyl sulphate (IS). IS levels were estimated by enzyme-linked immunosorbent assays (ELISAs) with kits obtained from Elabscience (USA) according to the manufacturer instructions.

Statistical analysis

Results obtained in the present study were analyzed using the SPSS software 20. Numerical variables were expressed as mean ± SD and all statistical comparisons were made by means of independent t-test and ANOVA test with P ≤0.05 was considered statistically significant. Categorical variables were expressed as numbers and analyzed by cross tabulation to assess the frequency and percentage of each variable among studied groups. The correlation was done between all parameters using Pearson correlation test and Chi square to test the relationships between categorical variables with the measurement of Phi that is considered as a chi-square based measure of association to indicate the strength of the association (given that values ranged from 0–0.5 considered as weak association while values above 0.5 considered as strong association) in addition to p values. The normality of distribution was checked using Shapiro- Wilk and Kolmogorov- Smirnov tests.

RESULTS AND DISCUSSION

In this study, some characteristics of the studied groups summarized in Table 1 and Figure 1 that assessed by analyzing questionnaire answered by a direct interview with all subjects as indicated in appendix 1.

Table 1 showed that the group of patients with chronic kidney disease and an evidence of left ventricular hypertrophy (LVH-CKD) were age, sex, BMI, and address matched to those with chronic kidney disease without an evidence of left ventricular hypertrophy (NLVH-CKD). Moreover, there were non significant differences in a duration of CKD and incidence of DM between the two studied groups whereas significant differences were obtained in the smoking habit, causes of CKD, pulse pressure, systolic and diastolic blood pressure in addition to hypertension incidence between the studied groups. According to results illustrated in Table 2 and Figure 2, Indoxyl sulfate (IS) levels in patients with chronic kidney disease and an evidence of left ventricular hypertrophy (LVH-CKD) group were significantly (p=0.001) higher than those of patients with chronic kidney disease without an evidence of left ventricular hypertrophy (NLVH-CKD). Results postulated in Table 2 also revealed that there were non-significant differences between male and female patients in the levels of Indoxyl sulfate (IS).

On the other hand, Indoxyl sulfate (IS) levels in diabetic group were significantly higher than those of non-diabetic group whereas non-significant differences were obtained between hypertensive and normotensive patients in the levels of Indoxyl sulfate (IS). Furthermore, there were non-significant differences between smoking habit patients groups in the levels of Indoxyl sulfate (IS). Geographical distribution showed non-significant effect on the levels of Indoxyl sulfate (IS).

Results illustrated in Table 3 revealed non significant differences in levels of Indoxyl sulfate among
Table 2: Comparison between all studied groups in the levels of Indoxyl sulfate (IS).

| Group           | Mean ± SD    | P*  |
|-----------------|--------------|-----|
| LVH-CKD (n=45)  | 767.98 ± 356.59 | 0.001 |
| NLVH-CKD (n=45) | 551.97 ± 575.35 | 0.89  |
| Male (n=65)     | 664.31 ± 511.02 | 0.01  |
| Female (n=25)   | 648.7 ± 5368.79 | 0.715 |
| DM (n=41)       | 789.86 ± 548.58 | 0.001 |
| Non-DM (n=49)   | 543.77 ± 376.29 | 0.715 |
| HTN (n=55)      | 674.65 ± 381.27 | 0.819 |
| Non-HTN (n=35)  | 636.92 ± 596.58 | 0.819 |
| Non smoker (n=24) | 645.6 ± 403.78 | 0.629 |
| Ex-smoker (n=48) | 679.87 ± 537.42 | 0.629 |
| Chronic smoker  | 747.37 ± 386.66 | 0.629 |
| Kerkh (n=31)    | 602.03 ± 444.22 | 0.001 |
| Resafa (n=45)   | 673.7 ± 513.31 | 0.001 |
| Outskirts (n=14) | 744.18 ± 416.6 | 0.001 |
| DM (n=22)       | 1000.99 ± 626.87 | 0.001 |
| HTN (n=32)      | 60.18 ± 325.32 | 0.001 |
| History (n=12)  | 576.07 ± 296.25 | 0.001 |
| Other (n=24)    | 469.06 ± 409.19 | 0.001 |

Table 3: Comparison between CKD stages in the levels of Alpha-Klotho (αKlotho), Indoxyl sulfate (IS) and Malondialdehyde (MDA).

| Group            | mean ± SD    | P*  |
|------------------|--------------|-----|
| CKD stages       |              |     |
| Stage 2 (n=2)    | 341.99 ± 132.03 | 0.106 |
| Stage 3a (n=12)  | 369.31 ± 227.02 | 0.028 |
| Stage 3b (n=8)   | 573.71 ± 415.78 | 0.028 |
| Stage 4 (n=24)   | 777.55 ± 637.13 | 0.028 |
| Stage 5 (n=44)   | 705.25 ± 407.86 | 0.028 |
| LHV severity     |              |     |
| Mild (n=35)      | 706.09 ± 327.98 | 0.028 |
| Severe (n=10)    | 984.60 ± 385.05 | 0.028 |

patients with different CKD stages while significant elevations were obtained in Indoxyl sulfate levels in CKD patients with severe LVH when compared with those of CKD patients with mild LVH. Results illustrated in Table 4 revealed that the levels of urea, creatinine, uric acid, calcium, potassium, phosphate and fasting blood sugar were significantly elevated in patients with chronic kidney disease with an evidence of left ventricular hypertrophy (LVH-CKD) and those without an evidence of left ventricular hypertrophy (NLVH-CKD). On the other hand, levels of estimated glomerular filtration rate (eGFR) and hemoglobin were reduced significantly in LVH-CKD patients than those of NLVH-CKD. Additionally, sodium and chloride levels showed non-significant difference between the studied groups. Results obtained in Table 5 demonstrated that there were negative significant correlations between the age and BMI whereas all other correlations were non-significant. Data illustrated in Table 6 showed that there were non-significant correlation between both of IS with renal function tests measured. Results illustrated in Table 7 revealed that there were non-significant correlations between Alpha-Klotho (αKlotho), Indoxyl sulfate (IS), Malondialdehyde (MDA) levels and the levels of serum ions (Ca, Na, Cl, PO4). Results postulated in Table 8 showed that the incidence of LVH was associated significantly with the levels of Indoxyl sulfate (IS). IS showed to be significantly associated with DM and
Table 4: Laboratory characteristics in patients with chronic kidney disease and an evidence of left ventricular hypertrophy (LVH-CKD) and patients with non LVH-CKD (NLVH-CKD).

| Variable | LVH-CKD (n=45) | NLVH-CKD (n=45) | p-value |
|----------|----------------|-----------------|---------|
| Urea     | 211.62 ± 67.66 | 115.42 ± 50.46  | <0.001  |
| Creatinine | 6.80 ± 2.59    | 2.99 ± 1.92     | <0.001  |
| eGFR     | 11.57 ± 6.68   | 31.84 ± 16.31   | <0.001  |
| UA       | 9.03 ± 2.13    | 4.94 ± 1.95     | <0.001  |
| Ca       | 10.92 ± 1.52   | 9.70 ± 0.84     | <0.001  |
| Na       | 138.88 ± 5.39  | 140.51 ± 4.18   | 0.114   |
| K        | 4.78 ± 0.75    | 4.18 ± 0.39     | <0.001  |
| Cl       | 101.44 ± 5.25  | 100.06 ± 4.53   | 0.186   |
| PO4      | 5.34 ± 0.83    | 4.72 ± 0.83     | 0.001   |
| HB       | 8.79 ± 1.58    | 11.74 ± 1.41    | <0.001  |
| FBS      | 161.80 ± 60.14 | 136.40 ± 47.41  | 0.029   |

Table 5: The correlations between Klotho, IS, MDA, hemoglobin (HB) and fasting blood sugar (FBS) levels and age, body mass index (BMI) and the duration of CKD in patients with LVH-CKD.

|          | HB      | Age | BMI   | FBS   | How long have CKD (months) |
|----------|---------|-----|-------|-------|----------------------------|
| IS       | r 0.117 | 0.004 | 0.061 | 0.169 | 0.002                      |
|          | p 0.445 | 0.979 | 0.689 | 0.267 | 0.990                      |
| HB       | r - | 0.144 | -0.124 | -0.055 | 0.017                      |
|          | p - | 0.346 | 0.416 | 0.719 | 0.911                      |
| Age      | r - | - | -0.446 | 0.011 | 0.118                      |
|          | p - | - | 0.002 | 0.945 | 0.441                      |
| BMI      | r - | - | - | -0.031 | -0.045                    |
|          | p - | - | - | 0.838 | 0.768                      |
| FBS      | r - | - | - | - | -0.149                    |
|          | p - | - | - | - | 0.329                      |

non-significantly associated with the stages of CKD and HTN. Indoxyl sulfate is considered as one of the most important uremic solute that derived from bacterial action on dietary protein specifically tryptophan. In CKD patients, the diminished GFR lead to a reduction in the rate of IS excretion, that causes a gradual elevation in serum IS levels with both pre and post dialysis which associated with CVD. It was reported that IS affect CVS by its role in the development of atherosclerosis due to its induction effect vascular endothelial cell that lead to a dysfunction as a consequence of enhancing oxidative stress (Yang et al., 2015) which is consistent with the results obtained in the present study that showed significant elevation in their levels in LVH-CKD patients in a comparison with those of NLVH-CKD. Several studies demonstrated that IS levels elevated significantly in CKD patients and the increment is always parallel to the severity of the disease and related to the complications occur such as the cardiovascular complication.

In addition to involvement in the progression of CKD, a several clinical researches supports the idea that indoxyl sulfate may also contribute to CVD in the CKD patients and proposed that IS levels can be considered as a valuable marker in predicting cardiovascular events in patients with advanced CKD (Lin et al., 2012; Hung et al., 2017). There are a growing number of clinical studies that supports the idea that IS regarded as a crucial factor which contributes to CVD in the CKD, and can be considered as the main link between these diseases (Kamiński et al., 2017; Hung et al., 2017). IS plays a major role...
Table 6: The correlations between Alpha-Klotho (αKlotho), Indoxyl sulfate (IS), Malondialdehyde (MDA) and renal function tests (urea, creatinine, uric acid and estimated GFR) inpatients with LVH-CKD.

|       | Urea      | Creatinine | eGFR     | UA        |
|-------|-----------|------------|----------|-----------|
| IS    | 0.862     | 0.042      | -0.098   | 0.119     |
| p     | 0.000     | 0.783      | 0.521    | 0.437     |
| Urea  | -0.022    | -0.751     | 0.000    |
| p     | 0.888     | 0.000      |
| Creatinine | -        | -0.840   |
| p     | 0.000     |
| eGFR  | -0.119    |
| p     | 0.437     |

Table 7: The correlations between Alpha-Klotho (αKlotho), Indoxyl sulfate (IS), Malondialdehyde (MDA) and Serum ions (Ca, Na, Cl, PO4).

|       | MDA       | IS         | Ca        | Na        | K       | Cl     | PO4    |
|-------|-----------|------------|-----------|-----------|---------|--------|--------|
| αKlotho | -0.415    | -0.336     | -0.263    | 0.159     | -0.216  | -0.086 | 0.031  |
| p     | 0.005     | 0.024      | 0.081     | 0.298     | 0.154   | 0.573  | 0.840  |
| MDA   | -0.328    |
| p     | 0.028     |
| IS    | -0.022    |
| p     | 0.002     |
| Ca    | 0.328     |
| p     | 0.084     |
| Na    | -0.084    |
| p     | 0.583     |
| K     | -0.501    |
| p     | 0.059     |
| Cl    | -0.008    |
| p     | 0.059     |
| PO4   | 0.785**   |
| p     | 0.000     |

Table 8: Association of Alpha-Klotho (αKlotho), Indoxyl sulfate (IS) and Malondialdehyde (MDA) with the Incidence of LVH, Stages of CKD, DM and HTN.

| Parameter | Incidence of LVH | Stages of CKD | DM | HTN   |
|-----------|------------------|----------------|----|-------|
| IS        | 0.03             | 0.077          | 0.01| 0.715 |
| Eta²      | 0.053            | 0.093          | 0.073| 0.002 |

in renal-induced systemic inflammation, immune system alteration, and oxidative stress generation in various tissues of the body (Karbowska et al., 2018).

Indoxyl sulfate showed non-significant differences in their levels between different genders and also showed that their levels were not affected by hypertension, cigarette smoking and geographic distribution of patients.

These findings are not fully agree with previous literature which demonstrated that there was a direct link between hypertension in CKD patients and IS levels as it seems to play modulatory roles in some cellular pathways in hypertensive rats through reducing Klotho expression (Adijiang et al., 2011; Yisireyili et al., 2013) which is consistent with the results discussed above about the role of Klotho in the cardiovascular complications of CKD. So, previous researches demonstrated that IS effect indirectly on blood pressure and the incidence of HTN via Klotho expression reduction expression (Adijiang et al., 2011).
CONCLUSIONS

Inoxyl sulfate can be used as a marker for early diagnosis of LVH in patients suffered from CKD and it can be used to determine the severity of LVH in those patients.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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