Int J Clin Exp Med 2018;11(9):9921-9930
www.ijcem.com /ISSN:1940-5901/IJCEM0059662

*Original Article*

# Analysis of risk factors for proximal tubule damages in 38 patients with hepatolenticular degeneration

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Received June 17, 2017; Accepted July 5, 2018; Epub September 15, 2018; Published September 30, 2018

**Abstract:** Aims: The present study is to investigate renal proximal tubule damages in hepatolenticular degeneration (HLD). Methods: A total of 38 patients with HLD who received treatment at Tianjin Medical University General Hospital between September 2003 and March 2005 were enrolled in the study. Routine tests included urine routine, renal function, electrolytes, alkaline phosphatase, calcium, phosphate, $CO_2$ combining power, and anion gap. At 24 h, urine amount, urine glucose, urine proteins, urine potassium, urine sodium, urine chloride, urine calcium, and urine phosphate were determined. Urine acidification was measured. Quantitative analysis of urinary amino acids was performed on 37 HLD patients. Results: HLD changes a number of health indexes in the patients. Urine routine test results of HLD patients are not dependent on gender. Only blood ALP and Phos levels in adult HLD patients are significantly different from youngster HLD patients. Renal tubular damages in HLD alter most renal tubular damage indexes and clinical manifestations of the patients. Conclusion: The present study demonstrates that specific indexes of renal and renal proximal tubular dysfunction may indicate renal proximal tubule dysfunctions in HLD, which are not dependent on age or gender. In addition, examination of urine amino acids at 24 h may help with the diagnosis of HLD renal tubular injuries.

**Keywords:** Hepatolenticular degeneration, renal tubule, aminoaciduria, bone metabolism, Fanconi syndrome

## Introduction

Hepatolenticular degeneration (HLD) is clearly described for the first time by Wilson in 1912, and is also named Wilson disease [1]. HLD is an autosomal recessive inherited disease that is characterized by disorders of copper metabolism. Abnormal copper metabolism results in the accumulation of copper in the body, and the amount of copper deposition in different tissues and organs is different, leading to different degrees of lesions and distinct clinical manifestations. Clinical manifestations of HLD involve multiple organs and systems [2, 3]. A report shows that 40% out of 80 HLD cases have renal involvement, and 3.99% cases have renal symptom as initial symptom [4]. Another study discovers that 33.3% out of 30 children with HLD have renal involvement [5]. In addition, copper content in renal tissues from HLD patients is 10-20 times higher than that in healthy subjects [6].

Most researchers believe that kidney damage is caused by the deposition of a large amount of copper in renal tissues. Wolff et al. demonstrate that epithelial cells in proximal and distal convoluted tubules and parietal layer of Bowman's capsule have copper particle pigmentation [7]. Yoshitoshi et al. discover that 7 out of 18 HLD patients have lesions in proximal convoluted tubules [8]. Reynolds et al. report that HLD renal damages are mainly in renal tubules, especially proximal convoluted tubules [9].

The main function of renal tubules is to reabsorb water, electrolytes, glucose, amino acids, bicarbonate, and low-molecule proteins (molecular weight < 50,000), and to excrete hydrogen, uric acid, and metabolites. One or more dysfunctions in renal tubular reabsorption or secretion can cause renal tubular diseases, including simple and complex dysfunctions. Patients with simple renal tubular diseases show reabsorption or secretion disorder of one substance, leading to nephrogenic diabetes insipidus, renal tubular acidosis, potassium losing nephropathy, salt-losing nephropathy, renal glycosuria,

vitamin D-resistant rickets, Liddle syndrome, Bartter syndrome or idiopathic hypercalcinuria, etc. Patients with complex renal tubular diseases have reabsorption or secretion disorder of multiple substances, leading to Fanconi syndrome, rickets, lithangiuria or kaliopenia, etc. HLD can cause both simple and complex renal tubular diseases. In the present study, we investigate renal proximal tubule damages in HLD.

## Materials and methods

### *Patients*

A total of 38 patients with HLD who received treatment at Tianjin Medical University General Hospital between September 2003 and March 2005 were enrolled in the study, including 17 males and 21 females. The age range of the patients was 13-55 years, and the average age was 25.13 ± 9.67 years. All patients were diagnosed to have HLD, and all cases met HLD diagnostic criteria [10, 11]. Other renal tubular diseases were excluded. The diagnostic criteria of HLD included: i) ceruloplasmin < 0.2 g/L; ii) serum copper < 11 μmol/L; iii) positive corneal Kayser-Fleischer ring; iv) urinary copper > 1.56 μmol/24 h; v) positive family history; vi) hepatic lesions; vii) extrapyramidal manifestations; viii) renal changes; ix) mental symptom; x) bone joint injuries. If a patient had three of items i)-iv), or two of items i)-iv) together with three of items v)-x), he/she was diagnosed with HLD. The exclusion criteria [12] included: i) primary renal diseases; ii) immune system diseases such as systemic lupus erythematosus; iii) endocrine diseases such as hyperthyroidism, diabetes mellitus, primary aldosteronism; iv) hepatitis or liver cancer; v) multiple myeloma; vi) heavy metal poisoning or amphotericin B poisoning. In control group, 31 healthy subjects were enrolled, including 18 males and 13 females (age range, 20-67 years; mean age, 39.87 ± 13.17 years). None of the healthy subjects had histories of chronic liver, kidney, stomach, and intestine diseases or autoimmune diseases.

### *Routine test of renal and renal tubular functions*

A vertical automatic biochemical analyzer (TBA-120FR; TOSHIBA, Tokyo, Japan) was used to detect the renal and renal tubular functions. Renal function (BUN and CREA) was tested using UV-GLDH method (UREA kit; Randox Laboratories Ltd., Crumlin, UK) and sarcosine oxidase-PAP method (Creatinine (Cr) Assay Kit; Elabscience, Houston, TX, USA), respectively. Blood electrolytes (K, Na and Cl) were tested by EasyLyte PLUS REF D2121 Na/K/Cl kit (Medica, Bedford, MA, USA). Alkaline phosphatase (ALP), carban dioxide-combining power ($CO_2CP$), anion gap (AG), Ca and Phos levels were determined by respective kits (Sigma-Aldrich, St. Louis, MO, USA).

### *Determination of 24 h urine protein (Pro)*

At 24 h, urine was collected for colorimetry. Proteins were deposited in urine by tungstate, and quantified by biuret method using ADVIA 1650 (Siemens, Berlin, Germany).

### *Evaluation of urine acidification function*

After three days of vegetarian diets, automatic potentiometric titrator (Baoshishan, Shanghai, China) was used to evaluate urine acidification function of the patients. The initial pH value was determined. Bicarbonate, titratable acid, and ammonium ion were measured to evaluate urine acidification function.

### *Determination of amino acids in urine*

To determine the level of amino acids, sulfosalicylic acid method was performed. The reagents included Li-A, Li-B and Li-C ninhydrin chromogenic agents. Briefly, urine (3 ml) was collected at 24 h, and deproteinized solution was prepared. The samples were diluted at a ratio of 1:10 before examination on the amino acid analyzer (Beckman 6300 amino acid analyzer; Beckman Coulter, Brea, CA, USA).

### *Statistical analysis*

All results were analyzed using SPSS11.0 statistical software (IBM, Armonk, NY, USA). For quantitative data, t-test was used to analyze grouped or paired data. In case of heterogeneity of variance, grouped data were compared using t'-test. For qualitative data, frequency and percentage were measured, and compared using Chi square test or exact probability method.

**Table 1.** Blood biochemical indexes of HLD group and normal control group (means ± standard deviations)

| Indexes | HLD (n = 38) | Control (n = 31) | T | P |
|---|---|---|---|---|
| ALP (U/L) | 107.79 ± 51.26 | 59.77 ± 21.32 | -5.188 | 0.000 |
| BUN (mmol/L) | 5.32 ± 1.41 | 5.53 ± 1.36 | 1.811 | 0.075 |
| CREA (umol/L) | 71.72 ± 23.08 | 81.77 ± 28.33 | 1.625 | 0.109 |
| Blood Ca (mmol/L) | 2.30 ± 0.16 | 2.43 ± 0.16 | 3.285 | 0.002 |
| Blood Phos (mmol/L) | 1.23 ± 0.29 | 1.41 ± 0.18 | 3.086 | 0.003 |
| Blood K (mmol/L) | 3.96 ± 0.37 | 4.34 ± 0.47 | 3.438 | 0.001 |
| Blood Na (mmol/L) | 140.67 ± 3.18 | 140.55 ± 4.49 | -0.136 | 0.893 |
| Blood Cl (mmol/L) | 105.33 ± 4.03 | 105.55 ± 3.92 | 0.225 | 0.823 |
| $CO_2$ combining power (mmol/L) | 25.40 ± 2.88 | 26.10 ± 3.32 | 0.934 | 0.354 |
| Anion gap | 13.92 ± 4.87 | 14.16 ± 4.20 | 0.216 | 0.829 |

**Table 2.** Urine biological indexes in HLD group and normal control group (means ± standard deviations)

| Indexes | HLD (n = 35) | Control (n = 31) | T | P |
|---|---|---|---|---|
| Urine amount (L/24 h) | 1678.57 ± 662.67 | 1567.74 ± 364.59 | -0.854 | 0.397 |
| Urine protein (mg/24 h) | 356.03 ± 334.45 | 166.13 ± 91.89 | -7.482 | 0.000 |
| Urine glucose (mg/24 h) | 847.99 ± 1357.18 | 131.05 ± 98.22 | -3.116 | 0.004 |
| Urine Ca (mmol/24 h) | 5.22 ± 3.57 | 4.20 ± 1.42 | -1.556 | 0.127 |
| Urine K (mmol/24 h) | 50.32 ± 16.73 | 40.25 ± 19.04 | -2.270 | 0.027 |
| Urine Na (mmol/24 h) | 193.83 ± 90.14 | 165.77 ± 55.71 | -1.539 | 0.129 |
| Urine Cl (mmol/24 h) | 172.51 ± 80.94 | 195.84 ± 40.95 | 1.502 | 0.139 |
| Urine Phos (mmol/24 h) | 19.60 ± 8.21 | 34.94 ± 8.58 | 7.416 | 0.000 |

**Table 3.** Urine routing pH values, specific gravity and acidification function (means ± standard deviations)

| Indexes | HLD (n = 36) | Control (n = 31) | T | P |
|---|---|---|---|---|
| PH value | 6.17 ± 0.71 | 5.74 ± 0.31 | -3.253 | 0.002 |
| Urine specific gravity | 1.024 ± 0.005 | 1.021 ± 0.004 | -2.847 | 0.006 |
| Initial pH | 6.39 ± 0.59 | 5.94 ± 0.64 | -3.006 | 0.004 |
| Bicarbonate | 7.71 ± 7.80 | 8.06 ± 2.81 | 0.255 | 0.800 |
| Titratable acid | 11.07 ± 10.87 | 18.68 ± 11.77 | 2.752 | 0.008 |
| Ammonium ion | 34.40 ± 15.72 | 39.70 ± 15.62 | 1.379 | 0.173 |

## Results

### *HLD changes a number of health indexes in the patients*

To compare each index of HLD patients with control group, blood biochemical index, urine biochemical index, urine pH value, urine specific gravity, urine acidification function, urine routine, and urine amino acids were examined. Blood biochemical index tests showed that the level of ALP in the blood from HLD group was significantly higher than control group ($P < 0.05$), levels of Ca, K and Phos in the blood from HLD group were significantly lower than control group ($P < 0.05$), and BUN, Cr, Na, Cl, $CO_2$CP, and AG levels in HLD group were not significantly different from control group ($P > 0.05$) (**Table 1**). Urine biochemical index tests showed that urine protein, glucose and potassium levels in HLD group at 24 h were significantly higher than control group ($P < 0.05$), urine Phos level in HLD group was significantly lower than control ($P < 0.05$), urine Ca and Na levels in HLD group were higher than control group but without statistical significance ($P > 0.05$), and urine Cl level in HLD group was not significantly different from that in control group ($P > 0.05$) (**Table 2**). In addition, urine pH value, urine specific gravity, and initial pH value were significantly higher than control group ($P < 0.05$), the level of urine titratable acid in HLD group was significantly lower than

**Table 4.** Urine routine test results of HLD group and normal control group

| Indexes | Positivity | HLD | Control | $X^2$ | P |
|---|---|---|---|---|---|
| Urine occult blood | Positive | 20 | 3 | 14.840 | 0.000 |
| | Negative | 17 | 28 | | |
| Urine protein | Positive | 14 | 3 | 7.134 | 0.011 |
| | Negative | 23 | 28 | | |
| Urine WBC | Positive | 16 | 1 | 14.945 | 0.000 |
| | Negative | 20 | 30 | | |
| Urine RBC | Positive | 18 | 3 | 12.585 | 0.000 |
| | Negative | 18 | 28 | | |
| Urine acetone bodies | Positive | 2 | 0 | - | 0.496 |
| | Negative | 35 | 31 | | |
| Urine glucose | Positive | 6 | 0 | - | 0.027 |
| | Negative | 30 | 31 | | |

**Table 5.** Urine amino acids of HLD group (means ± standard deviations)

| Indexes | HLD (n = 37) | Normal values | T | P |
|---|---|---|---|---|
| Leucine (mg/24 h) | 121.85 ± 93.37 | 50 | 4.681 | 0.000 |
| Isoleucine (mg/24 h) | 86.53 ± 84.59 | 21 | 4.712 | 0.000 |
| Valine (mg/24 h) | 46.28 ± 72.18 | 69 | -1.914 | 0.064 |
| Phenylalanine (mg/24 h) | 254.58 ± 513.20 | 63 | 2.271 | 0.029 |
| Tyrosine (mg/24 h) | 303.98 ± 219.93 | 142 | 4.480 | 0.000 |
| Alanine (mg/24 h) | 556.85 ± 537.88 | 172 | 4.353 | 0.000 |
| Glycine (mg/24 h) | 2365.51 ± 2111.60 | 1797 | 1.638 | 0.110 |
| Lysine (mg/24 h) | 401.14 ± 543.60 | 139 | 2.933 | 0.006 |
| Histidine (mg/24 h) | 1524.41 ± 1188.73 | 1005 | 2.658 | 0.012 |
| Aspartic acid (mg/24 h) | 114.25 ± 95.38 | 109 | 0.335 | 0.740 |
| Glutamate (mg/24 h) | 255.22 ± 185.71 | 130 | 4.102 | 0.000 |
| Threonine (mg/24 h) | 403.15 ± 706.10 | 93 | 2.672 | 0.011 |
| Serine (mg/24 h) | 678.54 ± 632.62 | 471 | 1.996 | 0.054 |
| Methionine (mg/24 h) | 275.51 ± 293.42 | 51 | 4.654 | 0.000 |
| Cystine (mg/24 h) | 470.05 ± 580.01 | 87 | 4.017 | 0.000 |
| Ornithine (mg/24 h) | 162.93 ± 137.55 | 40 | 5.436 | 0.000 |
| Taurine (mg/24 h) | 1187.67 ± 721.27 | 959 | 1.928 | 0.062 |

control group ($P < 0.05$), and urine bicarbonate and ammonium ion levels in HLD group were nog significantly different from control group ($P > 0.05$) (**Table 3**). The positive rates of urine protein, urine glucose, urine OB, urine REC, and urine WBC in HLD group were significantly higher than control group ($P < 0.05$) (**Table 4**). The levels of urine leucine, isoleucine, phenylalanine, tyrosine, alanine, lysine, histidine, glutamic acid, threonine, methionine, cystine, and ornithine in HLD group were significantly higher than normal values ($P < 0.05$), while the levels of urine valine, glycine, aspartic acid, serine, and taurine in HLD group were not significantly different from control group ($P > 0.05$) (**Table 5**). The results suggest that HLD changes a number of health indexes in the patients.

*Urine routine test results of HLD patients are not dependent on gender*

To compare the differences in urine routine parameters between male HLD patients and female HLD patients, we tested positive rates of urine proteins, glucose, OB, REC and WBC. The data showed that these parameters were in male HLD patients were not significantly different from those in female HLD patients ($P > 0.05$) (**Table 6**). The result indicates that urine routine test results of HLD patients are not dependent on gender.

*Only blood ALP and Phos levels in adult HLD patients are significantly different from youngster HLD patients*

To test how age affects HLD, the patients were divided into adult subgroup (> 18 years old; 18 cases) and youngster subgroup (≤ 18 years old; 12 cases). Blood biochemical test results showed that blood BUN, CREA, Ca, K, Na, and Cl levels in adult HLD patients were not significantly different from those in youngster HLD patients ($P > 0.05$), while blood ALP and Phos levels in adult HLD patients were significantly lower than those in youngster HLD patients ($P < 0.05$) (**Table 7**). Urine biochemical test results showed that the levels of urine amount, proteins, glucose, Ca, urine K, Na, Cl and Phos in adult HLD patients were not significantly different from those in youngster HLD patients ($P > 0.05$) (**Table 8**). In addition, urine pH values, urine specific gravity, initial pH value, bicarbon-

**Table 6.** Urine routine indexes in males and females with HLD

| Indexes | Positivity | Male | Female | $X^2$ | P |
|---|---|---|---|---|---|
| Urine occult blood | Positive | 10 | 10 | 0.562 | 0.453 |
| | Negative | 6 | 10 | | |
| Urine proteins | Positive | 7 | 7 | 0.286 | 0.593 |
| | Negative | 9 | 13 | | |
| Urine WBC | Positive | 5 | 11 | 2.948 | 0.086 |
| | Negative | 12 | 8 | | |
| Urine RBC | Positive | 6 | 12 | 2.786 | 0.095 |
| | Negative | 11 | 7 | | |
| Urine acetone bodies | Positive | 1 | 1 | - | 1.000 |
| | Negative | 15 | 19 | | |
| Urine glucose | Positive | 3 | 3 | - | 1.000 |
| | Negative | 13 | 17 | | |

**Table 7.** Blood biochemical indexes in adult and youngster HLD patients (means ± standard deviations)

| Indexes | Adult (n = 26) | Youngster (n = 12) | T | P |
|---|---|---|---|---|
| ALP (U/L) | 88.32 ± 37.62 | 153.82 ± 51.03 | -4.347 | 0.000 |
| BUN (mmol/L) | 5.43 ± 1.22 | 5.08 ± 1.80 | 0.721 | 0.476 |
| CREA (μmol/L) | 75.29 ± 24.53 | 63.98 ± 18.12 | 1.424 | 0.163 |
| Blood Ca (mmol/L) | 2.30 ± 0.15 | 2.30 ± 0.17 | 0.035 | 0.972 |
| Blood Phos (mmol/L) | 1.14 ± 0.26 | 1.43 ± 0.28 | -3.103 | 0.004 |
| Blood K (mmol/L) | 3.94 ± 0.38 | 3.99 ± 0.37 | -0.361 | 0.720 |
| Blood Na (mmol/L) | 140.48 ± 3.19 | 141.08 ± 3.26 | -0.534 | 0.596 |
| Blood Cl (mmol/L) | 105.06 ± 4.09 | 105.92 ± 4.01 | -0.603 | 0.551 |

**Table 8.** Urine biochemical indexes in adult and youngster HLD patients (means ± standard deviations)

| Indexes | Adult (n = 25) | Youngster (n = 10) | t | P |
|---|---|---|---|---|
| Urine amount (L/24 h) | 1678.57 ± 662.47 | 1567.74 ± 264.59 | -0.854 | 0.397 |
| Urine proteins (mg/24 h) | 379.78 ± 373.40 | 296.67 ± 212.76 | 0.659 | 0.515 |
| Urine glucose (mg/24 h) | 99.01 ± 154.92 | 49.28 ± 60.20 | 0.979 | 0.335 |
| Urine Ca (mmol/24 h) | 3.40 ± 1.96 | 2.88 ± 1.81 | 0.751 | 0.458 |
| Urine K (mmol/24 h) | 37.49 ± 12.43 | 47.15 ± 29.71 | -1.374 | 0.179 |
| Urine Na (mmol/24 h) | 197.41 ± 85.76 | 184.87 ± 104.69 | 0.367 | 0.716 |
| Urine Cl (mmol/24 h) | 177.78 ± 82.63 | 159.36 ± 79.23 | 0.602 | 0.551 |
| Urine Phos (mmol/24 h) | 20.07 ± 7.19 | 18.43 ± 10.70 | 0.525 | 0.603 |

ate, titratable acid, and ammonium ion in adult HLD patients were not significantly different from youngster HLD patients (P > 0.05) (**Table 9**). Similarly, leucine, isoleucine, phenylalanine, tyrosine, alanine, lysine, histidine, glutamic acid, threonine, methionine, cystine, ornithine, valine, glycine, aspartic acid, serine, and taurine levels in adult HLD patients were not significantly different from youngster HLD patients (P > 0.05) (**Table 10**). The positive rates of urine proteins, glucose, OB, REC and WBC in adult HLD patients were not significantly different from youngster HLD patients (P > 0.05) (**Table 11**). These results suggest that only blood ALP and Phos levels in adult HLD patients are significantly different from youngster HLD patients.

*Renal tubular damages in HLD alter most renal tubular damage indexes and clinical manifestations of the patients*

To investigate the effect of renal tubular damages on HLD patients, percentages of patients with different renal tubular damage indexes and clinical manifestations were calculated. Regarding renal tubular damage indexes, the percentage of HLD patients with increased ALP was 39.5%, that with increased BUN was 10.5%, that with increased CREA was 0%, that with reduced blood calcium was 13.2%, that with reduced blood Phos was 10.5%, that with reduced K was 15.8%, that with increased Cl was 0%, that with increased urine was 25.7%, that with urine protein (quantitative) was 45.7%, that with renal glycosuria (quantitative) was 42.9%, that with increased urine calcium was 2.9%, that with increased urine Phos was 0%, that with increased urine K was 2.9%, that with reduced urine Cl was 20%, that with positive urine-glu-

**Table 9.** Urine routing pH values, specific gravity and acidification function in adult and youngster HLD patients (means ± standard deviations)

| Indexes | Adult (n = 25) | Youngster (n = 11) | T | P |
|---|---|---|---|---|
| pH | 6.12 ± 0.74 | 6.27 ± 0.65 | -0.591 | 0.558 |
| Urine specific gravity | 1.0253 ± 0.049 | 1.0226 ± 0.056 | -1.499 | 0.772 |
| Initial pH | 6.35 ± 0.59 | 6.46 ± 0.60 | -0.520 | 0.606 |
| Bicarbonate | 7.94 ± 8.06 | 7.18 ± 7.55 | 0.265 | 0.793 |
| Titratable acid | 11.71 ± 11.97 | 9.60 ± 8.15 | 0.531 | 0.599 |
| Ammonium ion | 35.50 ± 15.93 | 31.92 ± 15.68 | 0.624 | 0.537 |

**Table 10.** Urine amino acids in adult and youngster HLD patients (means ± standard deviations)

| Indexes | Adult (n = 26) | Youngster (n = 11) | T | P |
|---|---|---|---|---|
| Leucine (mg/24 h) | 116.51 ± 89.55 | 134.47 ± 105.29 | -0.530 | 0.600 |
| Isoleucine (mg/24 h) | 80.92 ± 62.69 | 99.77 ± 125.15 | -0.614 | 0.543 |
| Valine (mg/24 h) | 55.14 ± 79.39 | 25.35 ± 48.06 | 1.401 | 0.171 |
| Phenylalanine (mg/24 h) | 290.95 ± 608.74 | 168.62 ± 100.80 | 0.657 | 0.515 |
| Tyrosine (mg/24 h) | 259.89 ± 169.43 | 408.21 ± 292.17 | -1.575 | 0.139 |
| Alanine (mg/24 h) | 457.47 ± 436.93 | 791.76 ± 691.27 | -1.779 | 0.084 |
| Glycine (mg/24 h) | 2119.26 ± 1885.27 | 2947.57 ± 2576.04 | -1.094 | 0.282 |
| Lysine (mg/24 h) | 323.30 ± 401.09 | 585.12 ± 780.15 | -1.355 | 0.184 |
| Histidine (mg/24 h) | 1346.63 ± 1012.72 | 1944.62 ± 1498.88 | -1.418 | 0.165 |
| Aspartic acid (mg/24 h) | 114.14 ± 95.64 | 114.51 ± 99.42 | -0.011 | 0.992 |
| Glutamate (mg/24 h) | 259.56 ± 184.33 | 244.97 ± 235.62 | 0.215 | 0.831 |
| Threonine (mg/24 h) | 254.95 ± 184.33 | 753.44 ± 1232.01 | -1.336 | 0.211 |
| Serine (mg/24 h) | 506.07 ± 326.51 | 1086.19 ± 956.06 | -1.965 | 0.075 |
| Methionine (mg/24 h) | 277.89 ± 317.83 | 269.90 ± 239.52 | 0.075 | 0.941 |
| Cystine (mg/24 h) | 380.27 ± 483.71 | 682.23 ± 745.41 | -1.471 | 0.150 |
| Ornithine (mg/24 h) | 156.83 ± 117.57 | 177.34 ± 182.30 | -0.410 | 0.685 |
| Taurine (mg/24 h) | 1327.50 ± 675.21 | 857.16 ± 749.69 | 1.875 | 0.069 |

cose was 16.7%, that with positive urine occult blood was 55.6%, that with positive urine protein was 38.9%, that with white blood cell urine was 44.4%, and that with amino acid urine was 100% (**Table 12**). Regarding clinical manifestations, the percentage of patients with vitamin D-resistant rickets was 11.4%, that with renal tubular acidosis was 17.1%, that with potassium-losing nephropathy was 2.9%, that with salt-losing nephropathy was 0%, that with idiopathic hypercalcinuria was 8.6%, and that with Fanconi syndrome was 42.9% (**Table 13**). These results indicate that renal tubular damages in HLD alter most renal tubular damage indexes and clinical manifestations of the patients.

## Discussion

In HLD, synthesis disorder of copper blue protein is caused by autosomal recessive inheritance, and excretion of copper in biliary tract is affected. A decrease in the normal copper binding protein and a lack of normal copper containing enzymes increase the amount of copper intake in intestinal tract, but intake of copper blue protein is still low [12]. As a result, a large number of copper deposits in the liver, resulting in lobular cirrhosis. After lysosomes in hepatic cell cytoplasm cannot accommodate the copper, excessive copper spreads and deposits into various organs through the blood. The neurons in basal ganglia and the transport of their normal enzymes are particularly sensitive to the toxicity of copper, but the deposition of copper in cerebral cortex and cerebellum dentate nucleus also causes symptoms [13, 14]. Deposition of copper in the elastic layer of cornea produces Kayser-Fleischer ring, and its deposition in the skin leads to dark skin color.

**Table 11.** Urine routine test results in adult and youngster HLD patients

| Indexes | Positivity | Ault | Youngster | $X^2$ | P |
|---|---|---|---|---|---|
| Urine occult blood | Positive | 16 | 4 | 3.070 | 0.080 |
| | Negative | 9 | 8 | | |
| Urine proteins | Positive | 11 | 3 | - | 0.306 |
| | Negative | 14 | 9 | | |
| Urine WBC | Positive | 11 | 5 | - | 1.000 |
| | Negative | 14 | 6 | | |
| Urine RBC | Positive | 11 | 7 | 1.178 | 0.278 |
| | Negative | 14 | 4 | | |
| Urine acetone bodies | Positive | 2 | 0 | - | 1.000 |
| | Negative | 23 | 12 | | |
| Urine glucose | Positive | 3 | 3 | - | 0.367 |
| | Negative | 22 | 9 | | |

**Table 12.** Percentages of patients with different renal tubular damage indexes

| Indexes | Total No. of cases | No. of cases with specific indexes | Percentage (%) |
|---|---|---|---|
| Increased ALP | 38 | 15 | 39.5 |
| Increased BUN | 38 | 4 | 10.5 |
| Increased CREA | 38 | 0 | 0 |
| Low blood Ca | 38 | 5 | 13.2 |
| Low blood Phos | 38 | 4 | 10.5 |
| Low blood K | 38 | 6 | 15.8 |
| Low blood Cl | 38 | 4 | 10.5 |
| Excessive urine | 35 | 9 | 25.7 |
| Urine proteins (quantitative) | 35 | 16 | 45.7 |
| Renal glycosuria (quantitative) | 35 | 15 | 42.9 |
| Increased urine Ca | 35 | 1 | 2.9 |
| Increased urine Phos | 35 | 0 | 0 |
| Increased urine K | 35 | 1 | 2.9 |
| Increased urine Cl | 35 | 7 | 20 |
| Positive urine-glucose | 36 | 6 | 16.7 |
| Positive urine occult blood | 36 | 20 | 55.6 |
| Positive urine protein | 36 | 14 | 38.9 |
| WBC urine | 36 | 16 | 44.4 |
| Blood urine | 36 | 18 | 50 |
| Amino acid urine | 37 | 37 | 100 |
| Excessive urine | 35 | 6 | 17.1 |

In the meantime, cirrhosis can cause a series of changes in portal hypertension [1, 3].

Renal proximal tubule damages caused by copper produce acidaminuria, or even losses of glucose, phosphate, calcium and uric acid, leading to metabolic bone diseases [15].

Renal tubular disease is a group of renal diseases characterized by specific or universal renal tubular dysfunction. Clinically, the disease does not show specific or prominent symptoms, or easily progress into renal failure like renal glomerular diseases do. In recent years, HLD has attracted more and more concerns because it is easily misdiagnosed. Therefore, it is of great importance to understand the injuries in renal proximal convoluted tubule and distal convoluted tubule in HLD [16].

Some HLD patients have bone joint disorders. However, the abnormal changes of bone joint in HLD are not completely due to the direct deposition of copper. We consider that bone metabolic disorders in HLD patients cause bone joint abnormalities. In the present study, blood Ca and Phos in HLD patients are lower than control group, while ALP level in HLD patients is higher than control. Urine Phos level in HLD patients is significantly lower than control, suggesting abnormal metabolism of Ca and Phos in HLD patients. HLD causes renal tubular dysfunction, as well as high urine Phos and Ca excretion. A study shows that blood PTH level in HLD patients is significantly reduced and CT level in HLD patients is significantly elevated [2], suggesting that HLD patients have poor deposition of bone calcium salt and compensatory increase in calcitonin secretion. In addition, excessive copper deposits in the liver and kidney of HLD patients, resulting in degeneration and necrosis of hepatocytes, different degrees of renal proximal tubule epithelial edema, and interstitial vascular congestion and expansion [17, 18]. Reduced activity of 25-hydroxylase synthesized in hepatocytes and impaired 1α-hydroxylase activity in renal proximal tubule epithelial cells cause insufficient synthesis of 1,25-$(OH)_2$VitD3, leading to low blood Phos and secondary hyperparathyroidism [4]. When a patient has reduced 1, 25 $(OH)_2$D3, bone mineralization disorder will occur. Because 1α-hydroxylase is located in mitochondria of proximal tubule cells, damages and structural changes

**Table 13.** Percentages of patients with different clinical manifestations of renal tubular damages

| Clinical manifestations | No. of total cases | No. of cases with specific clinical manifestations | Percentage (%) |
|---|---|---|---|
| Vitamin D-resistant rickets | 35 | 4 | 11.4 |
| Renal tubular acidosis | 35 | 6 | 17.1 |
| Potassium-losing nephropathy | 35 | 1 | 2.9 |
| Salt-losing nephropathy | 35 | 0 | 0 |
| Idiopathic hypercalcinuria | 35 | 3 | 8.6 |
| Fanconi syndrome | 35 | 15 | 42.9 |

of proximal convoluted tubule cells may be a reason for decreased blood 1,25 $(OH)_2$D3 level [5, 19]. Consistently, our results in the present study have shown low blood Ca, low blood Phos and high urine Ca. Surprisingly, the urine Phos level in HLD is lower than control group. This may be due to several reasons: i) blood Phos and urine Phos are sensitive to VD deficiency; and ii) HLD can also cause hypoparathyroidism. Therefore, the inhibition effect of PTH on the reabsorption of phosphate and sodium bicarbonate by proximal renal tubules is weakened [20]. ALP level in HLD patients is higher than control, and ALP reflects bone cell activity, which suggests high turnover bone metabolism.

In normal conditions, more than 99% of the amino acids in the original urine are reabsorbed by specific amino acid transporters in renal tubular epithelial cells [21].

Therefore, the content of amino acids in the urine of normal people is little, and maintained at a relatively constant level. Under certain pathological conditions, the excretion of amino acids in urine is increased, resulting in aminoaciduria. In some cases, the abnormality is limited to a single amino acid or a group of amino acids, such as phenylketonuria. By contrast, a number of different amino acids may also be present in the urine, which is commonly referred to as "full aminoaciduria", for example HLD. The present study shows that aminoaciduria is common in HLD, and the amino acids are numerous and abundant. Because of copper deposits in renal tubular cells, HLD directly affects the transfer mechanisms by which amino acids are transferred. In addition, this impacts other material transfer, leading to common renal tubular damages.

The HLD patients included in the present study have no diabetes history, but the levels of urine glucose and positive rate of urine routine glucose are higher than control group. Glucose is a small molecule that can freely pass through the glomerular basement membrane, but is absorbed in proximal tubules. Therefore, it does not appear in the urine. When the blood glucose concentration is normal but urine glucose result is positive, renal tubular proximal reabsorption is decreased, and this is called renal glycosuria. HLD copper induces renal proximal tubule damage, resulting in decreased renal tubular reabsorption of glucose, but normal glomerular filtration rate. Because of reduced renal glucose threshold, glycosuria is present, which is often accompanied by amino acid, uric acid and bicarbonate reabsorption disorders [22, 23].

HLD induces renal damage, changes glomerular basement membrane permeability, and interrupts renal tubular reabsorption, leading to proteinuria. Hematuria is the most common symptom of HLD renal damage [9]. The mechanisms may include: i) coagulation factors, ii) hypercalciuria, and iii) secondary IgA nephropathy [11]. Compared with control group, HLD group has higher incidence of routine urine white blood cell (44.4%), suggesting that HLD copper induces tubulointerstitial lesions.

Many patients with HLD can show the reabsorption or secretion disorder of various materials, leading to Fanconi syndrome. Its main clinical manifestation include urine glucose, full aminoaciduria, different levels of phosphaturia, bicarbonaturia and uric acid and organic acid urine caused by disordered proximal tubular reabsorption of multiple substances. In the meantime, proximal and distal renal tubules may also be affected, and tubular proteinuria and excessive electrolyte loss may occur. The main manifestations of HLD in children include rickets and growth retardation, while adult

osteopathy mainly includes osteomalacia and osteoporosis. A total of 15 cases (42.9%) among HLD patients in the present study have Fanconi syndrome. In conclusion, the present study demonstrates that HLD can cause damages to renal proximal tubule and lead to Fanconi syndrome.

## Acknowledgements

This work was supported by The First Affiliated Hospital of Medical University of Tianjin.

## Disclosure of conflict of interest

None.

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## References

[1] Schmidt HH. Wilson's disease. Dtsch Med Wochenschr 2005; 130 Suppl 5: S208-210.
[2] Yang G, Hong M and Yang B. Research on the mechanism of abnormal bone metabolism in patients with hepatolenticular degeneration. Chinese Journal of Nervous and Mental Diseases 2003; 29: 201-203.
[3] Kitzberger R, Madl C and Ferenci P. Wilson disease. Metab Brain Dis 2005; 20: 295-302.
[4] Booth BE, Tsai HC and Morris RC Jr. Vitamin D status regulates 25-hydroxyvitamin D3-1 alpha-hydroxylase and its responsiveness to parathyroid hormone in the chick. J Clin Invest 1985; 75: 155-161.
[5] Bland R, Walker EA, Hughes SV, Stewart PM and Hewison M. Constitutive expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in a transformed human proximal tubule cell line: evidence for direct regulation of vitamin D metabolism by calcium. Endocrinology 1999; 140: 2027-2034.
[6] Schindler EA, Guo XM, Schrag M, Ghoshal S, Schilsky ML and Beslow LA. Neuropsychiatric presentation of wilson disease in an adolescent male. Neuropediatrics 2016; 47: 346-347.
[7] Wolff SM. Renal lesions in wilson's disease. Lancet 1964; 1: 843-845.
[8] Yoshitoshi EY, Takada Y, Oike F, Sakamoto S, Ogawa K, Kanazawa H, Ogura Y, Okamoto S, Haga H, Ueda M, Egawa H, Kasahara M, Tanaka K and Uemoto S. Long-term outcomes for 32 cases of Wilson's disease after living-donor liver transplantation. Transplantation 2009; 87: 261-267.
[9] Reynolds HV, Talekar CR, Bellapart J, Leggett BA and Boots RJ. Copper removal strategies for Wilson's disease crisis in the ICU. Anaesth Intensive Care 2014; 42: 253-257.
[10] Bao Y, Zhang B and Jiang T. Renal damages in 50 patients with hepatolenticular degeneration. Chinese Journal of Practical Internal Medicine 1997; 11: 653.
[11] Sarles J, Durand JM, Scheiner C and Picon G. Wilson disease, IgA glomerulonephritis and vascular purpura: an incidental association? Arch Fr Pediatr 1993; 50: 501-504.
[12] Vargas O, Faraoun SA, Dautry R, Guerrache Y, Woimant F, Hamzi L, Boudiaf M, Poujois A, Soyer P and Dohan A. MR imaging features of liver involvement by wilson disease in adult patients. Radiol Med 2016; 121: 546-556.
[13] Hachmoller O, Aichler M, Schwamborn K, Lutz L, Werner M, Sperling M, Walch A and Karst U. Element bioimaging of liver needle biopsy specimens from patients with wilson's disease by laser ablation-inductively coupled plasma-mass spectrometry. J Trace Elem Med Biol 2016; 35: 97-102.
[14] Khalaf R, Phen C, Karjoo S and Wilsey M. Cholestasis beyond the neonatal and infancy periods. Pediatr Gastroenterol Hepatol Nutr 2016; 19: 1-11.
[15] Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM and Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell 2000; 100: 197-207.
[16] Kelly D, Crotty G, O'Mullane J, Stapleton M, Sweeney B and O'Sullivan SS. The clinical utility of a low serum ceruloplasmin measurement in the diagnosis of wilson disease. Ir Med J 2016; 109: 341-343.
[17] Nomiyama K, Nomiyama H, Kameda N, Tsuji A and Sakurai H. Mechanism of hepatorenal syndrome in rats of long-evans cinnamon strain, an animal model of fulminant wilson's disease. Toxicology 1999; 132: 201-214.
[18] Yang R. Wilson disease. Anhui Science & Technolog Publishing House, 1995.
[19] Henry HL. Parathyroid hormone modulation of 25-hydroxyvitamin D3 metabolism by cultured chick kidney cells is mimicked and enhanced by forskolin. Endocrinology 1985; 116: 503-510.
[20] Kelly PJ, Morrison NA, Sambrook PN, Nguyen TV and Eisman JA. Genetic influences on bone turnover, bone density and fracture. Eur J Endocrinol 1995; 133: 265-271.
[21] Fang H, Gao Y, Wang H, Yin H, Li G and An T. Photo-induced oxidative damage to dissolved free amino acids by the photosensitizer polycy-

clic musk tonalide: transformation kinetics and mechanisms. Water Res 2017; 115: 339-346.

[22] Park H, Park DK, Kim MS and Yoon JH. Pseudodominant inheritance in wilson's disease. Neurol Sci 2016; 37: 153-155.

[23] Wilson BA, Cruz-Diaz N, Su Y, Rose JC, Gwathmey TM and Chappell MC. Angiotensinogen import in isolated proximal tubules: evidence for mitochondrial trafficking and uptake. Am J Physiol Renal Physiol 2017; 312: F879-F886.