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ORIGINAL RESEARCH

Renal Tubular Function, Bone Health and Body Composition in Wilson's Disease: A Cross-Sectional Study from India

Nitin Kapoor[1] · Kripa Elizabeth Cherian[1] · Kattiparambil G. Sajith[2] · Maya Thomas[3] · Chundamannil E. Eapen[2] · Nihal Thomas[1] · Thomas V. Paul[1]

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Abstract
There is limited literature from India with regard to the prevalence and magnitude of renal tubular and bone manifestations in Wilson's disease (WD). Thus, we studied the prevalence of renal tubular acidosis among Indian patients with WD and also evaluated bone health and body composition in them. It was a cross-sectional study conducted at a south Indian tertiary care center. Twenty-five consecutive patients with WD aged more than 12 years attending the hepatology and neurology departments and 50 age, sex and BMI-matched controls were recruited. After clinical assessment, they underwent biochemical testing to assess renal tubular dysfunction. Bone mineral density (BMD) and body composition were assessed using a dual energy X-ray absorptiometry (DXA) scanner. Fifty-six percent (14/25) of patients with WD had renal tubular acidosis (RTA). Of them, 24% were diagnosed to have distal RTA. RTA was more common in hepatic WD patients who had prolonged duration of illness. Patients with WD had significantly lower BMD as compared to control subjects ($p < 0.05$). Low BMI, low IGF-1 and a shorter duration of therapy were key determinants of low bone mass in them ($p < 0.05$). Patients with WD had significantly more body fat ($p = 0.01$) and lower lean muscle mass ($p = 0.03$) when compared to age, sex and BMI-matched controls. In conclusion, renal tubular acidosis was common in patients with Wilson's disease. These patients had a lower bone mineral density, higher body fat percentage and lower lean muscle mass as compared to controls.

Keywords Wilson's disease · BMD · Renal tubular acidosis · Body composition · India

Introduction

Wilson's disease is an inherited disorder of copper metabolism, which is characterized by an accumulation of copper that occurs predominantly in the liver and brain [1]. Although it is a rare disease, it appears to have gained significant recognition in countries such as India and its southern states such as Tamil Nadu, where consanguineous marriages amount to 20% of marriages, thereby increasing its prevalence [2–4]. Patients with Wilson's disease, who otherwise have a near-normal life span with the current treatment options, often may have some potentially treatable and frequently under-recognized factors which could impact the bone and endocrine system adversely [5].

Bone health is affected in subjects with Wilson's disease via many mechanisms such as the impact of underlying hepatic osteodystrophy, chelating medications, overall nutritional status, renal tubular dysfunction, less physical activity and lean mass. These factors largely affect the trabecular bone and is characterized by a low bone turnover state with reduced osteoblast function and lower osteocalcin levels [6, 7]. The additional factors like poor calcium and vitamin D nutrition especially in India may further contribute to low bone mass in these subjects [8].

The other endocrine manifestations of Wilson's disease may include disorders of growth and puberty, hypothyroidism and hypoparathyroidism [9]. However, there appears to be a paucity of the medical literature from India with regard to the prevalence and magnitude of these bone and endocrine manifestations in Wilson's disease. Addressing these issues

✉ Thomas V. Paul
thomasvpaul@yahoo.com

[1] Department of Endocrinology, Diabetes and Metabolism, Christian Medical College, Vellore, Tamil Nadu 632004, India

[2] Department of Hepatology, Christian Medical College, Vellore, India

[3] Department of Neurology, Christian Medical College, Vellore, India

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would further improve the quality of life in these patients who are now effectively managed for their primary manifestations [10]. Thus, in this study, we attempted to study the prevalence of renal tubular dysfunction in subjects with Wilson's disease. We also studied the bone mineral parameters, bone mineral density (BMD) and body composition (body fat percentage and lean muscle mass) in these subjects.

Subjects and Methods

Consecutive patients above the age of 12 years who attended the clinical hepatology and neurology outpatient departments at Christian Medical College & Hospital over a period of 1 year (January 2017–December 2017), and were diagnosed to have Wilson's disease (now or previously) with stable liver functions and no active infection were included in this cross-sectional study. All subjects were recruited following a detailed written informed consent, and in those below 18 years of age, an assent form was signed by their parents. To compare the parameters of BMD and body composition, a healthy control group comprising of 50 age, sex and body mass index-matched individuals was recruited. The control group were healthy individuals and were recruited from the community health database.

An ethical clearance was obtained from the Institutional Review Board prior to initiation of this study vide IRB Min No. 7917, dated 04.07.2016.

The diagnosis of Wilson's disease was confirmed as per the standard guidelines of the American Association for the Study of Liver Diseases [11]. These subjects were asked a detailed history to assess the duration, presentation, severity and treatment received for the management of Wilson's disease. They also underwent a detailed physical examination (including anthropometry/sexual maturity rating) to assess their growth and pubertal status. Weight was recorded in kilograms using an electronic scale, and standing height was measured to the nearest centimeter using a stadiometer, with subjects wearing light indoor clothing without shoes. Sexual maturity rating was done for all patients according to Tanner criteria [12, 13]. Blood biochemistry and urine tests were done to assess the renal tubular dysfunction as per a previously published protocol which has also been validated in India [14]. All patients were assessed for urine anion gap, phosphaturia (Bijvoet's nomogram), proteinuria, hypercalciuria, natriuresis, fasting urine pH and ultrasound abdomen (to document nephrocalcinosis). In those patients, who did not have baseline metabolic acidosis, a standard ammonium chloride loading test was done to exclude an underlying distal renal tubular acidosis. A diagnosis of distal renal tubular acidosis was made in the presence of a fasting urine pH $\geq$ 5.5 with concomitant metabolic acidosis and/or presence of hypokalemia, nephrocalcinosis, hypercalciuria, natriuresis and a high-urine anion gap. Proximal renal tubular acidosis was diagnosed in the presence of fasting urine pH < 5.5 with concomitant metabolic acidosis and/or presence of phosphaturia, aminoaciduria, glycosuria and a normal urine anion gap. Those subjects who had features of both proximal and distal tubular function were diagnosed to have mixed renal tubular acidosis.

Fasting (over-night for 8 h) venous blood samples were collected for the measurement of serum calcium (*N* 8.3–10.4 mg/dL), phosphorus (*N* 2.5–4.5 mg/dL), alkaline phosphatase (*N* 40–125 U/L), albumin (*N* 3.5–5.0 g/dL), creatinine (*N* 0.6–1.4 mg/dL), 25-hydroxy vitamin D (*N* 30–75 ng/mL), intact parathormone (*N* 8–50 pg/mL), sodium (*N* 135–144 mmol/L), potassium (*N* 3.5–5 mmol/L), bicarbonate (*N* 22–28 mmol/L) and chloride (*N* 95–104 mmol/L). Fractional excretion of phosphate ($FePO_4$) was calculated and TmP/GFR was derived using Bijvoet nomogram [15]. A TmP/GFR < 2.5 mg/dL was indicative of phosphaturia. Fasting urine pH was measured. The pH was measured using a manual pH meter using an ion-specific electrode. The urine sample was collected after a minimum of 8 h of over-night fast. Plasma anion gap is calculated as follows: Anion gap $= [Na^+] - \{[Cl^-] + [HCO_3^-]\}$. Baseline blood pH in venous blood gas sample (VBG) was measured. Ammonium chloride loading test was done when indicated, as an inpatient [8]. On the day of testing, ammonium chloride (NH_4Cl) was given orally in a dose of 0.1 g/kg body weight with a glass of juice (to be taken in sips over 15–30 min) on fasting state with hourly urine collections for 5 h for pH measurement with electrometer(pH meter). VBG is retested at 3 h post ingestion to assess systemic acidification.

Blood calcium, phosphate, albumin, creatinine and alkaline phosphatase were measured using a colorimetric method with a Beckman coulter (Beckman coulter AU 5800). An iced sample for intact parathormone (iPTH) was collected and estimated by a chemiluminescence assay (Advia Centaur XPT immunoassay system) and 25-hydroxyvitamin D (vitamin D) was measured using an electrochemiluminescence assay (Roche Cobas 6000- Immunoassay system). Vitamin D deficiency was defined as 25(OH) vitamin D level of less than 20 ng/mL, and severe vitamin D deficiency as less than 10 ng/mL.

The BMD at three sites [lumbar spine (LS), femoral neck (FN) and forearm] and body composition were assessed using Hologic (QDR 4500; Discovery A Hologic, Inc., Waltham, Massachusetts). DXA scanners' daily quality control (QC) was performed with a phantom provided by the manufacturer and calibrations as per standard protocol. A coefficient of variation (CV) of 2% was noted at both sites over the study period. A Z score ≤ -2 at either LS or FN or forearm was considered diagnostic of low bone mass. BMD measurement by DXA scan had a CV of 2% at three sites and 4% for body composition analysis.

Sample size

The required sample size to show an estimated risk difference of 35% in occurrence of low bone mass based on a previously published Portuguese study, was 27 subjects with 5% level of significance and 80% power [16].

Statistics

Statistical analysis was done using SPSS v.16.0 software (IBM, Corp, USA). Continuous variables were expressed as mean ± SD and categorical variables as proportions. Student's *t* test was used to compare the means of two continuous variables as they were normally distributed. Significance was defined when p value < 0.05.

Results

A total of 28 subjects of Wilson's disease were screened over a period of 1 year. Three subjects were excluded of which one had decompensated cirrhosis and was on diuretics, another had history of alcohol intake and a traumatic fracture with osteomyelitis and one patient was on diuretics with portal hypertension. Twenty-five subjects (16 males and 9 females) were enrolled for the final analysis. All patients fulfilled at least two criteria for the diagnosis of Wilson's disease.

The mean (SD) age of subjects with Wilson's disease was 19.9 (6.8) years, the age ranged from 12 to 35 years. However, the mean (SD) age at the time of diagnosis was 13.5 (12.3) years. Sixteen male and nine female subjects were recruited in this study of whom 18 subjects had completed puberty and were included for analysis of BMD and body composition. Forty percent (10/25) of subjects were born to a consanguineous marriage. The mean (SD) age of menarche among girls was 11.4 (1.14) years. Subjects with WD had low mean (SD) dietary calcium intake [329.3 (233.6) mg/day].

The mean (SD) height and weight in study subjects was 160 (14.6) cm and 51.3 (16.5) kg respectively. The mean (SD) body mass index was 19.9 (4.6) kg/m^2. Sixty-four percent of subjects with Wilson's disease had a predominant hepatic presentation, another 16% had neurological involvement and 20% had overlapping hepatic and neurological manifestations. Gonadal hormone status was assessed both clinically and biochemically by estimating the serum testosterone in men, estrogen in women (who had not completed puberty), FSH (follicular stimulating hormone) and LH (luteinizing hormone) in all subjects. The mean (SD) serum testosterone (in men), estrogen (in women), FSH and LH in subjects who had completed puberty was 463 (87.2) ng/dL, 93.6 (48.4) pg/mL, 3.5 (2.4) mIU/mL and 3.6 (2.5) mIU/mL, respectively.

Eighty-four percent (21 of 25) of patients with WD were already on treatment (16/25 on pencillamine and 10/25 on zinc). The median duration of therapy in these patients was 81.4 months. Seventy-six percent (19/25) had vitamin D deficiency among whom six subjects 24% (6/25) had severe vitamin D deficiency.

Fifty-six percent (14/25) of patients with WD were diagnosed to have renal tubular acidosis of which 24% (6/25) had distal renal tubular acidosis, 16% (4/25) had proximal renal tubular acidosis and 16% (4/25) had features of mixed (proximal + distal) renal tubular acidosis (Table 1). One patient declined consent for ammonium chloride loading test. The comparison of parameters among patients of Wilson's disease with RTA and without RTA is shown in Table 2. There was no significant difference in mean (SD) age in subjects with and without RTA [18.1 (6.8) vs. 21.9 (6.3) years; $p = 0.74$]. There was no significant difference in mean (SD) albumin-corrected calcium [9.1 (0.5) vs. calcium 8.8 (0.4) mg/dL; $p = 0.85$] and phosphorus [3.3 (0.6) vs. 3.5 (0.8) mg/dL; $p = 0.92$] in individuals with RTA as compared to those without. However, the mean (SD) serum 25(OH) vitamin D was significantly higher in subjects with RTA as compared to those without RTA [14.8 (5.8) vs. 9.6 (11.8); $p = 0.04$]. The WD subjects with RTA had significantly lower mean (SD) BMD at FN when compared to subjects without RTA [0.787 (0.132) vs. 0.938 (0.041) g/cm^2; $p = 0.03$]. Among the cases, 9/16 (56.2%) male subjects and 5/9 (55.5%) female subjects had renal tubular acidosis ($p = 0.97$).

Table 1 Renal tubular acidosis in study subjects

Parameter	No RTA ($n = 11$)	Proximal RTA ($n = 3$)	Distal RTA ($n = 6$)	Mixed RTA ($n = 5$)
Urine anion gap				
Normal (0–10)	11	Nil	Nil	Nil
Increased (> 10)	Nil	Nil	6	2
Decreased (< 0)	Nil	3	Nil	3
Fasting urine pH (with acidosis)				
> 5.5	1*	Nil	6	1
≤ 5.5	10	3	Nil	4
Presence of hypokalemia	Nil	1	3	1
Presence of phosphaturia	Nil	3	Nil	5
Presence of nephrocalcinosis/hypercalciuria	Nil	Nil	6	Nil
Presence of natriurises	0	3	0	5
Presence of proteinuria	0	1	0	0

*Could not undergo NH_4Cl loading test

Table 2 Assessment of parameters among patients of Wilson's disease with and without renal tubular acidosis

Parameter mean (SD)	No RTA $n=11$	RTA present $n=14$	P value
*Height (cm)	167.8 (7.0)	162.6 (10.6)	0.24
*Weight (kg)	61.1 (17.9)	49.2 (8.9)	0.33
*Body fat percentage (%)	21.9 (10.1)	17.7 (6.4)	0.92
*Lean mass/height (kg/m^2)	28.2 (42.2)	15.3 (3.7)	0.06
Duration of illness (months)	66.2 (78.1)	136.7 (92.4)	**0.026**
Duration of therapy (months)	61.3 (88.1)	132.2 (90.2)	**0.038**
*Albumin-corrected calcium (mg/dL)	8.78 (0.4)	9.13 (0.5)	0.85
*Phosphorus (mg/dL)	3.47 (0.8)	3.25 (0.6)	0.92
*Magnesium (mEq/L)	2.1 (0.6)	2.2 (0.4)	0.62
*PTH (pg/mL)	57 (23.8)	34.15 (11.2)	0.23
*25(OH) vitamin D (ng/mL)	9.6 (11.8)	14.8 (5.8)	**0.04**
*Alkaline phosphatase (U/mL)	123 (73)	158.4 (69)	0.30
*BMD(g/cm^2)- femoral neck	0.938 (0.041)	0.787 (0.132)	**0.03**
*BMD(g/cm^2)- lumbar spine	0.912 (0.942)	0.823 (0.132)	0.06
*BMD(g/cm^2)- forearm	0.575 (0.032)	0.515 (0.072)	0.33
*Low BMD (Z score ≤ -2 at any one site)	22%	67%	**< 0.001**

Statistically significant values are highlighted in bold

*Excluded seven subjects who have not completed puberty in this analysis

It was seen that patients with Wilson's disease had much lower BMD at all three sites when compared to controls ($p<0.05$). Fifty-six percent of subjects (10 of 18 who completed puberty) had low BMD at any site. The differences in various parameters between subjects with low BMD and normal BMD are shown in Table 3. Among the 18 subjects who had completed puberty, 6/13 (46.1%) males and 2/5 (40%) females had low BMD ($p=0.83$). It was found that the patients with low BMD were older, had a lower BMI, a shorter duration of therapy, low body mass index

Table 3 Factors affecting BMD in patients with Wilson's disease who have completed puberty

Parameter	Low BMD ($N=10$)	Normal BMD ($N=8$)	P-value
Age	23.4 (6.9)	21.2 (5.8)	0.41
Sex			
Male:Female	7:3	6:2	0.20
Male (%)	70%	85.4%	
Female (%)	30%	14.6%	
Duration of Wilson's disease(months)	62.8 (59.7)	124.2 (122.7)	0.171
Median (range)	42 (8–200)	96 (4–300)	
Predominant hepatic involvement (%)	9 (90%)	5 (62%)	0.32
Duration of therapy(months) -mean(SD)	46.6 (33.5)	130.57 (104.9)	**0.013**
Median (range)	37 (8–108)	96 (26–300)	
Dietary calcium intake	364 (246)	318 (286)	0.9
Body mass index(kg/m^2) -mean(SD)	17.9 (3.1)	22.1 (4.8)	**0.04**
Body fat percentage mean(SD)	14.8 (4.7)	22.7 (10.5)	0.88
Lean mass/height^2-mean(SD)	16.3 (3.2)	31.1 (45.9)	0.23
Presence of renal tubular acidosis (%)	6/10 (60%)	3/8 (37%)	0.41
Serum calcium-mean(SD) mg/dL	8.91 (0.5)	8.9 (0.2)	0.97
Serum phosphorus-mean(SD) mg/dL	3.4 (0.87)	3.7 (0.69)	0.32
Serum magnesium-(mean(SD) mEq/L	2.0 (0.4)	2.1 (0.5)	0.43
25(OH) vitamin D(ng/mL)-mean(SD)	18.3 (11.5)	15.9 (6.7)	0.962
Serum iPTH-mean(SD) pg/mL	46.5 (22.1)	40.9 (19.2)	0.58
Low-insulin-like growth factor-1	9/10 (90%)	1/8 (13%)	**0.002**

Statistically significant values are highlighted in bold

and much lower insulin like growth factor-1. Among these factors, lower BMI, shorter duration of therapy and lower IGF-1 emerged significantly different. We found no significant difference in the mean (SD) 25(OH) vitamin D levels in individuals with lower BMD as compared to those with normal BMD. [18.3 (11.5) vs. 15.9 (6.7) ng/mL; $p = 0.96$] An univariate regression analysis was performed to study the impact of low duration of the illness, low duration of therapy, low BMI, low vitamin D, presence of RTA, low IGF-1, hypogonadism, and less dietary calcium intake on BMD at any site. However, none of these factors was found to be significant. Table 4 shows body composition of subjects with Wilson's disease who had completed puberty versus controls. Patients with Wilson's disease had a significantly higher body fat percentage and lower lean mass when compared to age and sex-matched controls ($p < 0.05$).

Discussion

This is the first Asian study to evaluate the prevalence of renal tubular acidosis, low bone mass and assess the body composition in patients with Wilson's disease. This study was beneficial to understand the impact of this disease and its therapy on the skeleton and renal tubules, especially in Tamil-speaking south Indian population, where consanguinity is highly prevalent [17]. We found that renal tubular acidosis was seen in about half of the patients with Wilson's disease especially in those with longer duration of disease and treatment. Subjects with WD had significantly lower mean BMD at all sites and had higher body fat. A lower BMI, low IGF-1 and a shorter duration of therapy were the key determinants of low bone mass in this cohort. Overall, patients with Wilson's disease were found to have higher adiposity indices and lower lean mass than age, sex and BMI-matched controls.

In this study, we found that a longer duration of therapy may have a detrimental effect on tubular function but rather may be protective for BMD. This may suggest a role of chelating agents per se in worsening renal tubular function. Moreover, RTA may manifest as sclerotic bone disease which may falsely increase the BMD. Further, besides tubular dysfunction other factors like low BMI, low IGF-1 and a short duration of therapy were the key determinants of low bone mass in this cohort.

Both proximal and distal RTA have been reported with Wilson's disease but there is paucity of consolidated prospectively evaluated data in this regard [5, 18]. In a previously published study, one-third of subjects with Wilson's disease were diagnosed to have distal renal tubular acidosis and 8.3% of subjects had proximal RTA [19]. This is similar to our study, in which it we found that distal RTA occurred more commonly than proximal RTA.

In our study, patients with renal tubular acidosis were found to have a significantly longer duration of illness and duration of therapy when compared to those subjects without renal tubular acidosis. This is probably due to more number of patients with liver involvement in our study cohort and presentation of hepatic predominant Wilson's disease occurring at an earlier age in the natural history [20]. In a previous study from our center, it was shown that the duration of an untreated copper over load was responsible for the development of renal tubular acidosis in families having the same mutation [21]. It was also found that patients who had renal tubular acidosis had a slightly higher 25-hydroxy vitamin D level and a significantly lower BMD at the FN than those without RTA. One plausible explanation for the high 25-hydroxy vitamin D in patients with renal tubular acidosis is the fact that a lower 1-alpha hydroxylase activity exists in subjects with proximal renal tubular acidosis [22, 23]. Mild elevation in 25 hydroxy vitamin D levels in individuals with RTA is akin to individuals with hereditary vitamin D-dependent type 1 rickets (VDDR-1) with 1-α-hydroxylase deficiency, wherein, there is not only low plasma concentrations of 1,25(OH) vitamin D but also a modestly increased level of 25(OH) vitamin D [24]. However, 1,25 dihydroxy vitamin D was not measured in our study.

Defective mineralization is more commonly reported in patients with proximal RTA [6]. The cause of rickets in patients with distal RTA is not clear. It has been suggested that chronic metabolic acidosis may affect vitamin D metabolism. Rachitic deformities in children may resolve following alkali supplementation [25]. Subjects with both proximal and distal RTA have a lower BMD when compared to normal age and sex-matched controls [7]. This is probably due to suppression of bone formation, which in turn may contribute to the development of low bone mass [26].

In our study, it was seen that patients with Wilson's disease had a much lower BMD at all three sites. More than half of subjects had low BMD, which is similar to previously

Table 4 Assessment of body composition

Body composition analysis	Cases ($n = 18$)	Controls* ($n = 90$)	*P* value
Total body fat % (mean)	20.92(2.2)	15.3(2.1)	0.016
Fat mass/height^2 kg/m^2 (mean)	6.85(2.9)	3.1(2.15)	0.017
Lean mass/height^2 kg/m^2 (mean)	15.6(2.3)	18.5(2.8)	0.03
Trunk:limb fat mass ratio (mean)	3.59(1.0)	0.79(0.9)	0.006

published data, where 58% of subjects had low BMD [6]. In a recent meta-analysis of limited studies, the pooled prevalence rates of osteopenia and osteoporosis in WD patients were 36.5% and 27.7%, respectively [27].

Low body mass index is well known to be associated with low bone mineral density. IGF-1 deficiency, alterations in calcium and vitamin D metabolism, hypovitaminosis-K and release of inflammatory cytokines such as IL-1, IL-6 and TNF-alpha in the background of liver disease is known to adversely impact BMD [28]. Selimoglu et al. [29] showed no beneficial effect of penicillamine and zinc therapy on BMD in patients with WD. However, we found that shorter duration was associated with a lower BMD. So we suggest a long-term follow-up study to understand the interplay between chelating agents and the underlying systemic illness on skeletal health. In addition, poor dietary calcium intake was also noted in our study subjects as in other Indian cohorts [30, 31]. In the Indian context, there have been conflicting literature on the association of dietary calcium intake and its influence on bone health [32, 33].

It was found that patients with Wilson's disease who had completed puberty, had a significantly higher body fat percentage and lower lean muscle mass when compared to matched controls. This finding is in keeping with previously published literature by Peng et al. [34], who found a significantly higher body fat percentage (35.7%) in patients with liver disease. They also found that there was no significant difference of body fat percentage amongst different etiologies of chronic liver disease. The limitation of our study were many and include small sample size, cross-sectional design, non-measurement of 1,25 dihydroxy vitamin D, bone formation markers(PINP/Bone specific alkaline phosphatase) and lack of objective physical activity assessment. The strength of this study is that it is the first of its kind from India to evaluate renal tubular function, bone health and body composition in a cohort of subjects with Wilson's disease.

The key message from this study is that 56% of Indian subjects with Wilson's disease had a low BMD and this is not addressed as per current clinical practice. In view of multiple players affecting BMD in these patients and rare occurrence of this disease, we may not be able to point out with certainty the predominant factors affecting BMD in this study. Finding no significance in factors impacting on BMD in regression analysis in the current study could be probably attributed to small number of study subjects. However, ensuring optimal calcium and vitamin D nutrition in these subjects as in other populations [35], may play a beneficial role in improving bone health, and this has to be prospectively studied.

In conclusion, renal tubular acidosis was common in patients with Wilson's disease. These patients had a lower bone mineral density, higher body fat percentage and lower lean muscle mass as compared to controls. Hence all patients with Wilson's disease may need screening and treatment for RTA and bone health, to improve their quality of life.

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Author Contributions NK, KGS, MT, CEE and TVP coordinated and conducted the study. NK, KEC and TVP wrote the first draft. CEE and NT revised the draft. All authors reviewed and approved the final version of the draft.

Compliance with Ethical Standards

Conflict of interest Nitin Kapoor, Kripa Elizabeth Cherian, Kattiparambil G. Sajith, Maya Thomas, Chundamannil E. Eapen, Nihal Thomas, Thomas V. Paul confirm that they have no conflict of interest related to this study.

Human and Animal Rights and Informed Consent All tests on participants were performed in accordance with ethical standards stipulated by the CMC Research ethics committee. This study protocol was approved by the institutional review board prior to initiation of this study vide IRB Min No. 7917, dated 04.07.2016. No amimals were used in this study. All subjects were recruited following a detailed written informed consent, and in those below 18 years of age, an assent form was signed by their parents.

References

1. Patil M, Sheth KA, Krishnamurthy AC, Devarbhavi H (2013) A review and current perspective on Wilson disease. J Clin Exp Hepatol 3(4):321–336
2. Tarnacka B, Rodo M, Cichy S, Członkowska A (2000) Procreation ability in Wilson's disease. Acta Neurol Scand 101(6):395–398
3. Kapoor N, Chapla A, Furler J, Paul TV, Harrap S, Oldenburg B et al (2019) Genetics of obesity in consanguineous populations—a road map to provide novel insights in the molecular basis and management of obesity. EBioMedicine 40:33–34
4. Mamidala MP, Kalikiri MK, Praveen Kumar PTV, Rajesh N, Vallamkonda OR, Rajesh V (2015) Consanguinity in India and its association with autism spectrum disorder. Autism Res 8(2):224–228
5. Kelly C, Pericleous M (2018) Wilson disease: more than meets the eye. Postgrad Med J 94(1112):335–347
6. Weiss KH, Van de Moortele M, Gotthardt DN, Pfeiffenberger J, Seessle J, Ullrich E et al (2015) Bone demineralisation in a large cohort of Wilson disease patients. J Inherit Metab Dis 38(5):949–956
7. Domrongkitchaiporn S, Pongsakul C, Stitchantrakul W, Sirikulchayanonta V, Ongphiphadhanakul B, Radinahamed P et al (2001) Bone mineral density and histology in distal renal tubular acidosis. Kidney Int 59(3):1086–1093
8. Binu AJ, Cherian KE, Kapoor N, Thomas N, Paul TV (2018) Referral pattern for DXA scanning in a tertiary care centre from southern India. Arch Osteoporos 13(1):133

9. Ghosh L, Shah M, Pate S, Mannari J, Sharma K (2012) Wilson's disease presenting with hypokalemia, hypoparathyroidism and renal failure. J Assoc Phys India 60:57–59
10. Roberts EA (2018) Update on the diagnosis and management of Wilson disease. Curr Gastroenterol Rep 20(12):56
11. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML (2007) Wilson's disease. Lancet Lond Engl 369(9559):397–408
12. Marshall WA, Tanner JM (1969) Variations in pattern of pubertal changes in girls. Arch Dis Child 44(235):291–303
13. Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. Arch Dis Child 45(239):13–23
14. Bagga A, Sinha A (2007) Evaluation of renal tubular acidosis. Indian J Pediatr 74(7):679–686
15. Walton RJ, Bijvoet OL (1975) Nomogram for derivation of renal threshold phosphate concentration. Lancet Lond Engl 2(7929):309–310
16. Teixeira S, Domingos J, Carvalho R, Miranda HP, Magalhaes M (2011) Prevalence of metabolic bone disease in Wilson. In: BioScientifica. https://www.endocrine-abstracts.org/ea/0026/ea0026p535. Accessed 19 Jan 2019
17. Krishnamoorthy S, Audinarayana N (2001) Trends in consanguinity in south India. J Biosoc Sci 33(2):185–197
18. Tesar V, Mokrejsová M, Marecek Z, Petrtýl J (1991) Distal renal tubular acidosis in patients with Wilson's disease. Sb Lek 93(9–10):315–323
19. Dastur DK, Manghani DK, Wadia NH (1968) Wilson's disease in India. I. Geographic, genetic, and clinical aspects in 16 families. Neurology 18(1):21–31
20. Boga S, Ala A, Schilsky ML (2017) Hepatic features of Wilson disease. Handb Clin Neurol 142:91–99
21. Santhosh S, Shaji RV, Eapen CE, Jayanthi V, Malathi S, Chandy M et al (2006) ATP7B mutations in families in a predominantly southern Indian cohort of Wilson's disease patients. Indian J Gastroenterol 25(6):277–282
22. Reddy GS, Jones G, Kooh SW, Fraser D (1982) Inhibition of 25-hydroxyvitamin D3-1-hydroxylase by chronic metabolic acidosis. Am J Physiol 243(4):E265–E271
23. Palkar AV, Shrivastava MS, Padwal NJ, Padhiyar RN, Moulick N (2011) Renal tubular acidosis due to Wilson's disease presenting as metabolic bone disease. BMJ Case Rep [Internet]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3158360/. Accessed 19 Jan 2019
24. Kim CJ (2011) Vitamin D dependent rickets type I. Korean J Pediatr 54(2):51–54
25. Cunningham J, Fraher LJ, Clemens TL, Revell PA, Papapoulos SE (1982) Chronic acidosis with metabolic bone disease. Effect of alkali on bone morphology and vitamin D metabolism. Am J Med 73(2):199–204
26. Quemeneur A-S, Trocello J-M, Ea H-K, Ostertag A, Leyendecker A, Duclos-Vallée J-C et al (2014) Bone status and fractures in 85 adults with Wilson's disease. Osteoporos Int 25(11):2573–2580
27. Chenbhanich J, Thongprayoon C, Atsawarungruangkit A, Phupitakphol T, Cheungpasitporn W (2018) Osteoporosis and bone mineral density in patients with Wilson's disease: a systematic review and meta-analysis. Osteoporos Int 29(2):315–322
28. Handzlik-Orlik G, Holecki M, Wilczyński K, Duława J (2016) Osteoporosis in liver disease: pathogenesis and management. Ther Adv Endocrinol Metab 7(3):128–135
29. Selimoglu MA, Ertekin V, Doneray H, Yildirim M (2008) Bone mineral density of children with Wilson disease: efficacy of penicillamine and zinc therapy. J Clin Gastroenterol 42(2):194–198
30. Raj JP, Oommen AM, Paul TV (2015) Dietary calcium intake and physical activity levels among urban south Indian postmenopausal women. J Fam Med Prim Care 4(3):461–464
31. Balk EM, Adam GP, Langberg VN, Earley A, Clark P, Ebeling PR et al (2017) Global dietary calcium intake among adults: a systematic review. Osteoporos Int 28(12):3315–3324
32. Paul TV, Thomas N, Seshadri MS, Oommen R, Jose A, Mahendri NV (2008) Prevalence of osteoporosis in ambulatory postmenopausal women from a semiurban region in southern India: relationship to calcium nutrition and vitamin D status. Endocr Pract 14(6):665–671
33. Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D, Srinivasarao PVLN, Sarma KVS et al (2007) High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians. Am J Clin Nutr 85(4):1062–1067
34. Peng S, Plank LD, McCall JL, Gillanders LK, McIlroy K, Gane EJ (2007) Body composition, muscle function, and energy expenditure in patients with liver cirrhosis: a comprehensive study. Am J Clin Nutr 85(5):1257–1266
35. Rizzoli R, Bischoff-Ferrari H, Dawson-Hughes B, Weaver C (2014) Nutrition and bone health in women after the menopause. Womens Health Lond Engl 10(6):599–608