KIREPORTS
KIReports.org
CLINICAL RESEARCH

Changes in Kidney Function Among Malaysian Adolescents and Its Determinants

Fathhullah Azmie Nawawi[1,2], Wan Ahmad Hafiz Wan Md Adnan[3], Maslinor Ismail[1], Muhammad Yazid Jalaludin[4] and Hazreen Abdul Majid[2,5,6]

[1]Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; [2]Centre for Population Health, Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; [3]Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; [4]Department of Pediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; [5]School of Health and Rehabilitation Sciences, AECC University College, Parkwood Campus, Bournemouth, UK; and [6]Department of Nutrition, Faculty of Public Health, Universitas of Airlangga, Surabaya, Indonesia

Introduction: The health and wellbeing of adolescents are often neglected, including the knowledge of chronic kidney disease (CKD), especially in its early stages.

Methods: A total of 607 adolescents were recruited from the Malaysian Health and Adolescents Longitudinal Research Team (MyHeART) study, a prospective cohort study conducted from March 2012 to May 2016 that explored the noncommunicable diseases (NCDs) risk factors among 13 to 17 years old students in 3 states of Peninsular Malaysia. Students who participated in all 3 data collection periods in 2012, 2014, and 2016 with kidney function assessment across all 3-time points were included in the current study. The students' estimated glomerular filtration rate (eGFR) was calculated from isotope-dilution mass spectrometry-traceable Schwartz's equation and categorized based on Kidney Disease: Improving Global Outcomes (KDIGO) classification. Changes in kidney function were examined, and the longitudinal relationship between eGFR and multiple NCD risk factors was analyzed using the generalized estimating equation (GEE).

Results: The prevalence of decreased eGFR (60–89 ml/min per 1.73 m^2) among the students increased from 6.1% (2012) to 30.0% (2014) and 40.2% (2016). Based on the GEE, the student's eGFR decreased over time, with a steeper decline during early to midadolescence. Males and rural students had lower eGFR compared to their counterparts. Students who are morbidly obese had lower eGFR than those with normal body mass index (BMI). Protein consumption also has a potential moderating effect on eGFR in adolescents.

Conclusion: Kidney function changes can be detected as early as adolescence and are likely attributable to multiple NCD risk factors. Therefore, more comprehensive prevention efforts from various stakeholders are needed to identify health issues like CKD.

Kidney Int Rep (2023) **8,** 1965–1977; https://doi.org/10.1016/j.ekir.2023.07.028
KEYWORDS: adolescent health; chronic kidney disease; glomerular filtration rate; obesity; public health

Adolescence is a unique stage of life for human development and a foundation for good health. Nearly 20% of the population is comprised of adolescents.[1] Despite being a vital part of the world population, inequality regarding the efforts and management of adolescents' health and wellness, including CKD, can still be seen worldwide.[2] The efforts for early disease detection of CKD were notably less among adolescents, probably due to a higher disease burden in adults.[3] As a result, little knowledge of the disease burden, primarily the earlier stage CKD, can be found worldwide and in Southeast Asia countries like Malaysia.

In Malaysia, the number of children on dialysis at the end of 2016 remains low compared to adults (2.2%, 889/39,711 total patients).[4] However, in the last decade (2007–2016), a higher increment rate in dialysis was observed among younger adults aged 25 to 34 years compared to those ≥65 years (57% and 35%, respectively).[4,5]

Correspondence: Hazreen Abdul Majid, School of Health and Rehabilitation Sciences, AECC University College, Parkwood Campus, Parkwood Rd, 13-15 Parkwood Rd, Bournemouth BH5 2DF, UK. E-mail: hazreen.abdulmajid@gmail.com or hmajid@aecc.ac.uk

Received 24 January 2023; revised 18 June 2023; accepted 31 July 2023; published online 5 August 2023

Considering that CKD and end-stage renal disease are long-standing diseases, this group of young adults may have started earlier during adolescence. Nevertheless, no such evidence is available in the country. Therefore, this study was conducted. It is the first study to investigate kidney function status among adolescents in communities of middle-income countries. It also determines the potential lifestyle and health factors associated with kidney function changes during adolescence.

METHODS

Participant Recruitment

The MyHeART study was an open dynamic prospective cohort study conducted between March 2012 and May 2016. The study explored the risk factors for NCDs in adolescents aged 13 to 17 years from 15 schools in 3 Peninsular Malaysia states, namely Selangor, Perak, and Kuala Lumpur. These schools were selected randomly from 595 schools that have been stratified between urban and rural. A total of 1361 students aged 13 years were voluntarily recruited in the MyHeART study in 2012, followed up with in 2014 and 2016. The current study, which served as a substudy of MyHeART, applied a closed cohort analysis, selecting only 607 of the 1361 students who had participated in all 3-time data collection points and had kidney function assessment across all 3-time points (Figure 1). A large proportion of students were excluded because of the following 2 reasons: (i) students did not return in the subsequent data collection year (e.g., already moved to a different school, attending other curricular activities), and (ii) due to the nature of the MyHeART study itself, which was an open dynamic cohort, a small group of students did not have a complete kidney function assessment across all 3-time points. However, the *post hoc* power analysis[6] for this study was 82.2%, based on the prevalence of

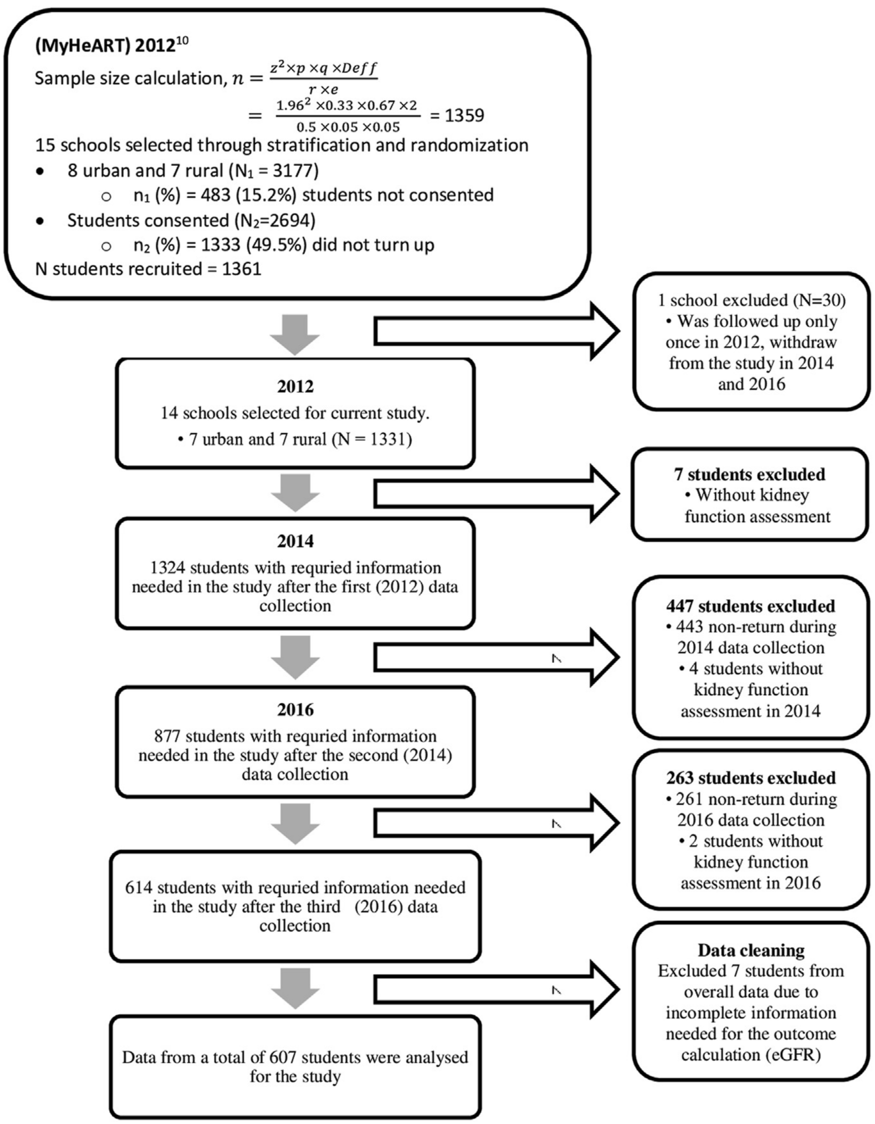

Figure 1. Sampling flow in the study.

adolescents with eGFR <90 ml/min per 1.73 m^2 in the United States of 34.6%.[7] The study protocol for the MyHeART study has been explained by Hazreen *et al.*[8] Ethics approval was granted by the University Malaya Medical Centre's Ethics Committee (MEC ref. no. 896·34).

Kidney Function Assessment

The creatinine-based eGFR was utilized to determine the kidney function of the students. Serum creatinine from venous blood sampling was measured by the enzymatic method on the Atellica clinical chemistry analyzer as part of the Total Lab Automation System. The enzymatic method is based on Fossati, Prencipe, and Berti: Creatinine is converted to creatine by the action of creatininase and expressed in μmol/l. The creatinine estimate was an isotope-dilution mass spectrometry standardized. The eGFR was then calculated using the isotope-dilution mass spectrometry-traceable Schwartz equation,[9] as follows:

$\text{eGFR} = 0.413 \times (\text{height}/\text{Scr})$ for height expressed in centimeters

eGFR - expressed in ml/min per 1.73 m^2.

Scr - standardized serum creatinine, expressed in mg/dl (to convert Scr in μmol/l to mg/dl, multiply Scr in μmol/l by 88.4)

This formula was selected because it is the current practice in Malaysia and other countries in the Asia Pacific region, such as Thailand.[10] Multiple other community-based studies have also utilized similar equations in their study.[7,11] The calculated eGFR was then classified based on the cut-off values by the KDIGO classification.[12] An eGFR of >90 ml/min per 1.73 m^2 was considered normal, whereas an eGFR of <90 ml/min per 1.73 m^2 was considered a decreased eGFR (Supplementary Table S1). Those with a decreased eGFR were classified accordingly using the KDIGO staging value range.

Sociodemographic Measures

A translated and validated self-administered questionnaire adapted from the Young Heart Study, a previous cohort study conducted in Northern Ireland,[13] was given to students and their parents to collect information on their socioeconomic background, birthing history, physical and nutritional habits, as well as high-risk behavior histories such as smoking, vaping, alcohol consumption, and illicit drug use.

Anthropometric Measurements

The study measured the waist circumference, body fat percentage, and BMI of the students. Due to the multicollinearity issue between BMI, waist circumference, and body fat percentage, BMI was selected as the body composition measurement for the longitudinal relationship. Body weight was measured on a digital electronic scale (Seca 813, UK) and recorded to the nearest 0.1 kilograms. A calibrated vertical stadiometer (Seca Portable 217, UK) measured height in centimeters. BMI was calculated from weight/height (in meters) squared. It was classified according to the Extended International Obesity Task Force cut-off (Supplementary Table S2).[14]

Level of Physical Activity

The student's physical activity level was measured using the physical activity questionnaire for older children, a self-reported physical activity questionnaire for older children.[15] Based on the classification by Kowalski *et al.*,[15] the physical activity questionnaire for older children score was categorized as either low physical activity (<2.33), moderate physical activity (2.33–3.66), or high physical activity (>3.66). This questionnaire was translated into Malay, tested, and proven to have acceptable validity and good internal consistency in the Malaysian population.[16,17]

Dietary Intake Assessment

A 7-day diet history was used to determine dietary intake because it is the most reliable method for estimating adolescents' intakes.[18] Qualified dietitians performed the 7-day dietary assessment. The macronutrient and micronutrient intakes were calculated using the Nutrient Composition of Malaysian Foods and entered into the Nutritionist Pro database (Axxya Systems, Stafford, TX). Students' energy and protein intake were dichotomized using the Recommended Nutrient Intake guideline for the Malaysian population (2017).[19] Intakes of sodium exceeding 2000 mg/d were considered excessive for both sexes.[20]

Other Clinical and Biological Evaluation

Using a stethoscope and a mercury sphygmomanometer (CK-101C Mercurial, Spirit Medical Co., Taiwan), trained personnel measured the systolic blood pressure (BP) and diastolic BP of the students at least twice. Abnormal BP was taken as mean systolic BP >120 mm Hg or diastolic BP >80 mm Hg.[21] The student's fasting blood sugar was evaluated from a venous blood sampling. An fasting blood sugar value of 3.9 to 5.6 mmol/l is considered normal in this study.[22]

Statistical Analysis

Analyses were performed using the SPSS software for Windows Version 26, IBM Corp, Armonk, NY and the significance level was set at a P value <0.05. Baseline characteristics of selected and excluded students were compared in the first part of the analysis. Each variable's continuous and categorical formats were compared using an independent sample *t*-test and χ^2 test, respectively. The first comparison used crude data, ignoring the existing missing values, and the second, was based on imputed data after the Little's missing completely at random test showed that the data was not missing completely at random ($P < 0.001$).

Once the differences have been determined, the main analysis of the 607 selected students commenced. Data imputation was conducted to overcome the missing values among the selected students because the data was not missing completely at random ($P <$ 0.001). In the multiple imputation model, outcome, exposure, and confounders were included. Eight separate data sets were generated from which pooled estimates were taken. The variables that were imputed in this study were the household income, parental education, birth weight, past medical history, high-risk behavior history (smoking, vaping, alcohol, and/or drug use), dietary intakes, systolic and diastolic BP, as well as the PAQ-C scores. The baseline characteristics of these students were explored, and the Mann-Whitney U test and Kruskal Wallis test were used to evaluate the differences between groups at baseline. A population estimate based on these characteristics was generated from a calculated weightage derived by multiplying the selected school weightage by the student weightage. Similar weightage was included during the study's complex sample analysis and the GEE method. Changes to the independent variables and the outcome of the study along the cohort were examined with Kendall's coefficient of concordance test, followed by a pairwise comparison with an adjusted significance level of $P < 0.05$ (adjusted by the Bonferroni correction for multiple tests) was considered as significantly different. Differences in the proportion of students with decreased kidney function based on various formulas were examined using the z-score for proportion differences. The cut-off value of z-score <1.96 ($P < 0.05$) determined a significant difference between the 2 proportions. A higher z-score value indicates more significant deviations from the reference formula.

Finally, the data was modeled in 2 models and analyzed with the GEE to determine the longitudinal relationship between the independent variables and the outcome. This exploratory model was built based on the theoretical framework suggested by Schnaper *et al.*[23] as the central construct, that also incorporates other knowledge for known risk factors for kidney disease development in children.[2,3] Variable with a *P* value of <0.05 was considered significant in the "main effect" and "interaction effect." The interaction effect was done simultaneously for all variables while applying a backward elimination procedure to drop the interaction with the highest *P*-value from the model before the GEE was rerun. This process was repeated until the model contained only significant interactions. Finally, a stratified analysis comparing differences in factors related to longitudinal eGFR changes based on sex was carried out with GEE.

RESULTS

Analysis of the Selected and Excluded Students

There was no significant difference between the median eGFR of the selected and excluded students for both the imputed and nonimputed data (Table 1). No significant difference was observed for the eGFR category based on the KDIGO classification between these 2 groups.

For the other variables, there were slight differences in the proportion of fasting blood sugar category between the selected and excluded students at baseline. However, the differences were small ($P < 0.047$). More apparent differences were observed between the selected and excluded students for their sex and place of residence (Table 2).

Table 1. Comparison of the KDIGO staging distribution between the selected and excluded students

	Without imputation			With imputation		
	Selected	Excluded	χ^2	Selected	Excluded	χ^2
Classification	*N* (%)	*N* (%)	*P*-value	*N* (%)	*N* (%)	*P*-value
KDIGO 2012						
G1	570 (93.9)	645 (91.2)	$P = 0.067$	570 (93.9)	1215 (92.5)	$P = 0.253$
G2	37 (6.1)	62 (8.8)		37 (6.1)	99 (7.5)	
KDIGO 2014						
G1	424 (69.9)	174 (67.2)	$P = 0.581$	424 (69.9)	598 (69.1)	$P = 0.914$
G2	182 (30.1)	85 (32.8)		182 (30.1)	267 (30.8)	
G3	1 (0.2%)	0 (0.0)		1 (0.2%)	1 (0.1)	
KDIGO 2016						
G1	360 (59.3)	48(54.5)	$P = 0.535$	360 (59.3)	408 (58.7)	$P = 0.959$
G2	244 (40.2)	40 (45.5)		244 (40.2)	284 (40.9)	
G3	3 (0.5)	0 (0.0)		3 (0.5)	3 (0.4)	

KDIGO, Kidney Disease: Improving Global Outcomes.

Table 2. Distribution of the baseline characteristics of the selected and excluded students between non-imputed and imputed data

	Without imputation			With imputation		
	Selected	Excluded	χ^2	Selected	Excluded	χ^2
Classification	*N* (%)	*N* (%)	*P*-value	*N* (%)	*N* (%)	*P*-value
Sex						
Male	169 (27.8)	340 (47.0)	$P < 0.001$	169 (27.8)	340 (47.0)	$P < 0.001$
Female	438 (72.2)	384 (53.0)		438 (72.2)	384 (53.0)	
Place of residence						
Urban	318 (52.4)	463 (64.0)	$P < 0.001$	318 (52.4)	463 (64.0)	$P < 0.001$
Rural	289 (47.6)	261 (36.0)		289 (47.6)	261 (36.0)	
FBS 2012						
Low	15 (2.5)	8 (1.1)	$P = 0.047$	15 (2.5)	8 (1.1)	$P = 0.039$
Normal	574 (94.6)	676 (94.2)		574 (94.6)	681 (94.1)	
High	18 (3.0)	34 (4.7)		18 (3.0)	35 (4.8)	

FBS, fasting blood sugar.

Sociodemographic Characteristics at Baseline

This study recruited more female students (72.2%) than males (27.8%) at a median (interquartile range) age of 12.9 (0.5) years (Table 3). Urban students outnumbered rural students by 4.8%. More than half of the student's parents had at least a secondary education, and only 4.4% had no formal education during childhood. The majority (66.7%) of households had a median (interquartile range) income of RM1200 (900) (equivalent to 388.68 US Dollars in 2012), whereas only 8.7% had a high income.

Kidney Function at Baseline

The median (interquartile range) eGFR was significantly different between males and females ($P < 0.001$). Male students had 105.2 (22.1) ml/min/1.73 m^2, while females had 119.4 (32.5). The urban and rural students had similar eGFRs (115.6 [32.9] and 113.6 [26.3] ml/min per 1.73 m^2, respectively ($P = 0.237$).

Kidney Function Changes Along the Cohort

The median (interquartile range) eGFR reduced from 115.1 (29.0) ml/min per 1.73 m^2 in 2012 to 93.5 (25.0) ml/min per 1.73 m^2 in 2016. At the end of the cohort, male students remained to have lower eGFR than females ($P < 0.001$), and students in rural areas had lower eGFR than those in urban ($P = 0.001$) (Figure 2).

Along the cohort, the percentage of students with decreased eGFR (60–89 ml/min per 1.73 m^2) increased from 6.1% in 2012 to 30.0% in 2014 and 40.2% in 2016. Few students already had eGFR <60 ml/min per 1.73 m^2 in 2014 and 2016 (Figure 3).

Longitudinal Analysis of the Kidney Function Changes

Model 1

As the students' age, their eGFR reduces significantly with time (Table 4). A more significant eGFR reduction was seen during the first half of the study, where the eGFR reduced by 8.2 ml/min per 1.73 m^2 per year from 2012 to 2014 compared to 4.2 ml/min per 1.73 m^2 per year, which was observed from 2014 to 2016.

Model 2

Model 2 also showed decreasing eGFR over time. As presented in Table 5, male and rural students had lower

Table 3. Sociodemographic characteristics of students

Sociodemographic characteristics		Number of students (unweighted)	Estimation in the population (weighted)
Total		*N* (%)	*N* (%)
		607 (100)	696,048 (100)
Sex	Male	169 (27.8)	217,344 (31.2)
	Female	438 (72.2)	478,704 (68.8)
Ethnicity	Malay	464 (76.4)	437,629 (62.9)
	Chinese	53 (8.7)	52,314 (7.5)
	Indian	58 (9.6)	183,450 (26.4)
	Others	32 (5.3)	22,654 (3.3)
Place of residence	Urban	318 (52.4)	503,156 (72.3)
	Rural	289 (47.6)	192,892 (27.7)
State	Selangor	160 (26.4)	145,985 (21.0)
	Kuala Lumpur	121 (19.9)	161,904 (23.3)
	Perak	326 (53.7)	388,159 (55.8)
Father's education	No formal education	27 (4.4)	31,170 (4.5)
	Primary	80 (13.2)	92,279 (13.3)
	Secondary	385 (63.4)	451,024 (64.8)
	Tertiary (non-university)	44 (7.3)	55,451 (8.0)
	Tertiary (university)	71 (11.7)	66,123 (9.5)
Mother's education	No formal education	28 (4.6)	22,142 (3.2)
	Primary	69 (11.4)	85,104 (12.2)
	Secondary	393 (64.8)	432,427 (62.1)
	Tertiary (non-university)	51 (8.4)	76,177 (10.9)
	Tertiary (university)	66 (10.8)	80,198 (11.5)
Household income[a]	Low income (<988 USD)	405 (66.7)	467,566 (67.2)
	Middle income (988–2251 USD)	149 (24.6)	159,183 (22.9)
	High income (>2251 USD)	53 (8.7)	69,299 (10.0)

[a]Based on the 2012 Malaysian Ringgit (RM)-US Dollar (USD) exchange rate.

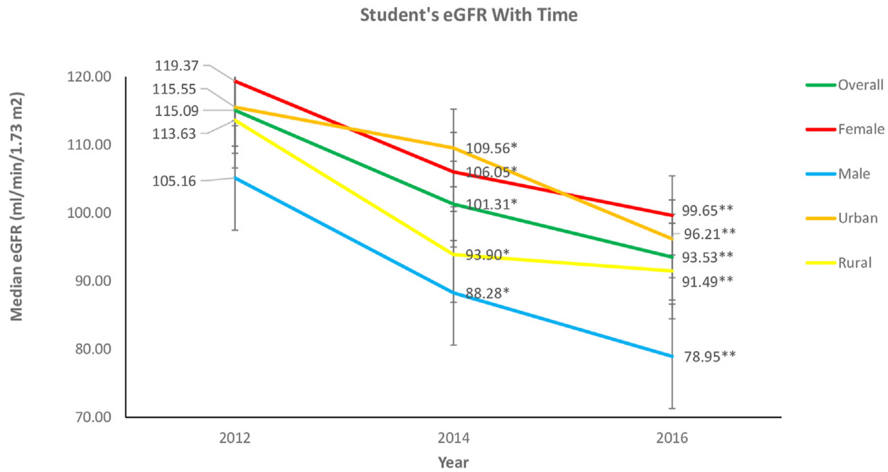

Figure 2. The eGFR of the students throughout the cohort. Data are the median eGFR of the students based on different characteristics throughout the cohort. Statistically significant differences are indicated with (*) and (**) at $P < 0.05$. Note: * compared with baseline; ** compared with second follow-up in 2016. eGFR, estimated glomerular filtration rate

eGFR ($P < 0.001$) than their counterparts. Indian students had the highest eGFR reduction by 8.6 ml/min per 1.73 m^2 (95% confidence interval: 4.4–12.9, $P < 0.001$) by the end of the study. In this model, students with more than 3.5 kg birth weight had significantly 4.7 ml/min per 1.73 m^2 higher eGFR ($P = 0.021$) than the normal birth weight group. As for the BMI, thinness grade 1 and 2 students had higher eGFR than students with normal BMI by 5.6 and 6.2 ml/min per 1.73 m^2, respectively. Students who are morbidly obese had 11.4 ml/min per 1.73 m^2 lower eGFR than those with normal weight ($P = 0.003$). No other factors were associated with eGFR changes in the main effects of model 2.

In Table 6, we present the significant interaction effect with time after the backward elimination process. In 2014, we observed that the eGFR of Chinese students were significantly higher than Malay students by 23.1 ml/min per 1.73 m^2 (95% confidence interval: 16.6–29.6). This difference remains significant in 2016 ($P < 0.001$) (Figure 4). Despite rural students having generally lower eGFR than urban (main effect), the interaction with time was only significant for the differences seen in 2014 (Figure 5). Students with high physical activity in 2016 had a lower eGFR by 35.4 ml/min/1.73 m^2 compared to those with moderate physical activity in the same year ($P = 0.009$) (Figure 6).

Table 4. Generalized estimating equation between eGFR and time (crude)

Variables	β	Standard error	*P*-value	95% CI
Time				
2012	Reference			
2014	−16.4	1.010	<0.001	(−18.4, −14.4)
2016	−24.8	1.007	<0.001	(−26.8, −22.9)

CI, confidence interval; eGFR, estimated glomerular filtration rate.

Surprisingly, overweight students had 7.2 ml/min/1.73 m^2 higher eGFR than normal weight students ($P = 0.012$) in 2014, and morbidly obese students had higher eGFR compared to normal weight students in 2016 ($P = 0.005$) (Figure 7). Students who consumed above the recommended protein in 2016 had a 7.2 ml/min/1.73 m^2 (95% confidence interval: 1.7–12.8) lower eGFR than those who consumed within the recommended level ($P = 0.010$).

Stratified Analyses of Model 2 Based on Sex

Given that there were differences in the proportion of male and female students between the selected and excluded students, a stratified analysis was conducted based on this covariate. In these 2 separate analyses between male and female students, the eGFR significantly reduced with time in both groups ($P < 0.001$). A slightly bigger reduction of eGFR was seen in males than in females. Indian students showed significantly lower eGFR by 9.1 ml/min per 1.73 m^2 compared to Malay students, but only among females ($P = 0.001$). Similarly, only the female students in the rural area were associated with significantly lower eGFR compared to those in the urban area.

Surprisingly, none of the BMI categories was associated with the eGFR changes in male students. However, female students with thinness grade 1 had 6.7 ml/min per 1.73 m^2 higher eGFR than those with normal BMI ($P = 0.002$). In addition, morbidly obese female students had lower eGFR by 11.8 ml/min per 1.73 m^2 than those with normal BMI ($P = 0.021$). We also observed that female students who consumed sodium above the recommended level had significantly higher eGFR than those who consumed sodium within the recommended level ($P = 0.021$). On the other hand, male students who consumed protein above the

Table 5. Generalized estimating equation for the longitudinal relationship between eGFR, sex, ethnicity, place of residence, birth weight, and BMI among adolescents in Peninsular Malaysia over 5 years (model 2: main effect)[a]

Classification	eGFR (ml/min per 1.73 m²)		
Variables	β	95% CI	P[b]
Time			
2012	Reference		
2014	−15.9	(−18.1, −13.7)	<0.001
2016	−24.6	(−26.7, −22.5)	<0.001
Sex			
Male	Reference		
Female	19.2	(16.3, 22.1)	<0.001
Ethnicity			
Malay	Reference		
Chinese	−1.2	(−6.3, 3.9)	0.684
Indian	−8.6	(−12.9, −4.4)	<0.001
Others	−6.4	(−13.1, 0.3)	0.048
Place of residence			
Urban	Reference		
Rural	−9.7	(−12.6, −6.9)	<0.001
Birth weight status			
Normal weight	Reference		
Low birth weight	−3.4	(−7.1, 0.4)	0.079
High birth weight	4.7	(0.7, 8.7)	0.021
BMI (IOTF class)			
Normal	Reference		
Thinness grade 3	6.8	(−2.1, 15.7)	0.133
Thinness grade 2	6.2	(1.2, 11.2)	0.015
Thinness grade 1	5.6	(2.2, 8.9)	0.001
Overweight	−0.1	(−3.2, 3.1)	0.965
Obesity	2.0	(−3.6, 7.5)	0.490
Morbid obesity	−11.4	(−19.0, −3.9)	0.003

CI, confidence interval; BMI, body mass index; eGFR, estimated glomerular filtration rate; IOTF, International Obesity Task Force.
[a]Adjusted for household income, parental education level, history of high-risk behavior (smoking, vaping, alcohol consumption, and illicit drug use), level of physical activity, systolic BP, diastolic BP, fasting blood sugar and dietary intake.
[b]Statistically significant ($P < 0{\cdot}05$).

Table 6. The effect of time interaction between the student's eGFR and variables in model 2 based on GEE (eGFR * main effect * time interaction)[a]

Classification	eGFR (ml/min per 1.73 m²)		
Variables	β	95% CI	P[b]
Main effects (model 2)	Interaction		
Time*ethnicity			
2014*Malay	Reference		
2014*Chinese	23.1	(16.6, 29.6)	<0.001
2016*Malay	Reference		
2016*Chinese	20.4	(15.0, 25.9)	<0.001
Time*Place of residence			
2014*urban	Reference		
2014*rural	−7.2	(−11.4, −3.0)	0.001
Time*Physical activity level			
2016*Moderate physical activity	Reference		
2016*High physical activity	−35.4	(−62.0, −8.8)	0.009
Time*BMI status (IOTF)			
2014*Normal weight	Reference		
2014*Overweight	7.2	(1.6, 12.7)	0.012
2016*Normal weight	Reference		
2016*Morbid obesity	12.8	(3.9, 21.7)	0.005
Time*FBS category			
2016*Normal	Reference		
2016*Low FBS	−17.6	(−33.8, −1.4)	0.033
Time*Protein intake status			
2016*within recommendation	Reference		
2016*above recommendation	−7.2	(−12.8, −1.7)	0.010

BMI, body mass index; BP, blood pressure; CI, confidence interval, eGFR, estimated glomerular filtration rate; GEE, generalized estimating equation; FBS, fasting blood sugar; IOTF, International Obesity Task Force.
[a]Post backward elimination procedure. Results displayed were only for significant interaction effect with $P < 0.05$. All interaction effects have been adjusted for sex, household income, parental education level, birthweight category, history of high-risk behavior (smoking, vaping, alcohol consumption, and illicit drug use), systolic BP, diastolic BP, fasting blood sugar and dietary intake.
[b]Statistically significant ($P < 0{\cdot}05$)

recommended level had significantly higher eGFR than their counterparts. However, this association was weak ($P = 0.042$). In Table 7, we present the covariates with significant findings in the stratified analyses.

DISCUSSION

Kidney Function Trend Among the Students

Throughout the cohort, marked changes in the percentage of students with decreased eGFR were observed. It was higher than a report in Turkey, where only 2.92% of children aged 5 to 18 in their population have CKD stage 2 (eGFR 60–89 ml/min per 1.73 m^2).[24] However, in that study, the kidney function was assessed with urine protein and eGFR, which may have contributed to the lower prevalence reported. Although Saydah *et al*.[7] reported a similar prevalence for eGFR <90 ml/min per 1.73 m^2 among adolescents aged 12 to 18 years in the United States (34.6%), the data was collected over 15 years from 1994 to 2009, whereas our study was only for 5 years. Furthermore, it involved a series of cross-sectional data analyses, unlike ours, which was a cohort study. According to the Kidney Disease Outcomes Quality Initiative (KDOQI) guideline, the expected percentage of healthy adults in the population with eGFR 60 to 89 ml/min per 1.73 m^2 is 13.7% for those aged 20 to 39 years and increased to 42.7% for those aged 40 to 59 years.[25] Therefore, the percentage of students we observed in 2016, which has the same eGFR range as stated by the KDOQI guidelines, is already similar to the expected percentage for adults aged 40 to 59 years, which raised a concern.

Accuracy of the Findings

Although that was the report from the KDOQI guidelines, the provided estimate was based on the adult GFR estimation formula. However, none of the adult equations to estimate GFR is suitable for adolescents[26] because it tends to overestimate the eGFR in adolescents.[27,28] Therefore, the expected percentage deduced by KDOQI can only be applied to adults. In a separate

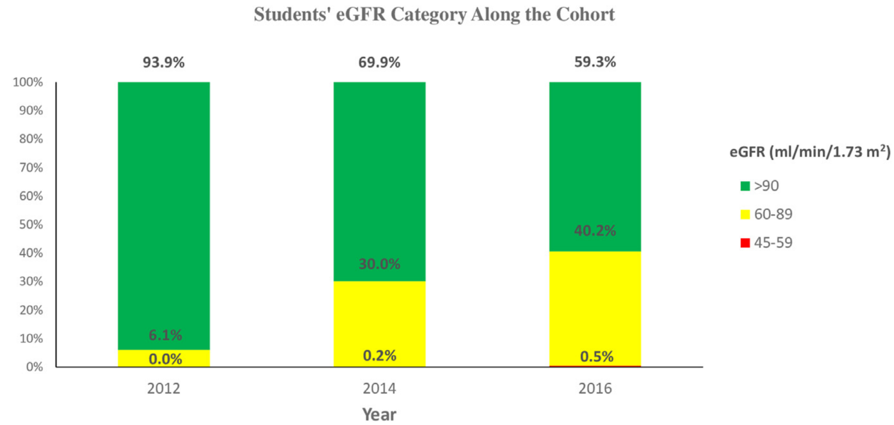

Figure 3. Students' eGFR category throughoutthe cohort. eGFR, estimated glomerular filtration rate.

analysis of the student's eGFR status using 4 different adult formulas, a higher percentage of adolescents with hyperfiltration (eGFR ≥140 ml/min per 1.73 m^2) were detected when both the Chronic Kidney Disease Epidemiology Collaboration formula was applied (Supplementary Table S3), suggesting the eGFR overestimation effect. In a similar analysis, significant differences in the prevalence of students' kidney function status were also observed, primarily when using the Chronic Kidney Disease Epidemiology Collaboration formula, the recommended formula for individuals aged ≥18 years old. The big difference in the percentage of students with decreased eGFR 60 to 89 ml/min per 1.73 m^2 remains even though this formula was used at the end of the adolescence phase when students were aged 17 years in 2016. Pottel *et al.*[27] found that the Chronic Kidney Disease Epidemiology Collaboration (2009) equation was the culprit for the implausible changes, where it overestimates GFR by 21 ml/min per 1.73 m^2 during the adolescent-adult transition phase. In a similar report, the Lund-Malmö-Revised equation outperforms other equations with higher $P_{30\%}$ and lower biases, especially for calculating eGFR of measured GFR >75 ml/min per 1.73 m^2 during that phase. This formula yielded the lowest z-value for proportion differences in our earlier separate analysis (Supplementary Table S3). Despite that issue with the adult equation, the Schwartz equation underestimates the "true" eGFR by 10%, especially among those with GFR ≥90 ml/min per 1.73 m^2,[9] which may have contributed to the high prevalence of students with eGFR 60 to 89 ml/min per 1.73 m^2 seen in this study.

Figure 4. Median eGFR of different ethnic groups at different years in the cohort. The differences in the eGFR between Malay and Chinese students observed from 2014 to 2016 resulted in a significant interaction effect in model 2. eGFR, estimated glomerular filtration rate.

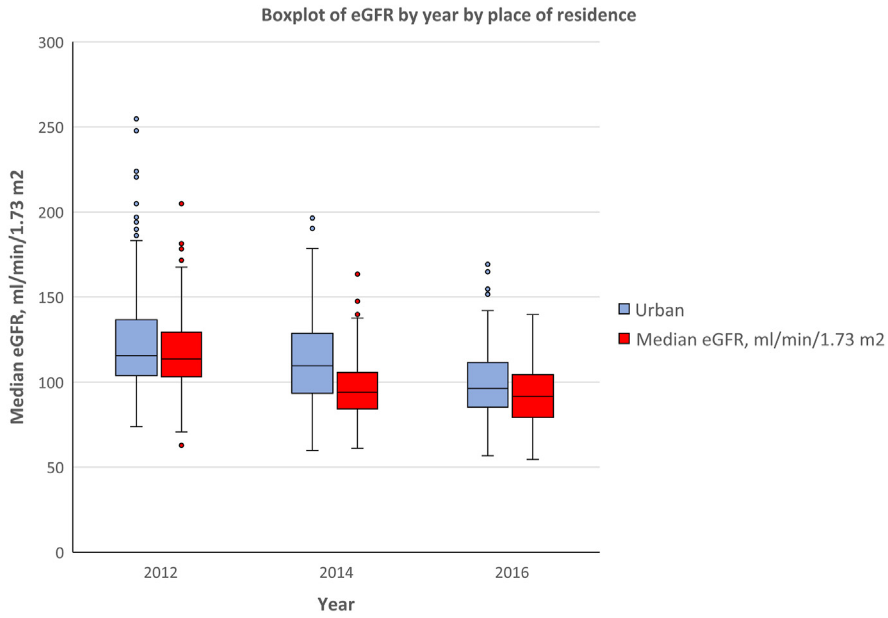

Figure 5. Box plot illustrating the eGFR of the students based on place of residence at different timelines. The data showed a marked difference between the upper quartile Q3 and median eGFR of urban and rural students in 2014 compared to the other 2 years. These differences may have contributed to the significant interaction effects seen in 2014. eGFR, estimated glomerular filtration rate.

Effects of Health and Lifestyle Factors on eGFR

The longitudinal analysis revealed greater eGFR changes in the first half of the study. This reduction rate exceeded the KDOQI guideline of 1 ml/min per 1.73 m^2 per year.[25] We hypothesized that it could be related to puberty because the Italkid project has reported that kidney function declines 10 times faster after the pubertal growth spurt.[29] The female students' eGFR was also significantly higher than males in this study, although it was expected to be 8% lower than males at all ages, according to KDOQI.[25] Two possible explanations were suggested. We argue that the students' eGFR changes may have started earlier, even before the study, because eGFR changes could be observed as early as 8 to 9 years old.[30] Considering that females attain menarche earlier than males,[31] female sex steroids protect the kidneys from further deterioration.[32] Furthermore, male students were more active throughout the study, resulting in increased creatinine excretion through protein catabolism,[33] which reduced the eGFR. The effect of physical activity also may have played a role in ethnic groups-eGFR changes seen in this study. Indian students were the most active, whereas Chinese students were the least active in the

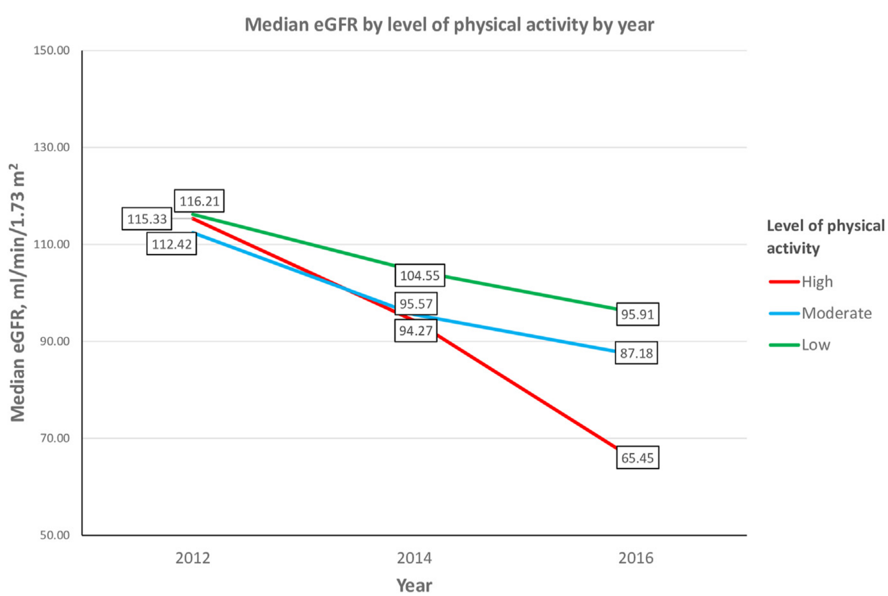

Figure 6. Median eGFR of the students based on the level of physical activity along the cohort. The marked reduction of eGFR among students with high physical activity in 2016 resulted in a significant interaction effect in model 2. eGFR, estimated glomerular filtration rate.

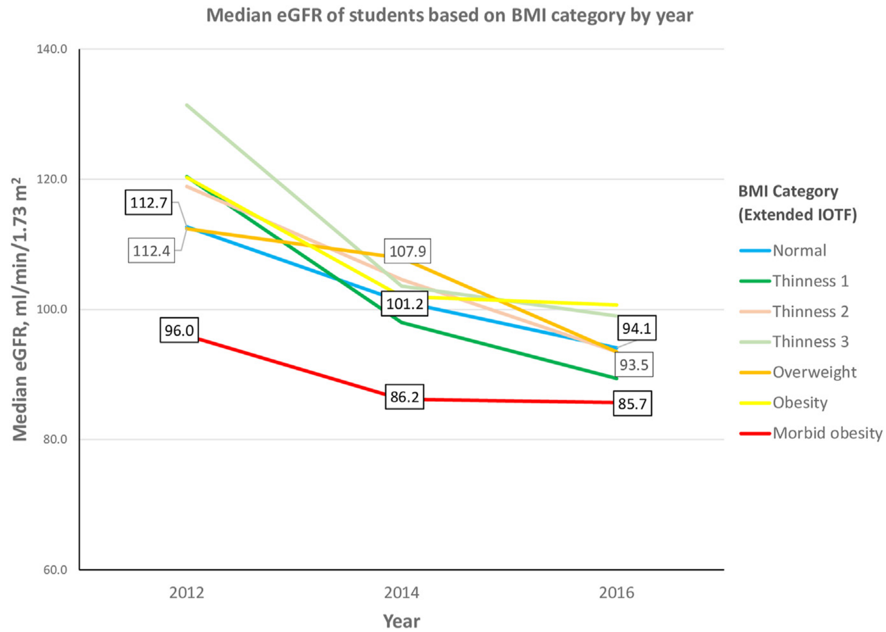

Figure 7. Median eGFR of the students based on body mass index category along the cohort. The marked reduction of eGFR seen among the students with high physical activity in 2016 resulted in a significant interaction effect in model 2. eGFR, estimated glomerular filtration rate.

Table 7. Generalized estimating equation for the longitudinal relationship between eGFR, ethnicity, place of residence, BMI, and dietary intake among adolescents in Peninsular Malaysia over 5 years, stratified by sex[a]

Classification	eGFR (ml/min/1.73 m^2)					
	Male			Female		
Variables	β	95% CI	*P*	β	95% CI	*P*
Time						
2012	Reference			Reference		
2014	−17.5	(−20.8, −14.2)	<0.001	−15.4	(−18.3, −12.6)	<0.001
2016	−27.2	(−30.7, −23.7)	<0.001	−23.5	(−26.2, −20.9)	<0.001
Ethnicity						
Malay	Reference			Reference		
Chinese	−0.5	(−7.4, 6.4)	0.885	−1.5	(−7.9, 5.0)	0.661
Indian	−6.0	(−12.9, 0.9)	0.088	−9.1	(−14.4, -3.8)	0.001
Others	0.4	(−7.9, 8.7)	0.917	−8.5	(−17.5, 0.4)	0.062
Place of residence						
Urban	Reference			Reference		
Rural	−3.3	(−8.2, 1.7)	0.194	−11.3	(−14.7, −7.9)	<0.001
BMI (IOTF)						
Normal	Reference			Reference		
Thinness grade 3	7.9	(−4.7, 20.5)	0.218	7.3	(−2.2, 16.8)	0.131
Thinness grade 2	2.2	(−4.7, 9.2)	0.533	6.9	(−0.1, 13.8)	0.053
Thinness grade 1	1.5	(−3.3, 6.4)	0.533	6.7	(2.4, 11.0)	0.002
Overweight	2.6	(−1.8, 7.0)	0.245	-0.8	(−4.8, 3.2)	0.691
Obesity	−5.5	(−14.6, 3.6)	0.235	3.6	(−2.9, 10.1)	0.274
Morbid obesity	−7.2	(−17.4, 2.9)	0.164	-11.8	(−21.7, -1.8)	0.021
Sodium intake						
Within recommendation	Reference			Reference		
Above recommendation	−2.1	(−5.5, 1.3)	0.219	3.4	(0.5, 6.2)	0.021
Protein intake						
Within recommendation	Reference			Reference		
Above recommendation	4.3	(0.2, 8.5)	0.042	−1.5	(−5.1, 2.1)	0.423

BMI, body mass index; BP, blood pressure; CI, confidence interval; eGFR, estimated glomerular filtration rate; IOTF, International Obesity Task Force.
[a]Adjusted for household income, parental education level, history of high-risk behavior (smoking, vaping, alcohol consumption, and illicit drug use), birth weight category, level of physical activity, systolic BP, diastolic BP, fasting blood sugar and dietary intake.

study, possibly leading to the observation seen in Indian and Chinese students.

In this study, rural students had significantly lower eGFR than urban students. The NHANES data and the Chronic Kidney Disease in Children study found that rural residents have a higher risk of CKD than urban residents, linked to lower socioeconomic status.[7,34] In addition, the rural students in the current study had a higher percentage of smokers, abnormal BP and obesity, which is known to lower eGFR among adolescents.[30,35,36] We suggested that obesity-related glomerulopathy resulting from overweight/obesity[37] coincided with the students in this study, which may explain why the eGFR of the overweight or obese students showed positive and negative changes in the longitudinal analysis. Contrarily, the students' eGFR changed in parallel to the birth weight in this study. Although low birth weight was not significantly associated with changes in eGFR in the longitudinal analysis, we observed that their eGFR was lower than that of students with normal birth weights. Evidence has shown that individuals with low birth weight had higher odds of albuminuria and low GFR,[38] which is closely related to the number of nephrons in their kidneys.[39] Diet also affected eGFR in this study. In 2016, students who consumed more protein had lower eGFRs. However, during the stratified analyses, males who consumed more protein were observed to have a higher eGFR. These variations in the effect of protein on the kidney have been reported in the populations elsewhere too.[40] We hypothesized that the variations were related to the type of protein intake, whereas animal protein is CKD-risky, plant-type protein is renoprotective.[41]

Although this study also found a significant relationship between high sodium intake and eGFR changes in female students potentially related to hyperfiltration,[42] this finding needs cautious interpretation. Because >20% of students were excluded and there were disparities in sex distribution, we acknowledge that the results may be biased and affect the internal validity of this and other findings.[43,44] Although the analysis showed that the data were not missing completely at random and imputation can be conducted to complete the data, our strategy of obtaining the student's actual eGFR without imputation led to the exclusion of a large number of students who did not undergo kidney function assessment in all 3 years. However, we did not observe any difference in the eGFR status between the selected and excluded students, which is the outcome of this study.

Strengths and Limitations

The study's power was maintained due to adequate sample size, with a *post hoc* power analysis of 82.2%. The students' selection bias was reduced by randomization and stratified sampling during the initial recruitment strategy in the MyHeART study. However, potential selection bias was introduced because many students were excluded from the current study, and there were disparities in the proportion of male and female students between the selected and excluded students. In addition, the study's kidney function assessment method was a limitation. Without urine protein evaluation in the study, the students' "actual" kidney function status cannot be determined. Our study did not assess the type of protein (animal-based or plant-based) consumed, which may explain the longitudinal relationship between kidney function changes and protein intake. Due to all these, we advise that the findings from the study not be generalized to all adolescent population worldwide, especially in providing a clearer picture of the CKD status among adolescents in the community. Having said that, we strongly believe that these findings will be a good start to provide some insight into the kidney status of adolescents, especially in the Malaysian community. Considering that this study was a prospective cohort study, a relationship between the progression of kidney function with its associated factors could be generated. More importantly, students with decreased kidney function were referred to a health clinic for reevaluation. This step was essential to ensure students' well-being. We also believe this study is novel because it is the first in Asia.

Recommendations

Current research finding suggests a high prevalence of decreased kidney function among adolescents with in the community. Therefore, it should be highlighted as a potential outcome of NCD risk factors even at a young age. High-risk adolescents, including males, those from rural areas, Indians and those who are overweight or obese, should be screened for possible "early stage CKD." Because school-aged children spend most of their time at school, implementing a robust integrated school-based health program is needed.[45] Enhancing collaboration between related ministries and NGOs is essential to ensure a properly monitored and evaluated program.[46] A pilot project to assess adolescent urine albumin is recommended for future research. The study's findings can be compared to the current study to assess the country's school-aged adolescent kidney status. The current practice of abrupt changes from the Schwartz equation to the Chronic Kidney Disease Epidemiology Collaboration formula during the adolescent-adulthood transition may require further evaluation.

CONCLUSIONS

A high percentage of Malaysian adolescents aged 13 to 17 years showed decreased kidney function. The kidney function declined faster than usual, and these changes are potentially associated with multiple nonmodifiable and modifiable NCD risk factors. Adolescents who are morbidly obese at 13 to 17 years old presented with lower eGFR, possibly related to obesity-related glomerulopathy. The kidney function changes during this period were also associated with the adolescent's dietary intake, including protein and sodium intake. These findings should alert the public, parents, and stakeholders that if children continue to adopt unhealthy lifestyles from a young age, their kidney health may be affected. Therefore, more comprehensive prevention efforts from various stakeholders are required to identify health issues such as CKD, not only among adults but also among children and adolescents, the world's future population.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

The authors thank the students, parents, guardians, and health care personnel who participated in the study. The authors also thank Professor Dr. Tin Su (Monash University) for her support during the data collection phase of the study. This study was supported by funding from the University of Malaya Research Programme (IIRG001B-2022SAH, RP022-14HTM) and the Vice-Chancellor Research Grant (UMQUB3D-2011).

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. The eGFR category based on KDIGO and the classification used in the study.

Table S2. The Extended International Obesity Task Force BMI cut-off used in the study.

Table S3. Prevalence of students with decreased eGFR and the z-score for proportion differences based on various eGFR equations.

Strobe Statement.

REFERENCES

1. UNICEF. Adolescents. Accessed July 3, 2022. https://data.unicef.org/topic/adolescents/overview/
2. Harambat J, Ekulu PM. Inequalities in access to pediatric ESRD care: a global health challenge. *Pediatr Nephrol*. 2016;31:353–358. https://doi.org/10.1007/s00467-015-3263-7
3. Geary DF, Schaefer F. *Pediatric Kidney Disease*. 2nd ed. Springer; 2016.
4. Wong HS, Goh B. 24th Report of the Malaysian Dialysis and Transplant Registry 2016. The National Renal Registry. Accessed October 15, 2021. https://www.msn.org.my/nrr/24th-report-of-the-malaysian-dialysis-and-transplant-registry-2016/
5. Lim YNTL. 14th Report of the Malaysian Dialysis and Transplant Registry 2006. The National Renal Registry. Accessed October 15, 2021. https://www.msn.org.my/nrr/14th-report-of-the-malaysian-dialysis-and-transplant-registry-2006/
6. Rosner B. *Fundamentals of Biostatistics*. 8th ed. Boston, MA: Cengage Learning; 2015.
7. Saydah SH, Xie H, Imperatore G, Burrows NR, Pavkov ME. Trends in albuminuria and GFR among adolescents in the United States, 1988-2014. *Am J Kidney Dis*. 2018;72:644–652. https://doi.org/10.1053/j.ajkd.2018.04.021
8. Hazreen MA, Su TT, Jalaludin MY, et al. An exploratory study on risk factors for chronic non-communicable diseases among adolescents in Malaysia: overview of the Malaysian Health and Adolescents Longitudinal Research Team study (The MyHeART study). *BMC Public Health*. 2014;14(Suppl 3):S6. https://doi.org/10.1186/1471-2458-14-S3-S6
9. Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. *Clin J Am Soc Nephrol*. 2009;4:1832–1843. https://doi.org/10.2215/CJN.01640309
10. Huong NTQ, Long TD, Bouissou F, et al. Chronic kidney disease in children: the National Paediatric Hospital experience in Hanoi, Vietnam. *Nephrology (Carlton)*. 2009;14:722–727. https://doi.org/10.1111/j.1440-1797.2009.01142.x
11. Abu OO, Raji YR, Amodu OK. Risk factors for chronic kidney disease among in-school adolescents in Ibadan, Southwest, Nigeria. *Sahel Med J*. 2019;22:64.
12. Inker LA, Astor BC, Fox CH, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. *Am J Kidney Dis*. 2014;63:713–735. https://doi.org/10.1053/j.ajkd.2014.01.416
13. Watkins D, Murray L, McCarron P, et al. Ten-year trends for fatness in Northern Irish adolescents: the Young Hearts Projects—repeat cross-sectional study. *Int J Obes (Lond)*. 2005;29:579–585. https://doi.org/10.1038/sj.ijo.0802945
14. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes*. 2012;7:284–294. https://doi.org/10.1111/j.2047-6310.2012.00064.x
15. Kowalski KC, Crocker PR, Donen RM. The Physical Activity Questionnaire for Older Children (PAQ-C) and Adolescents (PAQ-A) Manual. University of Saskatchewan: College of Kinesiology; 2004:1–38 pp. https://www.prismsports.org/UserFiles/file/PAQ_manual_ScoringandPDF.pdf
16. Zalilah M, Khor G, Mirnalini K, Norimah A, Ang M. Dietary intake, physical activity and energy expenditure of Malaysian adolescents. *Singapore Med J*. 2006;47:491–498.
17. Zaki NAM, Sahril N, Omar MA, Ahmad MH, Baharudin A, Nor NSM. Reliability and validity of the physical activity questionnaire for older children (PAQ-C) in Malay language. *Int J Public Health Research*. 2016;6:670–676.
18. Burrows TL, Martin RJ, Collins CE. A systematic review of the validity of dietary assessment methods in children when compared with the method of doubly labelled water. *J Am*

Diet Assoc. 2010;110:1501–1510. https://doi.org/10.1016/j.jada.2010.07.008

19. Ministry of Health Malaysia. Recommended nutrient intakes for Malaysia. Accessed January 23, 2019. http://nutrition.moh.gov.my/wp-content/uploads/2017/05/FA-Buku-RNI.pdf
20. World Health Organization. Guideline: sodium intake for adults and children. Accessed December 23, 2019. https://www.who.int/nutrition/publications/guidelines/sodium_intake_printversion.pdf
21. Flynn JT, Kaelber DC, Baker-Smith CM, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics*. 2017;140: e20171904. https://doi.org/10.1542/peds.2017-1904
22. Zimmet P, Alberti KGM, Kaufman F, et al. The metabolic syndrome in children and adolescents-an IDF consensus report. *Pediatr Diabetes*. 2007;8:299–306. https://doi.org/10.1111/j.1399-5448.2007.00271.x
23. Schnaper HW, Hubchak SC, Runyan CE, et al. A conceptual framework for the molecular pathogenesis of progressive kidney disease. *Pediatr Nephrol*. 2010;25:2223–2230. https://doi.org/10.1007/s00467-010-1503-4
24. Soylemezoglu O, Duzova A, Yalçınkaya F, Arınsoy T, Süleymanlar G. Chronic renal disease in children aged 5-18 years: a population-based survey in Turkey, the CREDIT-C study. *Nephrol Dial Transplant*. 2012;27(suppl 3):iii146–iii151. https://doi.org/10.1093/ndt/gfs366
25. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis*. 2002;39:S1–S266.
26. Selistre L, De Souza V, Cochat P, et al. GFR estimation in adolescents and young adults. *J Am Soc Nephrol*. 2012;23: 989–996. https://doi.org/10.1681/ASN.2011070705
27. Pottel H, Björk J, Bökenkamp A, et al. Estimating glomerular filtration rate at the transition from pediatric to adult care. *Kidney Int*. 2019;95:1234–1243. https://doi.org/10.1016/j.kint.2018.12.020
28. Selistre L, Rabilloud M, Cochat P, et al. Comparison of the Schwartz and CKD-EPI equations for estimating glomerular filtration rate in children, adolescents, and adults: a retrospective cross-sectional study. *PLoS Med*. 2016;13:e1001979. https://doi.org/10.1371/journal.pmed.1001979
29. Ardissino G, Testa S, Daccò V, et al. Puberty is associated with increased deterioration of renal function in patients with CKD: data from the ItalKid Project. *Arch Dis Child*. 2012;97: 885–888. https://doi.org/10.1136/archdischild-2011-300685
30. Correia-Costa L, Afonso AC, Schaefer F, et al. Decreased renal function in overweight and obese prepubertal children. *Pediatr Res*. 2015;78:436–444. https://doi.org/10.1038/pr.2015.130
31. Deng Y, Liang J, Zong Y, et al. Timing of spermarche and menarche among urban students in Guangzhou, China: trends from 2005 to 2012 and association with Obesity. *Sci Rep*. 2018;8:263. https://doi.org/10.1038/s41598-017-18423-6
32. Neugarten J, Acharya A, Silbiger SR. Effect of gender on the progression of nondiabetic renal disease: a meta-analysis. *J Am Soc Nephrol*. 2000;11:319–329. https://doi.org/10.1681/ASN.V112319
33. Calles-Escandon J, Cunningham JJ, Snyder P, et al. Influence of exercise on urea, creatinine, and 3-methylhistidine excretion in normal human subjects. *Am J Physiol Endocrinol Metab*. 1984;246:E334–E338. https://doi.org/10.1152/ajpendo.1984.246.4.E334
34. Hidalgo G, Ng DK, Moxey-Mims M, et al. Association of income level with kidney disease severity and progression among children and adolescents with CKD: a report from the Chronic Kidney Disease in Children (CKiD) Study. *Am J Kidney Dis*. 2013;62:1087–1094. https://doi.org/10.1053/j.ajkd.2013.06.013
35. García-Esquinas E, Loeffler LF, Weaver VM, Fadrowski JJ, Navas-Acien A. Kidney function and tobacco smoke exposure in US adolescents. *Pediatrics*. 2013;131:e1415–e1423. https://doi.org/10.1542/peds.2012-3201
36. Leiba A, Twig G, Vivante A, et al. Prehypertension among 2.19 million adolescents and future risk for end-stage renal disease. *J Hypertens*. 2017;35:1290–1296. https://doi.org/10.1097/HJH.0000000000001295
37. Salvatore SP, Chevalier JM, Kuo SF, Audia PF, Seshan SV. Kidney disease in patients with obesity: it is not always obesity-related glomerulopathy alone. *Obes Res Clin Pract*. 2017;11:597–606. https://doi.org/10.1016/j.orcp.2017.04.003
38. White SL, Perkovic V, Cass A, et al. Is low birth weight an antecedent of CKD in later life? A systematic review of observational studies. *Am J Kidney Dis*. 2009;54:248–261. https://doi.org/10.1053/j.ajkd.2008.12.042
39. Mañalich R, Reyes L, Herrera M, Melendi C, Fundora I. Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. *Kidney Int*. 2000;58:770–773. https://doi.org/10.1046/j.1523-1755.2000.00225.x
40. Hahn D, Hodson EM, Fouque D. Low protein diets for non-diabetic adults with chronic kidney disease. *Cochrane Database Syst Rev*. 2018;10:CD001892. https://doi.org/10.1002/14651858.CD001892.pub4
41. Mirmiran P, Yuzbashian E, Aghayan M, Mahdavi M, Asghari G, Azizi F. A prospective study of dietary meat intake and risk of incident chronic kidney disease. *J Ren Nutr*. 2020;30:111–118. https://doi.org/10.1053/j.jrn.2019.06.008
42. Boero R, Pignataro A, Quarello F. Salt intake and kidney disease. *J Nephrol*. 2002;15:225–229.
43. Kristman V, Manno M, Côté P. Loss to follow-up in cohort studies: how much is too much? *Eur J Epidemiol*. 2004;19:751–760. https://doi.org/10.1023/b:ejep.0000036568.02655.f8
44. Molenberghs G, Kenward M. *Missing Data in Clinical Studies*. Chichester, UK: John Wiley & Sons; 2007.
45. Jourdan D, Gray NJ, Barry MM, et al. Supporting every school to become a foundation for healthy lives. *Lancet Child Adolesc Health*. 2021;5:295–303. https://doi.org/10.1016/S2352-4642(20)30316-3
46. World Health Organization. Who guideline on school health services. Accessed January 24, 2022. https://www.who.int/publications/i/item/9789240029392