


# BMJ Open Comparison of three spot urine formulae and their validation using 24-hour urine sodium for estimation of daily salt intake: a cross-sectional study among Bangladeshi adults

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## ABSTRACT

**Objective** This study aimed to assess the validity of three commonly used (Tanaka, Kawasaki, INTERSALT) methods based on spot urinary sodium excretion against the 24-hour urinary sodium excretion to estimate the dietary salt intake in Bangladesh.

**Design** A population-based cross-sectional survey.

**Setting** A cross-sectional survey was done in an urban and a rural area of Bangladesh in 2012–2013.

**Participants** 418 community living residents aged 40–59 years participated in the survey and data of 227 subjects who had complete information were analysed for this validation study.

**Outcome measures** The Bland-Altman method was used to evaluate the agreement between the estimated and measured 24-hour urinary sodium. The estimated average salt intake from Tanaka, Kawasaki and INTERSALT methods were plotted against 24-hour urinary sodium excretion.

**Results** The mean 24-hour estimated salt intake was 10.0g/day (95% CI 9.3 to 10.6). The mean estimated urinary salt by Tanaka, Kawasaki and INTERSALT methods were 8.5g/day (95% CI 8.2 to 8.8), 11.4g/day (95% CI 10.8 to 12.0) and 8.8g/day (95% CI 8.6 to 9.0), respectively. Compared with the estimated mean salt intake from 24-hour urine collection, the Bland-Altman plot indicated the mean salt intake was overestimated by the Kawasaki method and underestimated by Tanaka and INTERSALT methods. The linear regression line showed the Kawasaki method was the least biased and had the highest intraclass correlation coefficient (0.57, 95% CI 0.45 to 0.67).

**Conclusion** Tanaka, Kawasaki and INTERSALT methods were not appropriate for the estimation of 24-hour urinary sodium excretion from spot urine samples to assess dietary salt intake in Bangladesh. Among the three methods, the Kawasaki method has the highest agreement with the 24-hour urinary sodium excretion concentration in this population.

## INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death globally.<sup>1</sup> Bangladesh, a

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ Estimation of salt intake in urban and rural population was done using 24-hour urine collection method as well as from spot urine sodium concentration using three globally validated equations.
- ⇒ The second day spot urine samples were not used.
- ⇒ The participants were volunteers with age 40–59 years from selected communities.

lower-middle-income country, has been undergoing epidemiological transition and, CVD has become the top cause of death.<sup>2–3</sup> High blood pressure is the most common CVD risk factor. Recent national and subnational estimates of the prevalence of hypertension among adults range from 6.5% to 48% in Bangladesh, with a lower prevalence in the rural population.<sup>4–8</sup> Habitual excess salt consumption is a well-established determinant of high blood pressure.<sup>9–11</sup> Prevention of high blood pressure by reducing the salt intake is one of the cost-effective measures for the prevention of CVD.<sup>12</sup> WHO has set a target of 30% relative reduction of salt intake by 2025.<sup>13</sup>

To have an appropriate national strategy for reducing salt intake, the current level of salt consumption needs to be known. The best estimate of the population distribution and average level of dietary salt intake is provided by measuring 24-hour urinary sodium excretion in a representative sample of the individuals.<sup>14</sup> However, collection of 24-hour urine is inconvenient and has a participant burden and is difficult to use repeatedly to monitor the trends of the population's salt intake to achieve the goal of reducing salt intake. To evaluate the effectiveness of country-level salt

reduction strategies, the use of spot-urine sodium concentration for estimation of daily salt intake was proposed as an alternative to 24-hour urine collection.<sup>14</sup> Several equations have been developed for estimating salt intake based on spot urine sodium concentration.<sup>15–17</sup> Few studies in Bangladesh used spot urine sodium concentration to estimate 24-hour urinary sodium excretion using different equations developed in other population.<sup>4 18</sup> However, those equations were not validated in Bangladesh in a single set of cross-sectional study where both methods were used to estimate salt intake.<sup>18 19</sup> In light of this, the aim of this study was to assess the validity of three commonly used (Tanaka, Kawasaki, INTERSALT) methods based on spot urinary sodium excretion against the 24-hour urinary sodium excretion to estimate the dietary salt intake in Bangladesh.

## METHODS

A cross-sectional study was done in an urban and a rural area of Bangladesh in 2012–2013 to estimate the dietary salt intake indirectly from urinary excretion of sodium measured from the collection of 24-hour urine in the community living residents. Invitations were sent to residents aged 40–59 years living in the selected areas to participate in the study, as they were readily available. Volunteers willing to participate were requested to attend study clinics at project areas for enrolment in the study and subsequent measurements.

All respondents provided informed consent to participate in the study. All the participants provided both verbal and written informed consent before participating in the study during sample collection, and the consent explicitly stated the purpose of the use of data. After taking the informed consent, a screening questionnaire was administered, and blood pressure, height and weight were measured by the trained and experienced data enumerators. Height and weight were measured in standardised way.

The individuals with kidney disease, heart failure or on diuretics were excluded, and participants who were pregnant at the time of the survey were also excluded.

## Patient and public involvement

No patient was involved.

## Urine collection

For the collection of 24-hour urine, participants were requested to void urine after coming to the clinic, and a five ml sample of that urine (spot urine) was taken for biochemical measurement. The time of voiding was recorded, and participants were instructed to collect all urine in a jar for the next 24 hours and visit the clinic the next day at the scheduled time. On the next day, respondents were requested to void urine in the clinic, and that urine was mixed with the collected urine for the last 24 hours. The total volume of the urine was measured by trained laboratory technicians, and then four aliquots of

5 mL urine (24 hours urine) were collected and kept in the freezer (–20°C) until measurement in the biochemical laboratory. Urinary sodium (Na), potassium (K) and creatinine (Cr) in spot and 24-hour collection samples were measured by using an auto-analyzer (Easylyte Plus, Medica, USA).

Twenty-four-hour Na excretion and salt intake per day were measured using WHO/PAHO protocol.<sup>14</sup> Following steps were used to measure the 24-hour salt intake from the reported Na (mmol/L) concentration of 24-hour urine samples.

1. Measured 24-hour urinary sodium excretion (mmol/day) = Concentration of 24-hour urinary sodium excretion (mmol/L) × 24-hour urine volume (L/day).
2. Measured 24-hour urinary sodium excretion (mg/day) = 23 × Measured 24 hours urinary sodium excretion (mmol/day).
3. Measured 24-hour urinary salt excretion (g/day) = 0.0025 × Measured 24-hour urinary sodium excretion (mg/day).<sup>14</sup>

Estimated sodium excretion (mmol/day) from spot urine samples was calculated by following three methods using, predicted 24-hour urinary creatinine (PrUCr24h), spot urinary sodium (Nspot), spot urinary potassium (Kspot) and spot urinary creatinine (Crspot).

### Tanaka method<sup>15</sup>

- ▶  $21.98 \times \{N_{\text{spot}} \text{ (mmol/L)} / (C_{\text{rspot}} \text{ (mg/dL)} \times 10) \times \text{PrUCr24 hour (mg/day)}\}^{0.392}$ .
- ▶  $\text{PrUCr24h} = 14.89 \times \text{wt (kg)} + 16.14 \times \text{height (cm)} - 2.04 \times \text{age (year)} - 2244.45$ .

### Kawasaki method<sup>16</sup>

- ▶  $16.3 \times \{N_{\text{spot}} \text{ (mmol/L)} / (C_{\text{rspot}} \text{ (mg/dL)} \times 10) \times \text{PrUCr24 hour (mg/day)}\}^{0.5}$ .
- ▶  $\text{PrUCr24h} = 15.12 \times \text{wt (kg)} + 7.39 \times \text{height (cm)} - 12.63 \times \text{age (year)} - 79.90$  (male).
- ▶  $\text{PrUCr24h} = 8.58 \times \text{wt (kg)} + 5.09 \times \text{height (cm)} - 4.72 \times \text{age (year)} - 74.50$  (female).

### INTER-SALT method<sup>17</sup>

- ▶  $25.46 + 0.46 \times N_{\text{spot}} \text{ (mmol/L)} - 2.75 \times C_{\text{rspot}} \text{ (mmol/L)} - 0.13 \times K_{\text{spot}} \text{ (mmol/L)} + 4.10 \times \text{body mass index (BMI) (kg/m}^2\text{)} + 0.26 \times \text{age (year)}$  (male).
- ▶  $5.07 + 0.34 \times N_{\text{spot}} \text{ (mmol/L)} - 2.16 \times C_{\text{rspot}} \text{ (mmol/L)} - 0.09 \times K_{\text{spot}} \text{ (mmol/L)} + 2.39 \times \text{BMI (kg/m}^2\text{)} + 2.35 \times \text{Age (year)} - 0.03 \times \text{age}^2 \text{ (year)}$  (female).

## Statistical analysis

Out of 418 respondents, 181 respondents were excluded due to creatinine excretions corrected for bodyweight outside the intervals of 14.4–33.6 mg/kg for men and 10.8–25.2 mg/kg for women,<sup>20</sup> and 6 respondents were excluded for either very low or high urine volume (<0.5 liter/day and/or >4.5 liter/day). Only four respondents were excluded due to incomplete or incorrect urine collection.

Mean and 95% CI were calculated for all variables. The differences of age, BMI, total urine volume, urinary parameters (urinary sodium, potassium, creatinine and sodium to creatinine excretion ratio), and salt measured

**Table 1** The mean of age, BMI, urinary sodium, urinary potassium, urinary creatinine and urinary Na/K ratio among respondents by residence

Variables	Total (n=227)		Urban (n=166)		Rural (n=61)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Age (years)	48.8	48.0 to 49.6	48.8	47.9 to 49.7	48.8	47.0 to 50.5
BMI (kg/m <sup>2</sup> )	24.8	24.2 to 25.4	26.1	25.5 to 26.7	21.3	20.3 to 22.4
Urine volume (L/day)	2.5	2.3 to 2.6	2.7	2.6 to 2.9	1.8	1.6 to 2.0
Sodium (g/day)	4.0	3.7 to 4.2	4.3	4.0 to 4.6	3.1	2.6 to 3.5
Potassium (g/day)	1.6	1.5 to 1.7	1.6	1.5 to 1.7	1.7	1.5 to 1.9
Creatinine (g/day)	1.2	1.1 to 1.2	1.3	1.2 to 1.3	0.9	0.8 to 1.0
Ratio of Na to K excretion	4.5	4.2 to 4.7	4.9	4.6 to 5.2	3.2	2.8 to 3.6

BMI, body mass index; K, potassium (mmol/l); Na, sodium (mmol/l).

from 24HUNa and three different spot urine methods (Tanaka, Kawasaki and INTERSALT) observed by the residence. Agreement between salt measured from 24-hour urinary sodium and salt estimated from each spot urine method was evaluated using Bland-Altman plots.<sup>21 22</sup> Mean of differences (bias) line with 95% limits of agreement were calculated and added to Bland-Altman plots to clarify the varying limits of agreement. Linear regression line of difference on average between two methods with 95% CI lines of predicted mean also added due to observe the presence of proportional bias. Intraclass correlation coefficient (ICC) was analysed to assess the validity of three methods between estimated and measured 24-hour urinary sodium. Statistical analyses were performed using statistical software SPSS V.26.0 and considered at a 95% confidence level.

## RESULTS

Out of 418 volunteers, 227 respondents were included in the final analysis, 73.1% were from urban, and 26.9% were from rural sites. The mean (95% CI) of age, BMI, urinary sodium, urinary potassium, urinary creatinine and sodium to potassium ratio according to urban and rural sites are presented in [table 1](#).

The mean age was similar in urban and rural participants. However, the mean BMI was higher in urban respondents. The mean daily urinary Na, K and ratio of Na to K excretion were 4.0 g/day (95% CI 3.7 to 4.2),

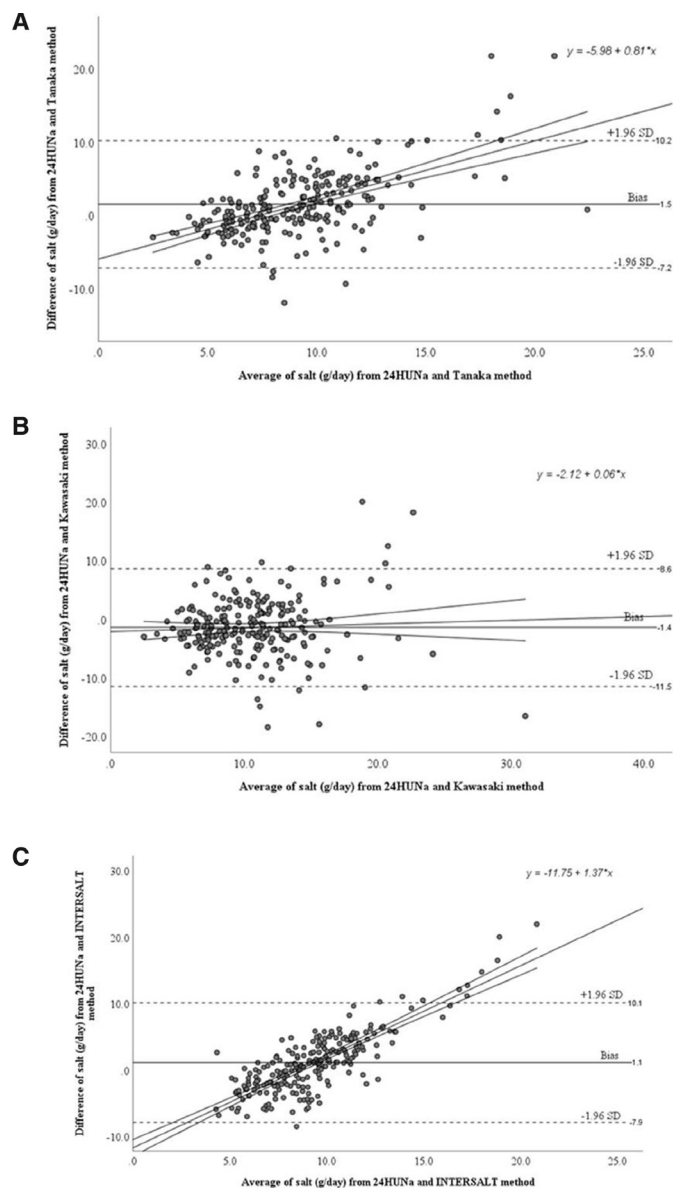
1.6 g/day (95% CI 1.5 to 1.7), and 4.5 (95% CI 4.2 to 4.7), respectively. The mean total urine volume was 2.5 L/day (95% CI 2.3 to 2.6), corresponding to a salt intake of 10.0 g/day (95% CI 9.3 to 10.6) measured from the 24-hour urinary sodium excretion method. From spot urine sodium concentration, the estimated mean salt intake was 8.5 g/day (95% CI 8.2 to 8.8), 11.4 g/day (95% CI 10.8 to 12.0) and 8.8 g/day (95% CI 8.6 to 9.0) by Tanaka, Kawasaki and INTERSALT methods, respectively. The mean salt intake by urban residents was more than that of rural residents (10.8 vs 7.6) g/day ([table 2](#)).

Bland-Altman plots have illustrated the agreement between salt intake measured from 24-hour urinary sodium and each of the three different spot urine methods. Compared with the estimated mean salt intake from 24-hour urine collection, the mean salt intake was overestimated by the Kawasaki method (11.4 g/day; 95% CI 10.8 to 12.0). Conversely, mean salt intake was underestimated by both the INTERSALT (8.8 g/day; 95% CI 8.6 to 9.0) and Tanaka methods (8.5 g/day; 95% CI 8.2 to 8.8). Furthermore, the regression coefficient (slope) of the linear regression line showed that the Kawasaki method (0.06) was the least biased, followed by the methods of Tanaka (0.81) and INTERSALT (1.37) ([figure 1A–C](#)).

The Kawasaki method had the highest (0.573, 95% CI 0.446 to 0.673) ICC, followed by the Tanaka method (0.510, 95% CI 0.364 to 0.623). The INTERSALT method

**Table 2** Salt consumption measured from 24-hour urine Na level and from spot urine Na level by three different methods

Variables	Total (n=227)		Urban (n=166)		Rural (n=61)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Salt (g/day) measured from 24-hour urine Na	10.0	9.3 to 10.6	10.8	10.1 to 11.5	7.6	6.5 to 8.8
Salt (g/day) estimated from spot urine by						
Tanaka method	8.5	8.2 to 8.8	8.6	8.2 to 9.0	8.1	7.4 to 8.8
Kawasaki method	11.4	10.8 to 12.0	11.6	10.9 to 12.3	10.9	9.8 to 12.1
INTERSALT method	8.8	8.6 to 9.0	9.1	8.9 to 9.4	8.0	7.6 to 8.4



**Figure 1** (A) Average of salt (g/day) from 24HUNa and Tanaka method. (B) Average of salt (g/day) from 24HUNa and Kawasaki method. (C) Average of salt (g/day) from 24HUNa and INTERSALT method.

had the lowest (0.295, 95% CI 0.084 to 0.457) ICC (table 3).

## DISCUSSION

This study revealed that the average salt intake estimated from the 24-hour urinary excretion of Na was 10.0 (95% CI 9.3 to 10.6) g/day, twice the maximum limit suggested by the WHO for adults. A few studies in Bangladesh have reported varying levels of population salt intake based on an assessment of urinary excretion of sodium either in spot urine (17 g/day, 2009), (9 g/day, 2018) or in 24-hour urine in the coastal rural population (6.8 g/day, 2011) or among pregnant women (9.4 g/day, 2009–10).<sup>18 19 23</sup> Our study showed the average salt intake estimated from spot urine by Tanaka, Kawasaki and INTERSALT methods

**Table 3** ICC of salt measured from 24-hour urine Na level and from spot urine Na level by three different methods

Method	ICC	95% CI
Tanaka	0.51	0.36 to 0.62
Kawasaki	0.57	0.45 to 0.67
INTERSALT	0.29	0.08 to 0.46

ICC, Intraclass Correlation Coefficient; Na, sodium.

were 8.5 g/day (95% CI 8.2 to 8.8), 11.4 g/day (95% CI 10.8 to 12.0), 8.8 g/day (95% CI 8.6 to 9.0), respectively.

The result of this study showed, among three methods, the INTERSALT method generated an average salt intake estimate (8.8 g/day; 95% CI 8.6 to 9.0), which was nearest to the measured salt intake by 24-hour urine (10.0 g/day; 95% CI 9.3 to 10.6) method. However, the Kawasaki method provided a relatively accurate estimate, as shown by Bland-Altman plots. The Bland-Altman plots indicated, of the three methods, both Tanaka and INTERSALT underestimated salt intake compared with salt intake measured by the 24-hour urine method, while the Kawasaki method overestimated depending on the bias.<sup>21 22 24</sup> This study also found significant biases for Tanaka and INTERSALT methods from estimating 24-hour urinary sodium, and the Kawasaki method was the least bias and highest agreement.<sup>24</sup> Other studies about validation on these three methods showed that the Kawasaki method was the least biased and had the most agreement with the concentration of measured 24-hour urinary sodium excretion conducted among the Chinese population and had an overestimation tendency in estimating 24-hour urinary sodium.<sup>25–29</sup>

The Kawasaki method showed no linear association between the mean and difference of measured and estimated values in our study. Nevertheless, Tanaka and INTERSALT were showed a positive association between mean and differences of measured and estimation values. By the regression analysis, according to the level of salt intake, underestimation or overestimation was confirmed in the plots that imply the intricacy of using the samples of a spot urine for estimation of salt intake. However, the mean bias line in the plots from the Kawasaki spot urine method was relatively close to zero, suggesting daily salt intake estimates from this spot urine method were comparable with the collection of 24-hour urine concentration.

This study found, the ICC between estimated and measured 24-hour urinary sodium was high with the Kawasaki method (0.57; 95% CI 0.45 to 0.67), while (0.51; 95% CI 0.64 to 0.62) with the Tanaka method and (0.29; 95% CI 0.08 to 0.45) with INTERSALT method. Here, the ICCs ranging from 0.29 to 0.57, which was higher than the ICCs of the PURE study, ranged moderate (0.21–0.29).<sup>27</sup>

The result showed that the estimated salt intake from the spot urine samples did not accurately match the estimates obtained from the 24 hours urine specimens. Further research is required to understand whether

this reflects random errors or biases. On the other hand, evidence showed that the INTERSALT method provided the least bias based on a first-morning urine sample among the young population compared with the Tanaka and Kawasaki methods.<sup>30</sup> It is noted that both the Tanaka and INTERSALT methods were developed using random urine samples in young populations. In contrast, the Kawasaki developed for fasting second-morning samples in a broader age group population.<sup>31–33</sup> The timing of the collection of spot urine samples is important, as it determines the formula that should work properly.<sup>34</sup> A study conducted in the USA among the adults found that the Kawasaki method was highly biased while the INTERSALT and Tanaka methods were the least biased when using the spot urine samples from several times a day and overnight samples.<sup>25 30</sup> In our study, we considered the first urine sample voided at the clinic as a spot urine sample to estimate 24 hours urinary sodium excretion. Furthermore, it has been reported that the INTERSALT and Tanaka methods tend to underestimate high salt intake and overestimate salt intake at a lower level of consumption.<sup>27 30 35 36</sup> The correlation between estimated and measured 24-hour urinary sodium excretion values was affected by the timing of spot urine sample collection.<sup>27 34</sup> Similar to our findings, Mente *et al* also found that the Kawasaki method had the best agreement and the least bias compared with the Tanaka and INTERSALT methods.<sup>28</sup> However, Cogswell *et al* reported that the INTERSALT method provided the least bias compared with Kawasaki and Tanaka methods.<sup>30</sup> Jędrusik *et al* found that the Kawasaki method was inadequate compared with the PAHO formula for estimating 24-hour urinary salt excretion.<sup>37</sup> Considering the issues a robust study where both 24-hour urine and spot urine samples at a point of time will be collected is needed to better understand the capacity of spot urine samples to estimate the dietary salt intake per day in Bangladesh's urban and rural sites.

The spot urinary Na/K ratio may be a useful and alternative method to 24-hour urine collection for estimation of the urinary Na/K ratio in the Bangladeshi people. In this study, the mean urinary Na/K ratio was 4.5 (95% CI 4.2 to 4.7); which is significantly higher than the WHO-recommended value of 1.5, which is considered beneficial for health.<sup>38 39</sup> A major strength of our study is that we used the 24-hour urine collection method to measure the level of urinary salt excretion as well as estimated salt intake by three different equations based on spot urine Na concentration in the same urban and rural respondents. This is the first study reporting estimation of daily salt intake by spot urine methods with a comparison of daily salt intake measured from 24-hour urine excretion in Bangladesh. Also, there are few limitations in our study. The study was conducted in 2012–2013 with an aim of estimating the mean salt intake at population level. A larger proportion of respondents, especially from the rural

sample, were excluded due to being outside of the normal range of urinary creatinine excretion. A sample size of 227 was not sufficient for the estimation of the sodium excretion at the population level, however, in the present analysis the ICC among the three methods ranged from 0.3 to 0.57. A sample of 227 provides about 48%–99% power at 5% precision level to detect this ICC. Furthermore, a single 24-hour urine collection is unable to assess the impact that day-to-day variability of salt consumption will have on urinary sodium excretion. Additionally, the Kawasaki method was formulated especially for spot urine samples of the second day. Moreover, urinary creatinine concentration should not be ignored as it is the most important reference index in both Kawasaki and Tanaka equations.

Though the Kawasaki method may estimate 24-hour urinary sodium excretion and mean salt intake, but the result of this study suggested that the Kawasaki, INTERSALT and Tanaka methods based on spot urine samples were not appropriate for assessing dietary salt intake levels in Bangladesh. Further research is required to modify accurate estimation methods for 24-hour urinary sodium excretion using spot urine samples with a large-scale survey for Bangladesh.

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**Contributors** SRC, MAA-M and MMZ contributed to the conception and study design. MAR, MAA-M and JUA implemented the research, coordinated field survey and biochemical analysis. SRC, MNNS, MAA-M, ZZ and JA did statistical analysis and interpretation of the data. SRC, MAA-M, JA, ZZ drafted, reviewed and edited the manuscript. SRC, MMZ, MAA-M, JA and MNNS provided critical input on the draft. All authors approved the final draft. SRC is acting as guarantor.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Consent obtained directly from patient(s)

**Ethics approval** This study involves human participants and was approved by the Ethics Review Committee of National Heart Foundation Hospital and Research Institute, Bangladesh (reference number: N.H.F.H&R. IERC-4-14/7Ad:12511). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available on reasonable request. The data reported in this paper are available at <https://data.mendeley.com/datasets/b4387jcczn/1>.

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