ORIGINAL ARTICLE

Biomonitoring Study of Urinary Bisphenol A Levels and Impact of Bottle-Feeding Practices Among Infants and Children from Northern India

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ABSTRACT

Objectives: To compare the urinary bisphenol A (BPA) levels in bottle-fed and never bottle-fed infants and under-five children and to determine the impact of bottle-feeding practices and sociodemographic factors on urinary BPA levels.

Methods: A community-based cross-sectional study was carried out on children aged between 2 to 60 months attending the Anganwadi centres in Chandigarh.

Results: Urine samples were collected from 184 children, out of which 94.56% (*n* = 174) children had detectable urinary BPA levels. The mean (SD) BPA level was 2.74 (2.60) ng/ml and BPA was detected in 93.9% of 'ever' bottle-fed children (*n* = 93/99) and 95.3% of 'never' bottle-fed children (*n* = 81/85) (*P* = 0.69). On multivariate regression analysis, there were no significant predictors for high (≥ 75th percentile) urinary BPA levels. Still, the odds of urinary BPA levels ≥75th percentile showed higher trend for significance among children from middle/higher socioeconomic background in reference to lower socioeconomic stratum (adjusted OR 7.02; 95% CI 1.24, 133.25; *P* = 0.07) and among children whose feeding bottles were brushed once or twice daily in reference to group with no daily brushing (adjusted OR 3.92, 95% CI 0.95, 20.56; *P* = 0.07).

Conclusions: Although feeding with plastic bottle did not emerge as a statistically significant risk factor for BPA exposure, yet detection of BPA levels among majority of study children signals urgent need for unmasking exposure to other sources given the potential long-term toxicity of BPA among infants and young children.

***Keywords*:** Children, Feeding, Plastic, Urine

INTRODUCTION

Breastmilk substitutes and bottle feeding have emerged as popular alternatives to breastfeeding in both developed as well as low middle-income countries with 59% of infants being fed using bottles by 5 months of age [1]. The global baby bottle market is expected to grow substantially at 4.7% annually from 2019 to 2025 with plastic bottle segment having the largest market share (44.1%) in year 2018 [2]. Every fifth child aged under 3 years in India is bottlefed according to the latest National Family Health Survey-5 [3]. Indeed, 'Feeding Bottles and Infant Foods (Regulation of Production, Supply and Distribution) Act 1992', is not adequately regulated in India (IMS Act,1992) and despite the recent proposal for prohibition of BPA in baby feeding bottles by Bureau of Indian Standard (BIS) regulations in 2015, compliance with ban is still nebulous [4].

This raises concerns regarding the childhood exposure to Bisphenol A (BPA), a chemical ingredient of polycarbonate plastic used for production of infant feeding bottles. BPA which is obtained from condensation between phenol and acetone is an endocrine disrupting chemical (EDC) and vulnerability of children to such environmental toxicants is well known. A study carried out by 'Toxics Links' organization to investigate BPA content in feeding bottles as well as sippy cups in India revealed higher BPA content despite

its prohibition in infant feeding bottles. Out of randomly collected 13 samples of sippy cups of various brands from different markets in Delhi, 10 (77%) were found to have high BPA [5]. A possibility of causal association of BPA levels with various childhood conditions like atopy, asthma, obesity and autism had also been reported [6,7]. Undeniably, the concerns of toxic exposure to BPA with use of plastic bottles for feeding have led many countries to ban sales of BPA containing plastic feeding bottles [7]. However, there are no bio-monitoring studies from India evaluating the urinary BPA levels in infants and importance of disaggregated regional data has already been highlighted for formulating evidence-based preventive strategies. We conducted this study with a primary objective of comparing the urinary levels of Bisphenol A (BPA) levels in bottle-fed and non-bottle-fed under-five children. We also assessed the impact of bottlefeeding practices and sociodemographic variables on urinary BPA levels in young children.

METHODS

This community-based cross-sectional study was conducted among children aged 2-60 months enrolled in the Anganwadi centres of Chandigarh city, India, from January 2019 to December 2019. An informed written consent was obtained from parents or legally acceptable representatives. Children suffering from chronic systemic illnesses like congenital or acquired heart disease, chronic renal or liver disease, malabsorption state, or immune deficiency diseases were excluded.

Out of the three 'Integrated Child Development Scheme' (ICDS) projects in Chandigarh covering 450 Anganwadi centers (with a total of 55,000 children), we randomly selected a cluster of 10 Anganwadi centers from each ICDS project (total 30 Anganwadi centers for the study). At least 6 children aged 2 months to 5 years were enrolled from each Anganwadi center. Children were randomly selected from pre-existing lists categorized by age at each Anganwadi center. Within each age group, children were assigned unique identification numbers. A random number generator was used to pick two numbers for each age category (2-12 months; 1-3 years, 3-5 years). In case of non-availability of the particular child, non-biased replacement of the candidate was done from the randomization list.

Demographic details, history and clinical examination were recorded for all children included in the study. Urine samples preferably, first morning sample, were collected from each participant in a sterile plastic container (BPA free) provided to the primary caregiver the previous day. Urine samples were transported from the Anganwadi center in cold chain in ice boxes.

Quantitative estimation of urinary BPA levels was performed using a Qtrap 4500 Tandem Mass Spectrometer (ABSciex) equipped with Electron Spray Ionization (ESI) coupled with ShimazduNexra X2 chromatographic instrument. Data processing and quantitation was done using Sciex Analyst® 1.6.2 software [8].

Sample size calculation was done using PASS software based on the effect size from a previous study [9] where BPA levels in never bottle-fed infants did not exceed 0.45 ng/ml (~0.5mg/ml) and that in bottle-fed infants was twofold higher (~1ng/ml). A sample size of 86 children per group was needed at 90% power to detect a mean BPA level difference of 0.5 ng/ml assuming the standard deviation of 1.0 in both the groups with error of 0.05 using a two-sided two sample equal variance t-test. Anticipating refusal or urine sample collection failure in 25% of children, a sample of 215 children was needed. We decided to enroll

220 children to attain the desired sample size.

Statistical analysis: Data was entered in Excel spreadsheet and analyzed using R software (R studio IDE) and STATA 14. Means, standard deviations, and frequencies were computed for variables as appropriate. 95% confidence intervals were also computed for various point estimates. Univariate analysis included t test/Mann Whitney U test and one-way ANOVA/Kruskal Wallis test and Chi-square test for association of categorical outcomes. Predictors of high urinary BPA levels (≥ 75th percentile) were analyzed using univariate and multivariate regression analyses. All statistical tests were two-tailed and *P* value less than 0.05 was taken as statistically significant.

RESULTS

We enrolled 220 children, out of which we could collect urinary samples for BPA estimation in 184 children (93 boys, 91 girls) see **Fig. 1.** The mean (SD) age of enrollment in our study cohort was 25.66 (16.88) months. **Table I** depicts the sociodemographic characteristics of the enrolled children and their parents.

Out of 184 children, 174 (94.56%) had detectable urinary BPA. The mean (SD) BPA level (ng/ml) was 2.74 (2.60) which ranged from 0.01 to 19.0. The geometric mean (95% CI) level of BPA (ng/ml) in our study cohort was 1.74 (1.47, 2.05). Nearly one fourth (24.71%) of the study children had urinary BPA levels less than 1 ng/ml whereas 13.79% children had urinary BPA levels more than 5 ng/ml. The median (25^{th}, 75^{th} percentile) urinary BPA level among girls and boys were 2.19 (0.81, 3.52) ng/ml and 2.05 (1.04, 4.04) ng/ml, respectively. BPA was present in detectable amounts in 93.9% of 'ever' bottle-fed children (*n* = 93/99) and 95.3% of never bottle-fed children (*n* = 81/85) (*P* = 0.69). The median (25^{th}, 75^{th} percentile) urinary BPA levels among the ever-bottle-fed children [2.19 (1.01, 3.42)] were statistically not different from never bottle-fed children [1.9 (1.01, 4.09)] (*P* = 0.99). The median (25^{th}, 75^{th} percentile) urinary BPA levels in children 'currently' taking bottle feeds [2.1 (0.97, 3.40) ng/ml] were also comparable to that in those 'not currently' taking bottle feeds [2.15 (1.01, 4.08) ng/ml] (*P* = 0.58).

The unadjusted effect sizes (odds ratios) for the outcome of high urinary BPA levels (≥ 75th percentile) for various bottle-feeding related characteristics as shown in **Table II**. There was statistically no significant difference in proportion of children with high urinary BPA levels (≥ 75th percentile) between 'ever bottle-fed' and 'never-bottle-fed' children (OR=0.76; 95% CI 0.37, 1.52) (*P* = 0.44). However, BPA levels (≥ 75 percentile) showed higher odds among children whose bottles were brushed more than 3 times daily as compared to children whose feeding bottles were never brushed daily (OR 3.24; 95% CI 0.87, 16.4) (*P* = 0.08). **Table III** depicts the association of high urinary BPA levels (≥ 75th percentile) with various sociodemographic, family and dietary factors. The odds of high urinary BPA levels (≥ 75th percentile) were decreased by 59% among families of lower socioeconomic status in comparison to middle or higher socioeconomic status [OR 0.41; 95% CI 0.11, 1.13] (*P* = 0.09). An interesting observation was related to family diet where odds of high urinary BPA levels (≥ 75th percentile) were increased by more than 2 times among children who were also eating family diet [OR = 2.02; 95% CI 0.90, 5.05] (*P* = 0.09).

On multivariate logistic regression the odds of urinary BPA levels ≥75th percentile were increased by 7 times among children belonging to middle/higher socioeconomic background in reference to those from lower socioeconomic strata (adjusted OR 7.02; 95% CI: 1.24, 133.25; *P* = 0.07). Brushing feeding bottles

once or twice daily also showed an independent trend for significance for higher urinary BPA levels in comparison to those children where feeding bottles were never brushed daily (adjusted OR 3.92, 95% CI: 0.95, 20.56; *P* = 0.07).

DISCUSSION

The present study is the first study to estimate the urinary BPA levels and assess their association with various feeding practices and socioeconomic characteristics among of infants and childrenfrom India. Among total children who underwent urinary Bisphenol A level estimation (*n* = 184), 94.56% (*n* = 174) had bisphenol levels above the limit of quantification.

In a study of 110 Portuguese children aged 4-18 years [10], 91% of morning urinary samples were positive for Bisphenol above the limit of quantification and their median urinary BPA levels was 1.89 µg/l (95th percentile 16.0 µg/l). In addition, detection of BPA in 94.56% of our study sample closely mirrors the Centers for Disease Control and Prevention (CDC) estimate of 93% reported in American children ≥ 6 years of age based 2003–2004 data from the National Health and Nutrition Examination Survey (NHANES) [11]. Their median BPA for all ages was 2.7 ng/mL which is quite close to median value of 2.13 ng/ml in our study. In a recent study, urinary BPA levels among preschool children from Ankara (*n* = 125) ranged from lower limit of detection to 18.36 µg/g creatinine without any significant difference between boys (1.26 µg/g creatinine) and girls (2.24 µg/g creatinine) (*P* value > 0.05) [12]. Even our study in children aged below 5 years did not show any statistically significant difference between girls vs boys (mean difference 0.07, 95% CI of mean difference 0.70, 0.85 ng/ml) (*P* value = 0.85). Surprisingly, bottle feeding did not emerge as a standalone risk factor for high urinary BPA levels in our study. One possible reason could be reported accumulation of BPA in body fat which may had concealed the association of plastic bottle feeding with urinary BPA levels in our study [13]. Also, substantial non-bottle exposures from other sources like food, air, water and soil may have masked the association in our present study. In this context, our unadjusted analysis revealed association of co-intake of family diet with high urinary BPA levels. It had been already shown that ready-to-eat and fast foods products wrapped in plastic bags and cans result in high exposure to BPA and other endocrine disrupting chemicals [14,15]. Interestingly higher, though not significant, odds of urinary BPA levels (≥ 75 percentile) among children of working mothers in our study may possibly points to the well reported over-reliance of working mothers on processed foods due to time constraints and easy affordability. Furthermore, children from lower socioeconomic stratum were less likely to fall in highest quartile urinary BPA (a trend for significance) in our study even after adjustment for other covariates in multivariate regression analysis. This points towards perhaps lesser opportunities for plastic mediated BPA exposure among lower socioeconomic stratum as suggested in a recent study among Egyptian children aged 2 to 18 years [16]. In their study, high BPA levels were reported from children of higher socioeconomic status possibly due to more frequent use of plastic bottles as well as plastic wares. As far as association between bottle brushing and urinary BPA levels was concerned, odds of high urinary BPA levels (≥ 75 percentile) showed a trend for higher odds among children whose bottles were brushed daily in comparisons to the children whose bottles were never brushed daily. This predictor of daily bottle brushing continued to retain its independent predictive trend in multivariate regression modelling. This finding mirrors the observations of a

migration study conducted in twelve PC baby bottleswhich showed that dishwashing, boiling and brushing, significantly bleach out BPA [17].

There was no significant association between urinary BPA levels and various other demographic characteristics like age or sex, akin to another multinational study [18]. Although many of the migration studies have shown an increase in BPA migration levels from feeding bottles with repeat use [19], our study failed to show statistically significant association between urine BPA levels and duration of bottle feeding.

However, there were a few limitations for our study. Urinary metabolite concentrations of BPA were measured from a single morning void urine sample in our study due to ease of collection as well as practicality. This may ignore the diurnal and day-to-day variability of exposure in chemicals like BPA which have very short half-life and do not bio-accumulate. However, previous studies indicated that a single sample provided substantial temporal reliability over 6 months and can be used as reliable biomarker in epidemiologic studies of association between exposure and health outcomes in children [20,21]. BPA was not creatinine-corrected to account for urinary dilution which could have increased observed variance in BPA measurements in our study. Our study sample was selected from Anganwadi centers where children are mostly from low to upper middle-class background. Hence, our study findings may not be generalized to uppermost strata of population. Considering that the majority of our subjects exhibited detectable levels of BPA and there was no statistically significant difference between infants who were ever bottle-fed and those who were not, a further study is urgently needed to investigate other potential sources of BPA exposure such as tethers, bottled water, pacifiers, environmental exposure by inhalation or solid foods given in plastic plates. Also, the effect sizes for multivariable logistic regression may be preliminary due to small sample size and further require validation in larger study. Importantly, Bisphenol F (BPF) and Bisphenol S (BPS) are increasingly used as replacements for Bisphenol A (BPA) in polycarbonates and epoxy resins. Therefore, biomonitoring studies to assess human exposure to these chemicals are urgently warranted.

Although, feeding with plastic bottle as such did not emerge as a statistically significant risk factor for BPA exposure, detection of BPA in majority of children signals urgent need for unmasking exposure to other sources given its potential long-term toxicity.

Ethics clearance: EIC, Postgraduate Institute of Medical Education and Research Chandigarh, No. INT/IEC/2018/001949 dated Nov 29, 2018.

Contributors: PG: Collection of data, drafting the manuscript; BB: Study design, data analysis and critical inputs; SVA: Study design, laboratory analysis and manuscript revision; VSM, AP: Data collection, laboratory analysis, manuscript writing.

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WHAT THIS STUDY ADDS?

The presence of Bisphenol A (BPA) in the urine of both bottle-fed and never bottle-fed infants and young children suggests multiple sources of exposure beyond just bottles. The

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Table I Sociodemographic Characteristics of the Study Cohort (*n* = 184)

Variable	***n (%)***
Gender	
Boys	93 (50.5)
Girls	91 (49.5)
Age distribution	
1-12 months	59 (32)
13-36 months	73 (39.7)
37-60 months	52 (28.3)
Socioeconomic Status (SES) (Modified Kuppuswamy scale)	
Upper middle & high SES status	83 (45.1)
Lower middle SES status	68 (37)
Lower SES status	33 (17.9)
Income groups (in Indian Rupees)	
<5000/month	40 (21.74)
5000-7000/month	47 (25.54)
>7000/month	97 (52.72)
Father's education	
Illiterate	19 (9.7)
Primary school	16 (8.2)
Middle school	35 (17.9)
Secondary	107 (54.6)
Graduate or more	17 (8.70)
Maternal education	
Illiterate	31 (16.85)
Secondary	48 (26.09)
Higher secondary	65 (35.33)
Graduate or more	40 (21.74)
Father's occupation	
Unemployed	02 (1.09)
Elementary (unskilled/semiskilled) workers	76 (41.30)
Skilled workers/ clerical /shopkeepers	101 (54.89)
Professional	05 (2.72)
Maternal occupation	
Homemaker	172 (93.5)
Employed	12 (6.5)

Table II Distribution of Bottle-Feeding Practices Between Low (<75th percentile) and High (≥ 75th percentile) Urinary Bisphenol A Groups (Total bottle-fed children = 99)

Variable	*≤75th percentile n (%)*	*>75th percentile n (%)*	*Odds Ratio (95% CI)*	*P value*
Bottle-feeding duration (months)				
< 6 months (*n* = 24)	20 (25.97)	4 (18.18)	Ref. group	
6 to 12 months (*n* = 22)	17 (22.08)	5 (22.73)	1.45 (0.32, 7.01)	0.62
≥12 months (*n* = 53)	40 (51.95)	13 (59.09)	1.58 (0.48, 6.40)	0.47
No. of feeding bottles used/day				
Single (*n* = 58)	43 (55.84)	15(68.18)	Ref. group	
Two (*n* = 34)	30 (39.96)	4 (18.18)	0.40 (0.10, 1.23)	0.11
>Two (*n* = 7)	4 (5.19)	3 (13.64)	2.14(0.36, 11.40)	0.38
Bottle brushing frequency				
Never daily (*n* = 28)	25 (32.47)	3 (13.64)	Ref. group	
1-2/day (*n* = 33)	25 (32.47)	8 (36.36)	2.56 (0.63, 14.5)	0.19
≥3/day (*n* = 38)	27 (35.06)	11 (50)	3.24 (0.87, 16.4)	0.08
Bottle cleaning method				
Boiling (*n* = 52)	41(53.25)	11 (50)	Ref. group	
Detergent (*n* = 33)	25 (32.47)	8 (36.36)	1.19 (0.41, 3.40)	0.74
Simple soap (*n* = 14)	11 (14.29)	3 (13.64)	1.04 (0.20, 4.18)	0.96
Use of left-over milk in bottles				
Discarded after feed (*n* = 65)	48 (67.61)	17 (77.27)	Ref. group	
Reused for feeding (*n* = 28)	23 (32.39)	5 (22.73)	0.59(0.17-1.71)	0.34
Source of milk				
Raw cow/buffalo milk (*n* = 46)	38 (49.35)	8 (36.36)	Ref. group	
Pasteurized milk in packets (*n* = 53)	39 (50.65)	14 (63.64)	1.68(0.64-4.72)	0.30
Exclusively bottle-fed				
No (*n* = 75)	60 (77.92)	15 (68.18)	Ref. group	
Yes (*n* = 14)	17 (22.08%)	7 (31.82)	1.65(0.55-4.67)	0.36
Currently on bottle feeds				
No (*n* = 21)*	15(19.48%)	6 (27.27%)	Ref. group	
Yes (*n* = 78)	62(80.52%)	16 (72.73%)	0.65 (0.22-1.93)	0.43

Table III Sociodemographic and Other Dietary Characteristics Between Low (<75th Percentile and High (≥75th percentile) Urinary Bisphenol A groups (*n* = 184)

Variable	*≤75th percentile n (%)*	*>75th percentile n (%)*	*Odds Ratio (95% CI)*	*P-value*
Gender				
Boys (*n* = 91)	67 (47.52)	24 (55.81)	Ref. group	
Girls (*n* = 93)	74 (52.48)	19 (44.19)	0.72(0.36-1.43)	0.34
Age groups				
1-12 months (*n* = 59)	47 (33.33)	12 (27.91)	Ref. group	
13-36 months (*n* = 73)	53 (37.59)	20 (46.51)	1.47 (0.65-3.42)	0.36
37-60 months (*n* = 52)	41 (29.08)	11 (25.58)	1.05 (0.41-2.68)	0.92
Maternal occupation				
Employed (*n* = 12)	7(4.96)	5(11.63)	Ref. group	
Homemaker (*n* = 172)	134(95.04)	38(88.37)	0.40 (0.12-1.45)	0.15
Socioeconomic status				
Middle/Upper (*n* = 151)	112(79.43)	39(90.70)	Ref. group	
Lower (*n* = 33)	29(20.57)	4(9.30)	0.41 (0.11-1.13)	0.09
Currently taking family diet also				
No (*n* = 53)	45 (31.91)	8 (18.60)	Ref. group	
Yes (*n* = 131)	96 (68.09)	35 (81.40)	2.02 (0.90-5.05)	0.09
Junk food intake				
No (*n* = 98)	78 (55.32)	20 (46.51)	Ref. group	
Yes (*n* = 86)	63 (44.68)	23 (53.49)	1.42 (0.71-2.85)	0.32

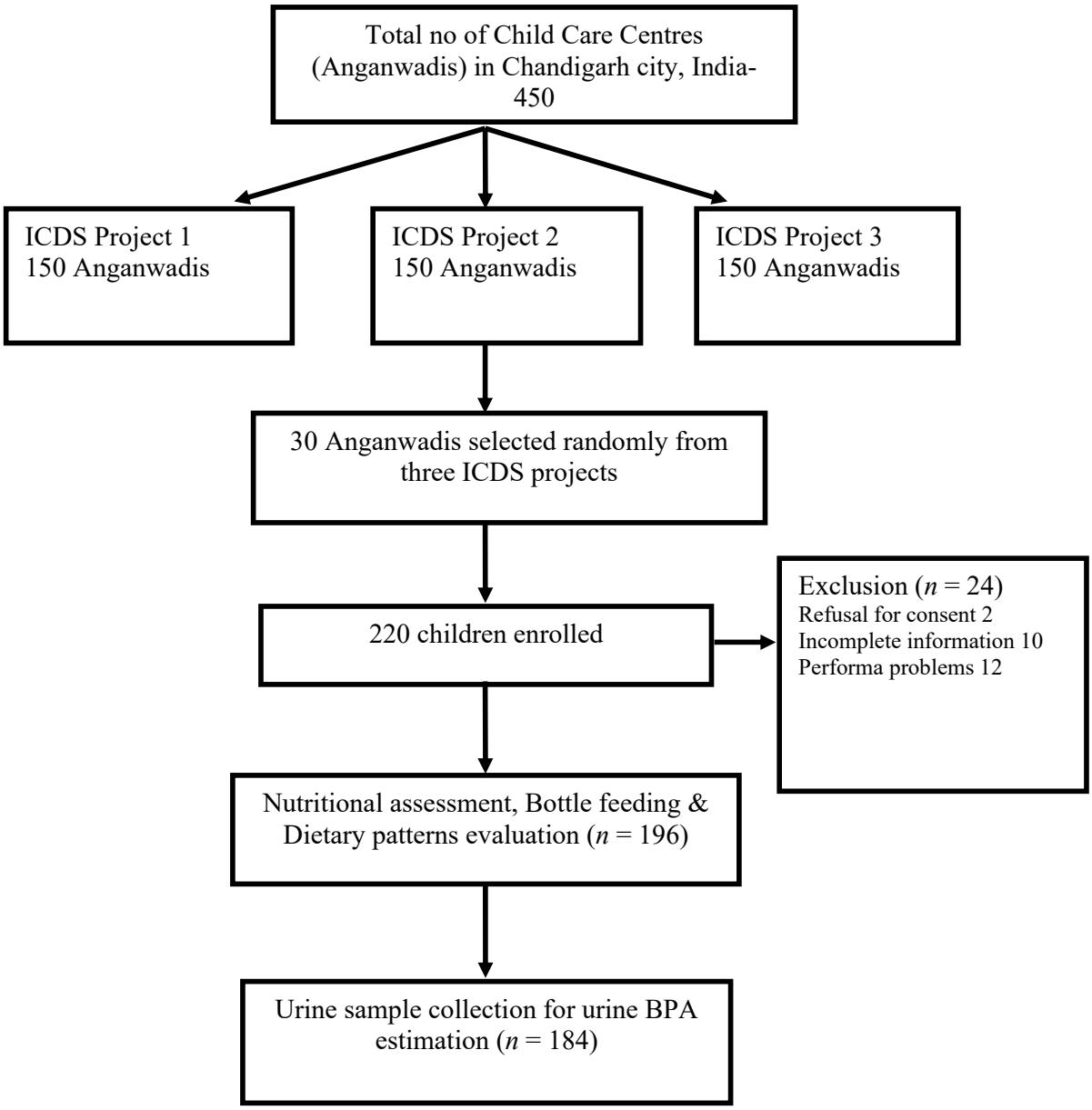

Fig. 1: Flow chart depicting study participants