Draft (25/09/11)

Childhood Rickets in Bangladesh: Newly emerged public health challenge from the first national survey

¹ SKRoy, ¹N.Alam, ¹R.Rakib, ²S.Haque, ²H.K.Das, ²M.Ali, ²T.H.Talukder, ²S.M.M.Rahaman, ² Faruque Ahmed, ²Selina Amin, ¹M.Iqbal, ¹A.Bhuiya, ¹Debjani Sarker

¹ICDDR,B, GPO Box 128, Dhaka 1212, Bangladesh

²Rickets Interest Group (RIG), Bangladesh (UNICEF, NNP, CARE, BRAC, SARPV, Plan BD)

25 September 2011

Address of Correspond:

Dr. S.K,Roy Senior Scientist, ICDDR,B GPO Box 128, Dhaka 1212 Bangladesh email: <u>skroy@icddrb.org</u> Cell: 01821998865

Abstract

Background: Rickets is among the most-frequent childhood diseases in many developing countries which is a condition of softening of bones in children, potentially leading to fractures and deformity. The National Rickets Prevalence Survey was conducted to explore the prevalence of rickets, determination of biochemical markers in patients with clinical rickets and identify dietary factors associated with rickets.

Objective: To conduct the first National survey of prevalence of rickets among children aged 1-15 years with their nutritional status and biochemical profiles.

Methodology: Twenty thousand children aged 1-15 years were randomly selected from rural and urban locations of 50% districts of 6 divisions in Bangladesh. Clinical diagnostic signs, radiological signs of active rickets and anthropometric measurements (weight, height, and mid-upper arm-circumference) were undertaken. Blood samples from rickets subjects were tested for biochemical markers.

Results: Rickets was found in each of 6 division of Bangladesh. A total of 197 rachitic cases were diagnosed with a prevalence rate of 0.99%. Radiologically, 24% were at active phase, 34% were in growing phase while 42% were not suggestive. The prevalence of severe stunting, underweight, and wasting were 53%, 40% and 1.4% respectively. About 98% of the children were vitamin D-deficient, and 48% were calcium-deficient. Dietary deficiencies had significantly associated with rachitic conditions.

Conclusion: The results of the first-ever national rickets survey indicated that about 550,000 children aged 1-15 years have been suffering from rickets at the rate of 0.99% in Bangladesh, which is a newly recognized public health problem, who need urgent attention for treatment and prevention. An appropriate policy and action for treatment and prevention formulation is strongly recommended in Bangladesh.

INTRODUCTION

The bone disorder most clearly related to nutrition is rickets. It was first reported from Europe in the mid-1600s¹. Rickets in the growing child or adolescent develop in a variety of clinical situations and have in common an absence or delay in the mineralization of growth cartilage and in newly formed bone collagen. Glisson and others have described typical findings of bony deformity with curving of the legs in rickets, which continued to be reported during successive centuries².

Re-emergence of nutritional rickets as an important and widely seen problem in the North America^{3,4} and secondly its higher prevalence in economically disadvantaged parts of the world where vitamin D insufficiency was uncommon⁵ were the two most striking things happened at the end of the last century. In United States and other western countries, rickets was most commonly seen in exclusively breastfed children with relatively darker pigmented skin^{3,6-12}.

In Australia and Europe, rickets was prevalent among migrated populations from the Middle East and the Indian subcontinent. In UK, majority of the patients were of black or Asian origin with darker skin colour for whom more exposure to ultraviolet light to synthesize vitamin D was required³³.Most affected patients develop the features within the first 6 to 12 months of life, with similar sex distribution. In the Middle East, rickets is often seen in sun-protected children of vitamin D-deficient mothers, but it also manifests as bone problems later in childhood. In sun-exposed regions of Asia and Africa, rickets typically manifests during the second or third year of life⁵. In Mongolia and China, the north Asian countries, lack of adequate UV-B rays during the coolest months of winter, a limited exposure of skin to sunlight in summer and traditional wrapping of infants for a maximum of one year was found to be a major contributing factor to the higher prevalence of rickets^{13,14}.

Causes of rickets:

By the 1800s, sunlight (UV radiation) and cod liver oil were found effective in treating rickets, and vitamin D was identified as the essential ingredient of this oil in the early 1900s². With the introduction of vitamin D supplementation, rickets became rare in the industrialized countries during the 20th century¹⁵.

Vitamin D, a fat soluble essential micronutrient can come either from diet or it can be synthesized under the skin when exposed to sunlight. This makes vitamin D deficiency ricket unusual in tropical or sub-tropical countries and shifts the focus on other causes of rickets like calcium deficiency ^{16,17}.

Since the 1600s, nutritional rickets has been traditionally attributed to vitamin D deficiency related to decreased exposure to sunlight resulting from crowded living conditions under skies polluted by the products of industrialization⁷. In the 1600s, wet nursing (use of mother substitutes to nurse infants) by women with calcium-poor breast milk had also been suggested as the primary cause of rickets⁸. Rickets associated with calcium deficiency has been reported from South Africa⁹ and Nigeria¹⁰.

By the late 1990s, the accumulated evidence suggested that low dietary calcium intake was indeed important in the pathogenesis of rickets^{22,23,24}. In fact, calcium insufficiency had also been suspected to contribute to some of the apparent vitamin D deficiency rickets seen in North America²⁵.

History and epidemiology of nutritional rickets in Bangladesh

Rickets has been recognized as a concealed health problem in Bangladesh. In 1991, an NGO called SARPV (Social Assistance and Rehabilitation of the Physically Vulnerable) brought it to greater attention during their visit of Chakaria region of South-Eastern Bangladesh after a devastating cyclone in the same year. Approximately 1% of the total children showed rachitic deformities in an informal village survey. Focus Group Discussions (FGD) suggested that rickets was "new" in that community and had not been seen before the early 1970s.

In 2000 and 2004, the Helen Keller International (HKI) conducted nationwide surveys in 28 upazilas in Bangladesh. Nationally, rachitic deformities were observed in 0.26% of the 21,571 children surveyed in 2000 and in 0.12% of the 10,005 children surveyed in 2004²⁶. The highest prevalence (1.4% in 1-15 year-old with visible rachitic deformities) was found in the Cox's Bazaar sub-district²⁶.

In 2003, BRAC conducted a survey among children and young people aged 1 to 20 years in Chittagong and noted rachitic deformities in 0.9% of the people surveyed²⁷. In 2004, the Institute of Child and Mother Health (ICMH) conducted a more detailed survey in the Chittagong Division and assessed 8.7% of the children to have at least one clinical finding consistent with rickets: 4% had lower limb deformities suggestive of rickets; 0.9% had radiological evidence of active rickets; and 2.2% had elevated levels of serum alkaline phosphatase²⁸. Interestingly, rickets was not identified among the indigenous populations of the hilly regions (Hill Tracts) of Chittagong district.

High prevalence of childhood rickets was reported from selected areas in Bangladesh in 2004. Therefore, a nation-wide survey along with detailed information was essential for determining the true prevalence of childhood rickets in Bangladesh.

METHODOLOGY:

Design: National Rickets Prevalence Survey in Bangladesh was a nation wide survey conducted to identify, diagnose and find out the prevalence of childhood rickets.

Population and area: Children aged 1-15 years were randomly selected from all socio-economic groups living in the rural and urban areas of the six divisions of Bangladesh. Half (32) of the 64 districts in Bangladesh were randomly selected for the survey. Stratified random sampling selected three upazila from each district, two unions from each upazila, and two villages from each union were randomly selected. In each village, 42 children between 1 and 15 years were randomly selected. For urban areas, 50 wards were selected in six divisional cities. From every ward, 4 slums were randomly selected and from every slum, 20 children were sampled.

Outcome variables: Identification of features of rickets and rachitic children, nutritional prevalence of rachitic children, biochemical markers of relevance in rachitic children and information on child feeding practice, disease control and caring practices are the outcome variables.

Sample size Estimation for Rural areas:

The minimum sample size was calculated to estimate the prevalence of rickets with precision at 5% statistical significance. Children, aged 1-15 years, were selected using stratified multistage cluster sampling to cover the whole country and regions and to facilitate supervision of field data collection. Sampling was proportionate to size (in terms of population in the 2002 census) of the division. The sample size was adjusted for stratified cluster sampling. The final sample size, accounting for the design effect for deviation from simple random sampling to stratified multistage random cluster sampling, was 7600 x 2.0 = 15200. The final sample size was rounded to 16000 children aged 1-15 years in rural areas.

Sample size Estimation for Urban areas:

In absence of prior estimate of the rickets prevalence in children living in poor areas of the cities in Bangladesh, the prevalence was assumed to be similar to the prevalence in rural children. For financial constraint, the prevalence was estimated with a precision of 0.05% (which was lower than 0.025% set for rural children) at 5% statistical significance.

The final sample size, accounting for the design effect for deviation from simple random sampling to stratified multistage random cluster sampling, was $1822 \ge 2.0 = 3644$. The estimated sample size for urban areas was $3644 \sim 4000$ children aged 1-15 years. Distribution of sample children across the cities was proportional to the population size of the cities.

List of slums/ villages in selected unions/ wards was used as sampling frame. From the list 384 clusters/villages in 6 administrative divisions and they were randomly selected. Number of children aged 1-15 years was 42 per cluster/village and they were selected systematically for interview. In 6 cities 50 wards was selected randomly. In each sampled ward, 4 slums were selected randomly and 20 children per slum were selected systematically for interview.

Method used for identification of the rachitic children

- a) Identifying present rickets patients: With the help of a multicoloured poster depicting features of costal, lower and upper limb clinical rickets deformities was diagnosed to identify present rickets patients.
- **b)** Clinical analysis of the deformities: The clinical examination showed one or more of the following signs of rickets^{5,32}:

For 1-5 years children	For 6-15 years children
Age less then 5,	Wrist Joint Swelling,
Height: <-2.0 SD,	Costal Rosary,
Wrist Joint Swelling,	Leg Pain during Walking,
costal rosary,	Bow Leg, Knock Knee,
Leg pain during walking,	Wind Swept,
Slight Bowing of whole leg	Sabre Tibia (Presence of any of the visible
Slight bowing between knee & ankle joint	symptoms indicate rickets)
(Presence of any 3 of them indicate rickets)	

Active Rickets	Growing phase of rickets	Normal findings
This group contains typical	This group shows widening of	No rachitic abnormalities seen
findings of rickets like splaying,	growth plate with long	in the X-ray.
fraying & cupping of	deformity. It does not show	
metaphyses of long bones with	typical radiological features of	
widening of growth plate.	rickets. But clinical features	
	suggest rickets. We need	
	biochemical test to confirm	
	rickets in this group.	

On the basis of radiological findings whole rachitic children were divided into 3 groups:

4. Dietary Assessment:

a) 24 hours recall method

Dietary intake of calcium and phosphorus were estimated by dietary recall method. An interviewer, trained in this method, asked the mothers/ primary caregivers (if different from mother) to recall all foods consumed by the children in the past 24 hours. The interviewer prompted respondents for collecting information on the types of food, portion sizes, recipe ingredients, cooking methods, condiments, and beverages. During interview, food models and household measuring utensils were used to improve the accuracy of portion sizes. Information on the types and proportion of food were recorded and their nutrient content will be estimated according to their proportions³⁸.

b) Food Frequency Questionnaire

A food frequency questionnaire (FFQ) was used to determine the frequency of consumption of certain foods. The questionnaire contained a list of foods and frequencies of intake during the last two weeks. The food frequency provided qualitative data on types and frequency of foods or food groups. A semi-quantitative food frequency questionnaire provided a ranking classification of individuals into low, medium, and high intakes of specific nutrients for assessing associations between nutrients and disease. The questionnaire included name of foods that were good sources of calcium to discriminate between low and high consumers³⁸.

5. Biochemical Assessment:

Children identified with clinically diagnosed rickets were taken into the biochemical study. One hundred and ninety seven subjects with rickets were identified in the survey. Blood samples of subjects with rickets were tested to see the levels of biochemical markers related with clinical rickets and interrelations among the biochemical markers^{39,40,41,42}. Vitamin-D level was categorized into three level, such as severely deficit (0-14.9 ng/ml), moderate deficit and (15.0-31.9 ng/ml) and normal level (32.0-100.0 ng/ml).

Serum calcium level was estimated using quanticrom calcium assay kit³⁹. Bioassay Systems' calcium assay kit is designed to measure calcium directly in biological samples without any pretreatment. A phenolsulphonephthalein dye in the kit forms a very stable blue coloured complex specifically with free calcium. The intensity of the colour measured at 612 nm, is directly proportional to the calcium concentration in the sample.

Serum concentration of 25-OH D is considered to be the most reliable measure of overall vitamin D status. Serum Vitamin D level was estimated using the IDS 25-hydroxy Vitamin D EIA kit⁴⁰. The kit is an enzymeimmunoassay intended for the quantitative determination of 25-hydroxy vitamin D (25-OH D) in serum or plasma⁴⁰.

Estimation of bone alkaline phosphatase was done using IDS Ostase BAP Immunoenzymetric Assay indicated for the quantitative measurement of bone-specific alkaline phosphatase which is an indicator of osteoblastic activity, in human serum⁴¹.

The DRG Intact PTH Immunoassay was used for serum PTH estimation which is intented for the quantitative determination of intact-PTH (parathyroid hormone) in human serum⁴². This Immunoassay is a two-site ELISA for the measurement of the biologically intact 84 amino acid chain of PTH.

Qualitative data collection:

The qualitative data were collected through In-depth interviews conducted by anthropologists. It was necessary for the parents/ primary caregivers of children to be involved in the discussion of the problem and to initiate remedial measures using resources that were available in their own households/environment. Information on feeding frequency, knowledge about calcium-rich food, dietary ingredients, frequency of feeding and constraints were recorded. Two caretakers of rickets affected children from each division and similar number from normal children were undertaken for in-depth interview. Topics for in-depth interview included mother's understanding and ability to identify rickets in children, consequences of rickets, their knowledge on feeding practices and source

of calcium-rich food, frequency of home feeding (breast-feeding, other source(s) of milk and intake of calcium phosphate rich diets), frequency of seeking medical care and referral to doctors. It also included adequacy of food in terms of energy, protein and calcium and parents'/ primary caregivers' perception on social/economical disability of the rickets affected child.

Data Analysis

Analysis of Quantitative data:

The cleaned data set was checked by the quality control team and analysed using SPSS windows version (12.0). Data were first entered using the CSPro 3.3 Designer (version 3.3.003) Database programme and then converted into SPSS (version 11.5) data file to analyze. Anthropometric indices were calculated using the WHO ANTHRO 2005 and Epi-Info 2000. Malnutrition was classified using the WHO standard cut-off values for different anthropometric indices (MGRS 2006). Cleaned data were used for generating tables of relevant variables. The cases of rickets would be related with other morbidity, dietary, nutritional and socio-economic variables. Analysis was carried out to estimate the prevalence of rickets.

Analysis of Qualitative data:

Data collected from the In-depth interview and observation was ordered, reduced and coded according to the qualitative data analysis method (Applied Health Research Manual: Anthropology of Health & Medical Care). All qualitative data was summarized and a detailed report was presented. Emphasis was given on changes in KAP (Knowledge, Attitude and Practice), BCC (Behavior Change Communication) among the mothers/primary caregivers of participating children.

RESULTS:

Prevalence of Rickets in different divisions:

Among 20,000 children of 1-15 years of age, 197 (0.99 %) were identified with rickets (Figure 1). The percentage of rickets was the highest in Chittagong division, (76.6%) while the percentage was the lowest in Khulna division (2.5%). In Dhaka division, the percentage of rickets was 7.1%, 3.6% in Barisal, 4.6% in Rajshahi and 5.6 % in Sylhet division.

Nutritional status of study participants:

Table 1 represents the nutritional status of rachitic study participants compared with the WorldHealth Organization (WHO) child growth standard.

In the survey 154 rachitic children aged 1-15 were measured for height, weight and MUAC by quality control team of ICDDR,B. The overall stunting prevalence (HAZ <-2 SD) was 75% and severe stunting was 53% (HAZ <-3 SD). The prevalence of underweight was 70% (WAZ <-2 SD) and severe underweight was 40% (WAZ <-3 SD). The prevalence of wasting was 17% (WHZ <-2 SD) and severe wasting was 1.4% (WHZ <-3 SD).

The relationship between biochemical findings and different grades of wasting:

Table 2 shows the relationship between biochemical findings and different grades of wasting. Patients with moderate wasting were found with the highest serum level of Alkaline Phosphatase $(87.12\pm58.16 \text{ mg/dl})$ and parathyroid hormone $(48.93\pm40.16 \text{ pg/ml})$. Level of Vitamin D were not different (ranging from 13.12 ± 5.56 to 14.42 ± 5.27 ng/l) among rachitic children with different grades of wasting. Serum calcium level, ranging from 2.19 ± 0.14 to 2.24 ± 0.14 (mmol/l) also didn't vary much among subjects with different grades of wasting.

Distribution of radiological status by vitamin D level:

Figure 2 shows the distribution of vitamin D level by radiological status of the rachitic children. Among 36 active rachitic cases 64% were severely vitamin-D deficient, 36% were moderately vitamin-D deficient and none were found normal. Prevalence of severe vitamin-D deficiency was a little less (49%) among children with growing phase of rickets, but was lowest among rachitic children with radiological normal status.

Association between calcium level and vitamin-D level:

Association between calcium level and vitamin-D level is shown in **figure 3.** Among severely vitamin D deficient patients (n=71), 44% had calcium deficiency. About 58% of the moderate Vitamin D deficient children had calcium deficiency, suggesting for negative association between calcium level and vitamin-D level.

Food Frequency of Rachitic children:

Food frequency of rachitic children in last 24 hours is shown in **figure 4.** About 42% children took small fish and less than one third took leafy vegetables whereas only 1% took egg and 5% took milk more than once a day. According to the list of food frequency in last 24 hours, consumption of carbohydrate rich food only once a day was highest (46%) while less than one third (28%)of the rachitic children consumed leafy vegetables. Consumption of small fish and egg only once was also found low (13% and 12% respectively).

Figure 5 shows the food frequency of dietary intake of 7 days of rachitic children. Only 41% of the rachitic children was found to consume leafy vegetables and 33% consumed fruits more than three days in a week. Consumption of milk, a calcium rich food was found low (>3 days 13% and 1-3 days 13%). Consumption of cereal more than three days in a week was more than 60% of the rachitic children in this study. About 50% of the rachitic children in this study took small fish more than three days.

Biochemical markers with clinical signs:

Figure 6 shows the distribution of rachitic children with calcium level by number of clinical signs.

Among 3 signs, 60.4% rachitic children were severely deficient vitamin D level, 35.8 % rachitic children were in moderate deficit level and only 3.8% children were in normal level. Among 4 signs, 50.8% children were found to be severely vitamin D deficient while 47.6% children were in moderate deficit level. About 52.6% rachitic children, showing five signs, were severely vitamin D deficient and 47.4% rachitic children were in moderate deficit level but no children were in normal level. Sixty percent children with 6 signs were found to be severely vitamin D deficient and 40% children were in moderate deficit level. Linear correlation between calcium and vitamin D was found significant.

DISCUSSION:

Rickets is a disorder of growing children associated with bony deformities resulting from inadequate mineralization of growing bones. Results of the initial studies in Bangladesh suggested that vitamin D deficiency was not a major cause for rickets, and calcium deficiency was assumed to be the primary etiologic factor¹⁷.

In the recent years, production of rice has substantially increased in Bangladesh; however, crop rotation and diversity, and availability of milk in poor segments have significantly reduced. Although childhood malnutrition is slowly decreasing in Bangladesh, the diets have become more monotonous than it was several decades ago; more importantly, the current common diets contain lesser amounts of calcium. Boys seem to be more likely to develop rachitic deformities than the girls²⁹ and rickets is associated with larger family sizes and less maternal education where as poverty has a cross cutting relationship. Rickets is associated with respiratory illness, but not with malaria or anemia. Similarly, toxins, food patterns, and overall nutritional status are not associated with the prevalence of rickets among Bangladeshi children²⁹. Genetic factors that potentially impact the risk of nutritional rickets have not been studied.

To satisfy the requirements for vitamin D, most humans depend on sun exposure. The cutaneous production of vitamin D3 is insufficient when body is covered from sun exposure due to ignorance or cultural/religious practice. Rickets in the growing child or adolescent develop in a variety of clinical situations and have in common an absence or delay in the mineralization of growth cartilage and in newly formed bone collagen. Classically, deficiency of vitamin D, which is essential for the absorption of dietary calcium, has been the major cause²⁴. Rickets is characterized by weak bones that become curved or misshapen from bearing the weight of the body. The causes of rickets are best understood against the background of our knowledge of vitamin D metabolism. Different studies re-emphasized the importance of ultraviolet light in vitamin D economy, and the widespread effect of this vitamin/hormone throughout the body.

The national survey showed that the prevalence rate of rachitic children was 0.99%. The southern parts of Chittagong division; Chittagong and Cox's Bazar districts had the highest prevalence. Interestingly, rickets was not identified among the indigenous populations of the hilly regions (Hill Tracts) of the district. But the parts where there was a lack of sun exposure showed a higher prevalence. Season, latitude, time of day, skin pigmentation all influence the cutaneous production of vitamin D3. People who stay inside and have poor diets often have at least subclinical deficiency

and in some cases lack of direct sun exposure may lead to serious consequences³⁰. Subclinical vitamin D deficiency can be common in sunny countries due to cultural practice of women who give heavy barrier with clothes on the body. Breast milk of such women may be deficient of vitamin D. In vitamin D deficiency, calcification of bone does not take place which causes the disruption of the orderly processes of bone formation.

Radiological findings of this national Rickets survey demonstrated that presence of more than five signs were common among active rachitic patients. Results of this study showed the prevalence of stunting around 75%. During anthropometric assessment the common scales were used to measure height. Rickets is characterized by weak bones that become curved or misshapen from bearing the weight of the body and rachitic children cannot stand properly. This could be the one of the reasons behind the stunting found among our study subjects. From the history of dietary intake it was revealed that patients with more number of signs had reduced intake of rich sources of vitamin D.

According to our findings of serum vitamin D level, most of the rachitic children (52%) were found to be moderately deficient (reference value 15.0-31.9%). Radiological findings of our study showed that presence of more than five signs were prevalent among patients at active phase. Biochemical markers confirmed the relationship of the disease with serum levels of vitamin D, Alkaline phosphatase and parathyroid hormone. There was a positive correlation between serum level of vitamin D and para thyroid hormone. The regression analysis of vitamin D level on Alkaline phosphatase of rachitic children also indicated the positive correlation between the levels of alkaline phosphatase and vitamin D level. A previous study aiming to test the relationship between vitamin D, calcium and parathyroid hormone showed that reduced serum level of 25-hydroxyvitamin D below 25 nmol/l caused a significant rise in PTH. It was evident that Vitamin D levels could be suboptimal even if calcium and parathyroid hormone (PTH) levels are within reference intervals. A raised PTH with a normal/decreased calcium can be a marker of more severe vitamin D deficiency³¹.

In Bangladesh, small fish and vegetables are good and readily available sources of calcium. Vegetables contain potassium, magnesium, beta carotene and vitamin C, nutrients useful for bone health ^{34,35,36,37}. In the present study, rachitic children were found to have cereal based diet, with an average consumption of leafy vegetables and fruits. Consumption of milk, a rich source of calcium, less available and costly to general people, was found very poor.

Most of the parents of rachitic patients were found to be illiterate and had no idea about the disease. It was revealed that mothers of non-rachitic subject had a better perception of the disease, its symptoms and its prevention compared to that of rachitic patients. These mothers of non-rachitic subjects were also found to have better knowledge about introduction of complementary feeding and nutritious food on time. They seemed to be aware of the importance of vaccination and exclusive breastfeeding. These mothers also confirmed that they had knowledge about food sources of calcium and vitamin D.

CONCLUSION:

Prevalence of childhood rickets in Bangladesh is as high as 1% which can be considered as a public health problem not only as an acute problem but leading to a permanent disability. The deficiencies in calcium and vitamin D reflect the impaired dietary intake of growing children and faulty sun exposure practice of breastfeeding women needs measures to improve the intake. A policy on treatment and preventive measures is urgently warranted. We recommend that

- Government policy should be developed to prevent rickets in children and take curative measures in hospital set up at district levels.
- Intervention programme should be incorporated for prevention and early detection.
- Nutrition education on dietary measures for prevention and treatment be undertaken in geographically high risk areas.
- A case control study for risk factors should be under taken to learn more about prevention.
- Provide education of families regarding the importance of breastfeeding after birth.

ACKNOWLEDGEMENT

The successful completion of the National Rickets Survey was accomplished by the generous contributions of a number of organizations and individuals The Survey was carried out in 2008 by investigators of (i) CARE Bangladesh (ii) UNICEF (iii) NNP (National Nutrition Program of the Bangladesh government) (iv) SARPV and (v) ICDDR,B. The authors gratefully acknowledge these investigators for their support and commitment to ICDDR,B's research efforts. We would like to express special thanks to UNICEF for their technical and financial support for successful conduction of the survey.

REFERENCES:

- Rajakumar K. Vitamin D, cod-liver oil, sunlight and rickets: a historical perspective. Pediatrics 2003; 112: e132-e135
- Combs GF. The Vitamins: Fundamental aspects in Nutrition and Health, 2nd edition, Academic Press, 1998
- Weisberg P, Scanlon KS, Li R, Cogswell ME. Nutritional rickets among children in the United States: review of cases reported between 1986 and 2003. Am J Clin Nutr 2004;80:1697S-1705S
- Gartner LM, Greer FR, American Academy of Pediatrics, Section on Breastfeeding and Committee on Nutrition. Prevention of rickets and vitamin D deficiency: new guidelines for vitamin D intake. Pediatrics 2003;111:908–910
- 5. Thacher TD, Fischer PR, Strand M, Pettifor JM. Nutritional rickets around the world: causes and future directions. Ann Trop Paediatr 2006;26:1-16)
- Mylott BM, Kump T, Bolton ML, Greenbaum LA. Rickets in the Dairy State. WMJ.2004;103 (5):84–87.
- Pettifor JM. Nutritional rickets: deficiency of vitamin D, calcium, or both? Am J Clin Nutr.2004;80 (6 suppl):1725S–1729S
- Pettifor JM. Rickets and vitamin D deficiency in children and adolescents. Endocrinol Metab Clin North Am2005;34 (3):537–553
- Kreiter SR, Schwartz RP, Kirkman HN, Charlton PA, Calikoglu AS, Davenport ML. Nutritional rickets in African American breast-fed infants. J Pediatr.2000;137 (2):153–157
- Pugliese MT, Blumberg DL, Hludzinski J, Kay S. Nutritional rickets in suburbia. J Am Coll Nutr.1998;17 (6):637–641
- 11. Sills IN, Skuza KA, Horlick MN, Schwartz MS, Rapaport R. Vitamin D deficiency rickets: reports of its demise are exaggerated. Clin Pediatr (Phila).1994;33 (8):491–493
- 12. Ward LM. Vitamin D deficiency in the 21st century: a persistent problem among Canadian infants and mothers. CMAJ.2005;172 (6):769–770
- Tserendolgor, U. Prevalence of rickets in Mongolia. Asia Pacific Journal of Clinical Nutrition. Vol 7, Issue 3-4, 1998, Pages 325-328

- David R Fraser. Vitamin D-deficiency in Asia. Journal of Steroid Biochemistry and Molecular Biology. Vol 89-90, May 2004, Pages 491-495
- 15. Chesney RW. Rickets: the third wave. Clin Pediatr 2002; 41:137-139
- 16. Thacher TD et al. Case-control study of factors associated with nutritional rickets in Nigerian children. J Pediatr 137: 367.
- 17. Fischer PR et al. (1999). Nutritional rickets without vitamin D deficiency in Bangladesh. J Trop Pediatr 45: 291-293
- 18. O'Riordan JLH. Perspective: rickets in the 17th century. JBMR 21:1506-1510, 2006
- 19. Thacher TD. Wet-nursing and rickets. J Royal Soc Med 2006;99: 545-546
- 20. Pettifor JM, Ross P, Wang J, Moodley G, Couper-Smith J. Rickets in children of rural origin in South Africa: is low dietary calcium a factor? J Pediatr 1978;92: 320-324
- 21. Okonofua F, Gill DS, Alabi ZO, Thomas M, Bell JL, Dandona P. Rickets in Nigerian children: a consequence of calcium malnutrition. Metabolism 1991; 40:209-213
- 22. Oginni LM, Worsfold M, Oyelami OA, Sharp CA, Powell DE, Davie MWJ. Etiology of rickets in Nigerian children. J pediatr 1996; 128: 692-694
- Thacher TD, Fischer PR, Pettifor JM, Lawson JO. Isichei CO, Reading JC, Chan GM. A comparison of calcium, vitamin D, or both for nutritional rickets in Nigerian children. N Engl J Med 1999; 341: 563-568.
- Bishop N. Rickets today- children still need milk and sunshine. N Engl J Med 1999; 341:602-604.
- DeLucia MC, Mitnick ME, Carpenter TO. Nutritional rickets with normal circulating 25hydroxyvitamin D: a call for reexamining the role of dietary calcium intake in North American infants. J Clin Endocrinol Metab 2003;88:3539-3545.
- 26. Rickets in Bangladeshi Children: a small focus or a widespread problem. Helen Keller Worldwide. Nutrition Surveillance Project Bulletin No.4, June 2001
- 27. Karim F, Chowdhury AM, Gani MS. Rapid assessment of the prevalence of lower limb clinical rickets in Bangladesh. Public Health 117(2003)135-144.
- Kabir ML, Rahman M, Talukder K, Hassan Q, Mostafa G, Mannan MA, Kumar S, Chowdhury AT. Rickets among children of a coastal area of Bangladesh. Mymensingh Med J 2004 Jan;13(1):53-58.
- 29. Combs GF, Hassan N. The Chakaria food system study: household-level, case-control study to identify risk factors for rickets in Bangladesh. Eur J Clin Nutr 2005; 59:1291-1301.
- Michael F Holick. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancer, and cardiovascular disease. American Journal of Clinical Nutrition, December 2004, Vol. 80, No. 6, 1678S-1688S.

- 31. D Haarburger, M Hoffman, R T Erasmus, T S Pillay. Relationship between vitamin D, calcium and parathyroid hormone in Cape town. J Clin Pathol 2009; 62:567–569.
- 32. Thacher TD, Fischer PR, Pettifor JM. The usefulness of clinical features to identify active rickets. Ann Trop Paediatr 2002; 22:229-237
- 33. Lo CW, Paris PW, Hollick MF. Indian and Pakistani immigrants have the same capacity as Caucasian to produce vitamin D in response to ultraviolet irradiation. American Journal of Clinical Nutrition 1986; 44:683-5
- 34. Souci SW Fachmann W, Kraut H. Food Composition and nutrition tables. Stuttgart: WVG, 1989.
- 35. Burns L, Ashwell M, Berry J, *et al.* UK Food Standards Agency Optimal Nutrition Status Workshop: Environmental factors that affect bone health throughout life. British Journal of Nutrition 2003; 89:835-40.
- 36. New SA, Intake of fruit and vegetables: implications for bone health. Proc Nutr Soc 2003;62:889-99.
- 37. New SA, Millward DJ. Calcium, protein and fruits and vegetables as dietary determinants of bone health. American Journal of clinical nutrition 2003;77:1340-1.
- 38. S.K.Roy, G.J. Fuchs, Zeba Mahmud, Gulshan Ara, Sumaya Islam, Sohana Shafique, Syeda Sharmin Akter, Barnali Chakraborty. Intensive nutrition education with or without supplementary feeding improves the nutritional status of moderately-malnourished children in Bangladesh. Journal of Health, Population and Nutrition 2005 December; 23(4):320-330.
- 39. Erzsebet Zavaczki, Viktoria Jeney, Anupam Agarwal, Abolfazl Zarjou, Melinda Oros, Monika Katko, Zsuzsa Varga, Gyorgy Balla, Jozsef Balla. Hydrogen sulfide inhibits the calcification and osteoblastic differentiation of vascular smooth muscle cells. Kidney International June 2011; 80, 731-739
- 40. Andrew M Wootton. Improving the measurement of 25-hydroxyvitamin D. Clinical Biochem Rev. 2005 February; 26(1):33-36
- Christopher P.Price, Thomas P. Milligan, Claude Darte. Direct comparison of performance characteristics of two immunoassays for bone isoform of alkaline phosphatase in serum. Clinical Chemistry 1997; 43(11):2052-2057
- Anson K. Abraham, Donald E. Mager, Xiang Gao, Mei Li, David R. Healy and Tristan S. Maurer. Journal of Pharmacology and experimental therapeutics. July 2009, Vol. 330 no.1:169-178

TABLES AND FIGURES:

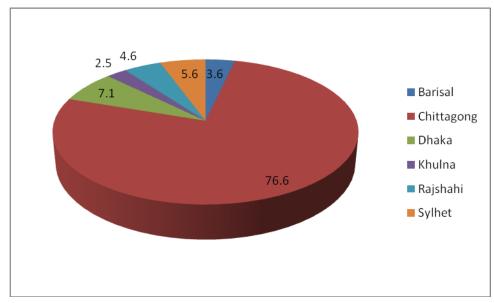


Figure 1: Distribution of rachitic children by division

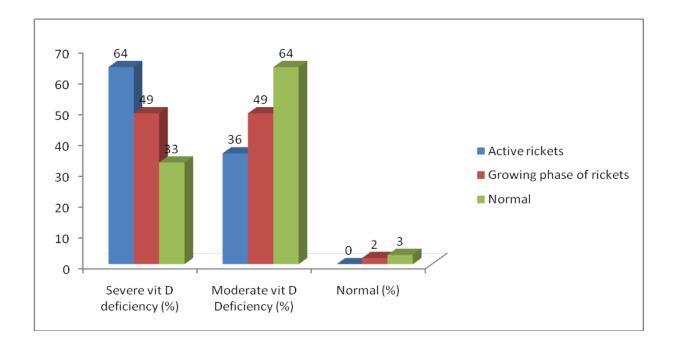
Table 1: Analysi	s of variance	of nutritional	l status with	biochemical	markers
------------------	---------------	----------------	---------------	-------------	---------

Variables	Nutritional Status(WHZ)		
	Normal	Moderate	Severe
	(-2.00 SD to above)	(-3.00 SD to -2.01 SD)	(<-3.00 SD)
Vitamin D level (ng/l)	14.37±5.23	13.12±5.56	14.42±5.27
Serum calcium level (mmol/l)	2.19±0.14	2.24±0.14	2.20±0.11
Para-thyroid hormone(pg/ml)	43.58±50.26	48.93±40.16	36.36±35.18
Alkaline phosphatase (mg/dl)	67.60±23.38	87.12±58.16	71.76±38.67

Indicator	Rachitic children		
	(n)	(%)	
HAZ			
<-3.00 SD	82	53.3	
-3.00 SD to -2.01 SD	33	21.4	
-2.00 SD to above	39	25.3	
Total (N)	154	100.0	
WAZ			
<-3.00 SD	61	40.1	
-3.00 SD to -2.01 SD	45	29.6	
-2.00 SD to above	46	30.3	
Total (N)	152	100.0	
WHZ			
<-3.00 SD	2	1.4	
-3.00 SD to -2.01 SD	21	15.1	
-2.00 SD to above	116	83.5	
Total (N)	139	100.0	

Table 2: Nutritional status of children by their rachitic status

Figure 2: Distribution of radiological status of the rachitic children with vitamin D level



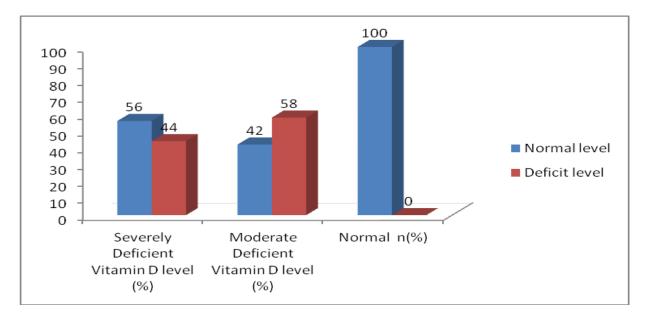
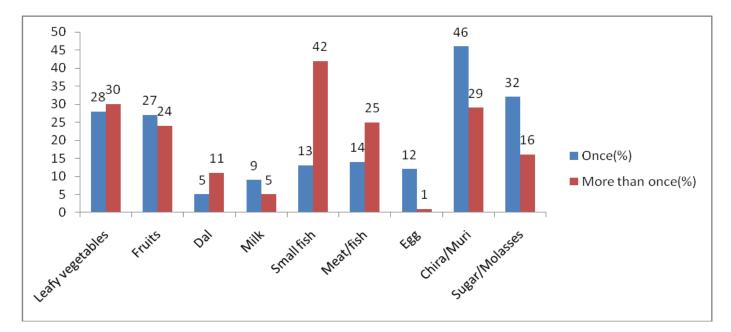


Figure 3: Percentage distribution of children by their serum Vitamin-D level and serum calcium level

Figure 4: Food Frequency of rachitic children in last 24-hours (n=197)



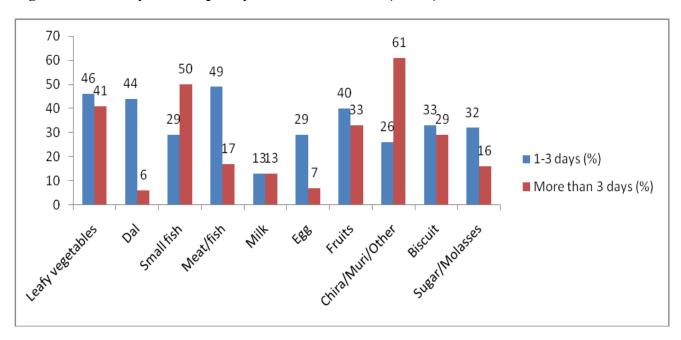


Figure 5: Seven day food frequency of rachitic children (n=197)

