http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (7): 1282-1286, 2004
ISSN 1028-8880

Serum Retinol Level and Nutritional Status of Rural Adolescent Children with or without Eye Changes

[1]A.F.M. Tariqul Islam, [1]Pricila Khan Moly, [2]Khaleda Islam and [3]H.K.M. Yusuf
[1]Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong,
[2]Institute of Nutrition and Food Science,
[3]Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh

Abstract: A study was conducted to find association between serum retinol level, blood haemoglobin level and Body Mass Index (BMI) of rural adolescent children (aged 10-17 years) having or not having eye changes. The study was carried out through a cross sectional survey on 906 adolescents from 803 households, sampled consecutively from four purposively selected villages of Rupganj Upazila (sub-district) under Narayanganj district, Bangladesh. An appropriate questionnaire was developed to obtain information on age, sex, height, weight, housing condition, sanitation and educational qualification of both the adolescents and their parents. Among 906 adolescents (47% male and 53% female), 2.1% (19) had eye changes that are known to occur due to Vitamin A deficiency (e.g., retinal, conjunctival and corneal changes). Eleven (57%) of them were male and 8 (42%) were female. Of the 19 adolescents who had eye changes, 9 (42%) had serum retinol level below the cut-off value of 20 μg dL^{-1}, 90% had BMI lower than 18.5 kg m^{-2} (i.e. suffering from chronic energy deficiency) and 100% were anaemic (Hb level <115 g L^{-1}). These figures were 25, 66 and 72%, respectively, in adolescents who had no eye changes. These data show strong association of malnutrition and anaemia with eye changes in adolescents. The national vitamin A supplementation programme should be extended from under-6 year old children to the adolescent population, who are yet another vulnerable group.

Key words: Adolescent children, eye changes, serum retinol, nutritional status, anaemia

INTRODUCTION

Adolescence is a period in life (10-19 years) when the last spurt in growth occurs for an individual to attain the near-adult height. This period is characterized by rapid changes in body physiology, particularly in the endocrine system that brings a child into the state called puberty. The health and nutrition issue of adolescents of developing countries has attracted world attention in the past decade. This attention was, until recently, mostly concentrated on issues such as sexually transmitted disease, reproductive health, substance misuse and violence. Limited work has been done on the nutrition of adolescents in the third world. In Bangladesh adolescents number some 31 million (23% of the estimated population in 2001)[1], 77% of whom live in rural areas. No national level intervention programme has even been undertaken on this population group in Bangladesh. Vitamin A deficiency is associated with a wide range of consequences, from blinding xerophthalmia to apparent compromises in growth, resistance to infection and survival.

Changes in the retina, conjunctiva and cornea (eye changes) are known to be produced by vitamin A deficiency. Night blindness is an early symptom of vitamin A deficiency. Prolonged nutritional deficiency of vitamin A results in xerophthalmia. Bitot's spots are an early manifestation of this condition. A loss of goblet cells in xerophthalmia alters the protective mucous layer resulting in an overlying accumulation of Gram positive bacilli and keratin debris on the paralimbal conjunctiva[2]. The World Health Organization (WHO) categorised the ocular manifestations of vitamin A deficiency as XN night blindness, X1A conjunctival xerosis, X1B Bitot's spot, X2 corneal xerosis, X3A corneal ulceration or keratomalacia involving one third or less of the cornea, XS corneal scar and XF xerophthalmia fundus[3].

The purpose of the present study was to find correlation between serum retinol level and eye changes in association with other factors like anaemia and general

Corresponding Author: A.F.M. Tariqul Islam, Lecturer, Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh Tel: 88 031 726311 14 Ext: 4334
E-mail: tariqdu@yahoo.com

nutritional status. An effort was made to find these correlations in adolescent patients having eye changes due to chronic vitamin A deficiency and those having no eye changes.

MATERIALS AND METHODS

For the study, a total of 1483 adolescents were initially recruited from 803 households of four villages of Rupganj Upazila under Narayanganj district, Bangladesh. Anthropometric measurements and clinical examinations were carried out on 906 adolescents from 597 households. Of these blood was sampled from 165. The subjects were aged 10-17 years.

Selection of study subjects: Apparently healthy unmarried adolescents children living in the surveyed households in the four purposively selected villages, of Rupganj upazila which has an area of 248 km^2 and an estimated population of 475,000 in 2001 [1]. This upazila was chosen for the study because the population there was acknowledged to be economically disadvantaged and it has not previously had any significant health and nutrition interventions. From within this upazila, the two most economically deprived unions (by criteria such as landlessness, occupation of head of household, building material of houses) were selected. From within each union two villages were selected for the study.

Demographic and socio-economic information: Households recruited for the study were visited with an initial interview involving the mother or other adults in the house. The following information was gathered on family members: age, sex, marital status, level of education, occupation M and relationship to household head. Socio-economic data included family monthly income, housing, water supply, sanitation, household possessions and transport vehicle ownership.

Anthropometry: Adolescents were weighed barefoot and in light clothes. Measurements were taken using a battery powered electronic digital scale (accurate to within 100 g). The machine was checked for calibration after every 50 measurements using a known measuring weight of 5 kg. Height was measured using a wooden scale, with accuracy nearest to 1 cm.

Clinical examinations: A physician examined the adolescents for features of nutritional deficiencies. The following were specifically looked for: pallor, eye changes of vitamin A deficiency, angular stomatitis, glossitis, goiter and scabies.

Biochemical analysis: Vitamin A (retinol) was estimated by High Performance Liquid Chromatography (HPLC).

Sample preparation: Blood samples (1 ml) of 165 adolescent children, 19 with eye changes and 146 with no eye changes, were collected by anticubital venipuncture and allowed to clot. The clotted blood was then centrifuged for 10 min at 12, 000 rpm. After centrifugation, the serum was collected by a Pasteur pipette and taken into Eppendrof tubes. All samples were stored at -28°C until analyzed.

Estimation of Vitamin A by HPLC: Vitamin A levels in serum was estimated by slight modification of the method of Bieri *et al.*[4].

Chromatographic condition: Methanol was prepared to 97% as the mobile phase for chromatography. The solution was filtered using Millipore filter (porosity 0.22) and degassed prior to use. The flow rate of the pump was 1.5 ml/min. The column effluent was monitored at 292 nm. The attenuation (AT), peak threshold level (PT), sensitivity and chart speed (CS) were 8, 100 and 0.01 AUFS (absorbance full length scale) and 0.5 cm/min, respectively.

Preparation of standard retinol solution and standard curve: 10 mg retinol was dissolved in 100 ml ethanol. The concentration of retinol in this solution was 100 μg ml^{-1}. 0.5 ml of this solution was diluted to 100 ml with ethanol. Now the concentration of retinol was 0.5 μg ml^{-1}. This was used as the working standard retinol.

Extraction of vitamin A from serum: Two hundred μl serum was taken into an eppendrof tube. Then 100 μl internal standard and 100 μl ethanol were added. After vigorous mixing 400 μl n-hexane was added. The mixture was mixed well with a vortex for 45 sec and centrifuged at 12,000 rpm for 10 min. The organic layer was collected and dried under nitrogen. The dried sample was dissolved with 100 μl ethanol, ready for injection. Serum retinol level of 20 μg dL^{-1} was taken as the cut off value for biochemical vitamin A deficiency [5].

Estimation of haemoglobin: Blood haemoglobin level was estimated according to the method of Cartwright[6] using a commercially available kit (Boehringer Mannheim, Germany). Haemoglobin level <115 g L^{-1} was diagnosed as anaemia[7].

Data analysis: Data was double entered into Epi-Info version 6.04 and checked for accuracy using the validate program.

RESULTS

Prevalence of eye changes in adolescent children was found to be 2.1% (19 out of 906). Of them 11 were male and 8 were female. Among the 19 eye change children, 8 (42%) had low serum retinol level (<20 μg dL^{-1}) and 11 (58%) had normal serum retinol level (>20 μg dL^{-1}). From this study it was found that serum retinol level of eye change patients was not always below the normal level. However, only 17% of adolescents with no eye change had low serum retinol level (Table 1).

All the eye change patients (100%) were anaemic (Hb <115 g L^{-1}), compared to only 72% of the adolescents with no eye changes (Table 2). The eye change patients thus appear to be more susceptible to anaemia.

All 8 (100%) adolescents with eye changes (who also had low serum retinol level) were anaemic; on the other hand, of the 25 adolescents who had low serum retinol level, 18 but had no eye changes (72%) were anaemic (Table 3). These results thus show a complex interplay between eye change, serum retinol and anaemia.

More than two-thirds of the total adolescents examined were thin, defined as BMI 5th centile of WHO[8] recommended reference i.e., <18.5 kg m^{-2}. Ninety percent of adolescents with eye changes had BMI less than 18.5, as against 66% with no eye changes (Table 4). Of the 8 eye change patients who had low serum retinol level, 7 (88%) had low BMI value, as opposed to 56% who had no eye changes (Table 5). The data suggested the interrelationship between low serum retinol level, eye change and nutritional status.

Table 1: Retinol level of eye change patients and the control individual

	Adolescents with eye change		Adolescents with no eye change	
Serum retinol level (μg dL^{-1})	Number	Percent	Number	Percent
<20	08	42	25	17
>20	11	58	121	83
Total	19	100	146	100

Table 2: Correlation between anaemia and eye change in adolescents

Anaemia	Adolescents with eye change	Percent	Adolescents with no eye change	Percent	Probability significance
Yes	19	100	105	71.9	
No	0	0	41	28.1	0.00
Total	19	100	146	100.0	

Table 3: Correlation between low serum retinol level, eye change and anaemia in adolescents

	Adolescents with no eye change				
Anaemia	Retinol (<20 μg dL^{-1})	Percent	Retinol (>20 μg dL^{-1})	Percent	Probability significance
Yes	18	72	92	76	
No	7	28	29	24	<0.05
Total	25	100	121	100	
Adolescents with eye change					
Yes	8	100	11	100	
No	0	0	0	0	<0.001
Total	8	100	11	100	

Table 4: Correlation between BMI and eye changes

BMI	Adolescent with eye change	Percent	Adolescent with no eye change	Percent	Probability significance
>18.5	02	10.5	300	33.8	
<18.5	17	89.5	587	66.2	<0.03
Total	19	100.0	887	100	

Table 5: BMI and serum retinol level in adolescents

	Adolescents with eye changes				
BMI	Retinol (<20 μg dL^{-1})	Percent	Retinol (>20 μg dL^{-1})	Percent	Probability significance
>18.5	01	12.50	01	9.10	
<18.5	07	87.50	10	90.90	<0.02
Total	08	100.00	11	100.00	
Adolescents with no eye changes					
>18.5	11	44.00	49	40.50	
<18.5	14	56.00	72	49.50	<0.07
Total	25	100.00	121	100.00	

DISCUSSION

Little study has been done on eye changes in adolescent children in Bangladesh. The present study focuses on the eye changes in adolescent children, although only 19 cases were found among 901 adolescents examined.

The presence of eye changes due to vitamin A deficiency in 2.1% (19 out of 901 adolescents) of this population was surprising as the national effort for vitamin A supplementation is restricted to the under 6 children. Serum retinol deficiency has previously been demonstrated[9] in 56% of 388 female Bangladeshi adolescent garments workers aged 12-19 years. The prevalence of clinical eye changes may indicate significant current vitamin A deficiency in the surveyed population and, given the availability of an intervention, further studies to identify the extent of the problem in rural adolescents are urgently called for.

Different studies showed a strong relationship between vitamin A deficiency and anaemia. There is clear evidence of an association between plasma serum levels of vitamin A and the level of haemoglobin in children[10-13] and in pregnant women[14]. It is thought that this association is likely to be causal, as there is evidence for an interaction between iron and vitamin A metabolism. Vitamin A deficiency has been associated with an impaired ability to utilize endogenous iron stores[15-17]. Vitamin A deficient subjects have been found to be unresponsive to dietary supplementation with iron[15].

In this study, it was found that 100% of the eye change adolescents were anaemic (hemoglobin level <115 g L^{-1}) compared to 71.9% who had no eye changes.

But retinol level of all 19 eye change patients was not below the cut-off level of 20 μg dL^{-1}. Only eight (42.1%) of them had low serum retional level (<20 μg dL^{-1}). It is possible that they were severely deficient of vitamin A sometime in the past and at that time the eye changes occurred which were not overcome. Among 146 adolescents with no eye changes 25 have low serum retinol level and 18 of them were anaemic.

Sixty-seven percent of the total adolescents examined were malnourished according to BMI. Nearly 90% of the adolescents with eye change were malnourished compared with 66% who had no eye changes. Malnutrition is one of the major problems in Bangladesh and this is highly prevalent among adolescents. This study thus showed that eye change adolescents were much more affected than the adolescents with no eye changes. Conversely, adolescents suffering from malnutrition are likely to have vitamin A deficiency as well, many having eye changes.

In this study it was found that 18 out of 19 eye change patients had only 3 years or less of schooling. So lack of education could be a cause of eye changes. In our country if individuals have proper knowledge, they have the likelihood of meeting their daily nutritional requirements from normal diet. For this purpose one has to get proper education. Studies have indeed shown that simple nutrition education intervention in the parents can dramatically reduce the prevalence of night blindness in the children[18,19].

Finally it can be concluded that eye change adolescents and also adolescents having no eye changes are suffering from low serum retinol level, high rate of malnutrition and anaemia. They are also lacking in education. So intervention programs for control of eye change in adolescent children should be taken immediately all over Bangladesh. Vitamin A deficiency, like PEM, arises mostly due to poverty and ignorance. We cannot remove poverty overnight but what we can do is to see that carotene-rich vegetables are grown by every household. To do this, the population at large, particularly in the rural areas, should be given nutritional knowledge. They should be given the understanding that their children need to eat Saaks (leafy vegetables) which are rich in vitamin A, otherwise they could go first night blindness and then totally blind.

ACKNOWLEDGMENT

The authors wish to thank Dr. Khurshid Talukder of the Institute of Child and Mother Health (ICMH), Matuail, Dhaka, Bangladesh, for his kind support and keen interest in the study.

REFERENCES

1. Anonymous, 2003. Bangladesh Bureau of statistics, Dhaka, Bangladesh.
2. Sommer, A., W.R. Green and K.R. Kenyon, 1981. Bitot's spots responsive and nonresponsive to vitamin A. Clinicopathologic correlations. Arch. Ophthalmol., 99: 2014-2027.
3. World Health Organization, 1994. Prevention of Childhood Blindness. Geneva: WHO, 1994.
4. Bieri, J.G., T.J. Tolliver and C.L. Catignani, 1979. Simultaneous determination of alpha tocopherol and retinol in plasma and red cell by high-pressure liquid chromatography (HPLC). Am. J. Clin. Nutr., 32: 2143-2149.
5. WHO/UNICEF/HKI/IVACG Meeting, 1982. Control of Vitamin A deficiency and erophthalmia. WHO Technical Report Series No. 672, Geneva.
6. Cartwright, G.E., 1968. Diagnostic laboratory haematology. 4th Edn. Grune and Strulton. New York, pp: 111.
7. WHO, UNICEF, UNU, 1998. Iron Deficiency Anemia: Prevention, Assessment and Control. In: Report of a joint WHO/UNICEF/UNU consultation. Geneva.
8. World Health Organization (WHO)1995. Physical Status: the Use and Interpretation of Anthropometry. Report of a WHO Expert Committee. Technical Report Series No. 854. Geneva: WHO, pp: 302-303.
9. Ahmed, F., N. Hasan and Y. Kabir, 1997. Vitamin A deficiency among adolescent female garment factory workers in Bangladesh. Eur. J. Clin. Nutr., 51: 698-702.
10. Mejia, L.A., R.E. Hodges, G. Arroyave, F. Viteri and B. Torun, 1977. Vitamin A deficiency and anaemia in Cental American children. Am. J. Clin. Nutr., 30: 1175-1184.
11. Mohanram, M., K.A. Kulkarni and V. Reddy, 1977. Haematological studies in vitamin A deficient children. Int. J. Vitamin Nutr. Res., 47: 389-393.
12. Ahmed, F., S. Barua, M. Mohiduzzaman, N. Shaheen, M.A.H. Bhuyan, B.M. Margeys and A.A. Jacson, 1993. Interaction between growth and nutrient status in school-age children of urban Bangladesh. Am. J. Clin. Nutr., 32: 2143-2149.
13. Wolde-Gebriel, Z., C.E. West, H. Gebru, A.S. Tadesse, T. Fisseha, P. Gabre, G. Ayana and J.G.A.J. Hautvast, 1993. Interrelationship between vitamin A, iodine and iron status in school children in Shoa Region, Central Ethiopia. Br. J. Nutr., 70: 593-607.

14. Suharno, D., C.E. West, M.H.G.M. Logman, F.G. de Waart, D. Karyadi and J.G.A.J. Hautvast, 1992. Cross sectional study on the iron and vitamin A status of pregnant women in West Java, Indonesia. Am. J. Clin. Nutr., 56: 988-993.
15. Mejia, L.A. and G. Arroyave, 1982. The effect of vitamin A fortification of sugar on iron metabolism in preschool children in Guatemala. Am. J. Clin. Nutr., 36: 87-93.
16. Mejia, L.A. and F. Chew, 1988. Haematological effect of supplementing anaemic children with vitamin A alone and in combination with iron. Am. J. Clin. Nutr., 48: 595-600.
17. Bloem, M.W., M. Wedel, R.J. Egger, M. Andries, A.J. Speek, J. Schrijver, S. Saowakontha and W.H.P. Schreurs, 1989. Iron metabolism and vitamin A deficiency in children in northeast Thailand. Am. J. Cliln. Nutr., 50: 332-338.
18. Islam, M.N. and H.K.M. Yusuf, 1992. Socio-economic correlates of child nightblindness in a coastal area of Bangladesh: a critical analysis. Ecol. Fd. Nutr., 28: 149-156.
19. Yusuf, H.K.M. and M.N. Islam, 1994. Improvement of night blindness situation in children through simple nutrition education intervention with the parents. Ecol. J. Nutr., 31: 247-256.