Prevalence of sub-clinical vitamin A deficiency in 2–5-year-old children in Tehran

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معدَّل انتشار عَوَز الفيتامين "أ" دون السريري في الأطفال بين الثانية والخامسة من العمر في طهران

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الخلاصة: قام الباحثون بتحليل 1257 طفلاً، تم اختيارهم عشوائياً من المناطق الريفية والحضرية في طهران، من أجل تحديد حالة الفيتامين "أ" باستخدام سيتولوجيا الانطباع الملتحمي لدى الأطفال ممَّن تتراوح أعمارهم بين 2 و5 أعوام. وقد سجّل الباحثون تاريخ استخدام مكملات الفيتامين "أ"، وحدوث العدوى التنفسية أو الإسهال خلال الأشهر الستة المنصرمة، ومحل الإقامة والوضع الاقتصادي والتعليمي للأسرة، وعمر الطفل، والجنس، والوزن. وقد وجد أن 23.6٪ من أفراد العيِّنة يعانون من عَوَز الفيتامين "أ" السريري (الذي يعرف بأنه سيتولوجيةٌ غير طبيعية في الانطباعات الملتحمية)، ويصنّف معدل الانتشار هذا أحد مشاكل الصحة العمومية المتوسطة. كما وجدت علاقة يُعتدُّ بها إحصائياً بين الجنس والعمر وبين سيتولوجيا الانطباعات الملتحمية غير الطبيعية ($P < 0.05$).

ABSTRACT To determine vitamin A status using conjunctival impression cytology (CIC) in children aged 2–5 years, we assessed 1257 randomly selected children in urban and rural areas of Tehran. History of using supplemental vitamin A, respiratory or diarrhoeal infection in the previous 6 months, residential location, parents' education, family economic status, and child's age, sex and weight were recorded. Sub-clinical vitamin A deficiency (defined as abnormal CIC) was found in 23.6% of the sample, a rate classified as a moderate public health problem. There was a statistically significant relationship between sex and age and abnormal CIC ($P < 0.05$).

Prévalence de la carence infraclinique en vitamine A chez l'enfant de 2 à 5 ans à Téhéran

RÉSUMÉ Afin de déterminer le statut vitaminique A par cytologie conjonctivale par empreinte (CIC pour *conjunctival impression cytology*) chez des enfants âgés de 2 à 5 ans, nous avons évalué 1257 enfants sélectionnés au hasard dans les zones urbaines et rurales de Téhéran. Les paramètres suivants ont été enregistrés : histoire d'une supplémentation vitaminique A, antécédents d'infection respiratoire ou de diarrhée infectieuse dans les 6 derniers mois, lieu de résidence, niveau d'instruction des parents, situation économique de la famille et âge, sexe et poids de l'enfant. Une carence infraclinique en vitamine A (définie par une CIC anormale) a été constatée chez 23,6 % des sujets de l'échantillon, taux qui place cette carence au rang des problèmes de santé publique modérés. Il est apparu une relation statistiquement significative entre le sexe, l'âge et une CIC anormale ($p < 0,05$).

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Introduction

Vitamin A deficiency (VAD) is one of the commonest nutritional deficiency disorders in the world [*1*]; the deficiency causes preventable blindness in children, increases the risk of severe illness, and can even be fatal owing to infections such as diarrhoea, measles and respiratory tract infection. VAD is a public health problem in 118 countries, especially in Africa and South-East Asia [*2,3*]. The World Health Organization has estimated that over 250 million children worldwide have deficient vitamin A stores [*1*]. The sub-clinical form of VAD has been seen in patients who, despite having no evident clinical signs or symptoms, suffer from many functional problems [*4*]. It is estimated that the ratio of clinical to sub-clinical forms of VAD is 1:10 [*5*].

Studies have been conducted in several provinces of the Islamic Republic of Iran to assess VAD. In Sistan-Baluchistan province, 20% of students had xerosis conjunctivae and xerosis corneae. In Tehran province, 35% of male infants and 21% of female infants had low serum retinol. In Semnan province, only 12% of rural and 40% of urban inhabitants received adequate daily vitamin A. In southern and western provinces, clinical manifestations of VAD were Bitot spots and xerophthalmia. In Kordestan province, prevalence of Bitot spots was 21% [*6*].

Prevalence of VAD differs in different countries, being high in Pakistan, Afghanistan and Iraq [*7,8*]. These countries have programmes implementing universal vitamin A supplementation. One study to assess the vitamin A status of 532 children with an age range of 6–60 months who lived in slum areas of Karachi, Pakistan found that a significant number had low vitamin A levels, and thus may have been at risk [*7*]. Another study in southern Iraq reported that most children 0–5 years of age had both acute and chronic malnutrition [*8*].

It is hoped that with improvements in the implementation of these programmes, annually around 1–2 million children aged 1–4 years will be saved [*5*]. Although the highest prevalence of VAD is seen in preschool children and in pregnant and lactating women, sub-clinical VAD is also common in schoolchildren and adolescents in some settings.

The main causes of VAD in the developing world are insufficient intake of vitamin A and poor bioavailability of provitamin A sources such as vegetables and fruits. In many of the developing countries, up to 90% of the vitamin A in the diet is of plant origin [*1*].

A number of different methods, clinical, biological and histological, have been used for evaluation of vitamin A status. Other methods not generally used include measuring retinol in teardrops and pupillary dark adaptation. Measurement of serum levels of vitamin A requires well-developed laboratory techniques and expert technicians. Since measurement of serum retinol concentration is carried out in the better laboratories in the large cities, it can be expensive, particularly during the hot months when transportation is an issue (retinol is unstable on exposure to heat and light). In healthy individuals, serum retinol concentrations are homeostatically controlled and do not begin to decline until liver reserves of vitamin A become dangerously low [*9*]. In addition, this method is not useful for diagnosis in the early stages of VAD [*10*].

Other methods, such as the relative dose response and modified relative dose response, can reflect liver reserves of vitamin A, but these are not good choices for assessment of vitamin A status in epidemiological surveys.

In developing countries, conjunctival impression cytology (CIC) is considered most appropriate for the diagnosis of VAD [*11*]. The most important advantage of the technique is its ability to demonstrate the condition in the early stages. In mild VAD it has been seen that histological abnormalities of the bulbar conjunctiva, including separation and distortion of epithelial cells and losses of mucin-secreting goblet cells, occur.

In this technique, a strip of cellulose acetate filter paper is gently applied to the temporal bulbar conjunctiva for 2–3 seconds. After removal, the specimen is placed in fixative and transferred to the laboratory for staining and assessment. The specimen is microscopically examined for the presence and density of goblet cells or mucin spots and density, size and shape of epithelial cells in relation to specimen area or number of microscopic fields. It has been demonstrated that CIC is a reliable and objective test for the assessment of vitamin A status. One study comparing impression cytology with serum vitamin A concentration reported CIC to be a simple and specific test for diagnosis of VAD [*4*].

Other studies that compared CIC results and other indices of vitamin A status confirm the validity of CIC [*12,13*]. An Indonesian study reported close correlation with serum vitamin A levels [*13*]. The evidence indicates that this technique is a feasible and reliable indicator of vitamin A status, appropriate for survey use.

It has been suggested that a prevalence of 20% for VAD constitutes a public health problem [*12*]. Studies from Asia using CIC suggest an abnormal 30% prevalence, comparable to the serological data [*12*].

Abnormal cytology, which is a common result of vitamin A deficiency, has also been associated with health risks such as respiratory infection, persistent diarrhoea, anaemia, mild xerophthalmia, otitis media and mild anthropometric deficit. The aim of this study is the evaluation of subclinical vitamin A deficiency in children aged 2–5 years in Tehran and its suburbs.

There are various methods for evaluation of VAD; in this study, CIC with a specificity of 94% and sensitivity of 93%, was used [*14*].

Methods

This research was conducted over a period of 9 months starting May 2002, on 1257 children aged 2–5 years with no history of conjunctivitis. The group most susceptible to VAD is children aged 6–60 months; CIC is not, however, usually used in children below 24 months due to the inability of the child to cooperate. Therefore, in this study we selected children aged 24–60 months.

The participants were selected from children in Tehran, Islamic Republic of Iran, who were receiving medical services provided by Shaheed Beheshti University of Medical Sciences, a total of 220 751 children aged 2–5 years, 201 101 from Tehran (urban) and 19 650 from the suburbs (rural). Systematic random sampling was used to select participants. Based on an estimated prevalence of VAD of 20% [*6*], and assuming 5% error and 95% confidence interval, sample size was estimated at 1190. To cover losses, a sample size of 1360 was used. There were 103 losses, leaving 1257 participants in the study.

Families of the children were invited by health workers to the health clinic for interviews and completion of information sheets, which included data on sex, age and weight of the child; history of using vitamin A supplements; history of respira-

tory tract infection and diarrhoea during the past 6 months; parent's education level; socioeconomic status of the family (based on household income, father's occupation, type of accommodation, per capita living space and number of children in the family) and location of residence.

After obtaining informed consent from all parents, the data sheets were completed by trained health workers. Two samples were obtained by a paediatrician for each eye. A 3–6 mm strip of cellulose acetate filter paper was applied gently to the temporal bulbar conjunctiva for 2–3 seconds. After removal, samples were placed in fixative and transferred to the laboratory in the Department of Health in the university within 2 hours. Filter paper specimens were stained with para-amino salicylic acid and haematoxylin eosin. Specimens were microscopically examined by a pathologist for presence of and density of goblet cells and shape, size and density of epithelial cells. Abnormal CIC findings were characterized by large, separated, partially keratinized epithelial cells and were devoid of goblet cells [*15,16*].

Data were analysed using *SPSS*, version 9. $P < 0.05$ was considered significant.

Results

A total of 1257 children were enrolled in the study; 637 (50.7%) were girls; 371 (29.5%) were from rural areas (Table 1).

Examination of the samples showed that 297 (23.6%) had abnormal CIC. Comparing the 2 residential areas, 22.3% of the urban children and 26.7% of the rural children had abnormal CIC; the difference was not statistically significant (Table 1).

In the 6 months before the survey, 30.9% (388) of the children in our sample had a history of using vitamin A supplements and 19.3% (243) had a history of respiratory tract infection and diarrhoea. Weight for age was below the 5th percentile in 8.8% (111) of the children we studied.

The rate of abnormal CIC in children under 3 years of age was significantly higher than in children aged ≥ 3 years ($P < 0.05$) (Table 1). There was a significant relationship between sex and abnormal CIC results,

Table 1 **Conjunctival impression cytology for children 2–5 years old in Tehran**

Characteristic	Total	Cytology				95% CI
		Normal		Abnormal		
		No.	%	No.	%	
*Sex**						
Female	637	472	74.1	165	25.9	22.5–29.3
Male	620	488	78.7	132	21.3	18.1–24.5
Residence						
Urban	886	688	77.7	198	22.3	–
Rural	371	272	73.3	99	26.7	–
*Age (years)**						
< 3	362	261	72.1	101	27.9	23.3–32.5
≥ 3	895	699	78.1	196	21.9	19.2–24.6
Total	1257	960	76.4	297	23.5	–

**Significant at* P < *0.05.*
CI = confidence interval.

higher in girls than in boys ($P < 0.05$). No significant relationship was found between abnormal CIC and economic status, history of respiratory tract infection and diarrhoea during the previous 6 months, weight, parents' education, history of using vitamin A supplements or residential location.

Discussion

In this study, prevalence of the sub-clinical form of VAD, based on CIC, among children aged 2–5 years was 23.6%. This is considered to be within the moderate deficiency range according to World Health Organization and UNICEF classifications [*17*]. Prevalence of VAD in children, evaluated on the basis of serum retinol level < 20 μg/dL, was 19% in central, 9% in western and 23% in eastern parts of the Islamic Republic of Iran [*6*]. According to World Health Organization and UNICEF evaluation criteria, serum retinol level < 20 μg/dL among 10% of children under 3 years of age, constitutes a public health problem [*17*]. It can be concluded, therefore, that VAD is a public health problem in the Islamic Republic of Iran. The difference in prevalence in various regions may be attributed to variations in living conditions, nutritional patterns and vitamin A supplementation.

The high prevalence of VAD deficiency in India is related to the nutritional habits and also the high incidence of infectious diseases among children, which in itself can be a cause. In neighbouring countries like Afghanistan, Iraq and Pakistan, prevalence of VAD is > 20% (classified as severe). In other countries, such as Syria and the Russian Federation, however, the sub-clinical form of VAD is rare [*5*].

Kurugöl et al. reported sub-clinical VAD to be a public heath problem in Izmir, Turkey: 15.6% of the children aged 6–59 months had the lower limit of serum retinol, and 42% of stunted children had marginal serum retinol levels [*18*]. Mi et al. reported moderate sub-clinical VAD did exist in some areas in China, and recommended vitamin A supplementation targeting children in those areas [*19*]. Munene et al. used CIC-transfer (CIC-T) to determine the prevalence of VAD among Kenyan children aged 4–7 years: only 23.1% of the children had normal CIC results, indicating that VAD was a significant health problem in Kenyan children [*20*].

A national survey to estimate the prevalence of VAD in Nigeria used CIC and a biochemical method (HPLC). A significant association between CIC and low serum retinol levels was seen [*21*]. Another study in Nigeria evaluated vitamin A status by CIC-T and serum retinol concentration in children aged less than 6 years [*22*]. The results showed that CIC-T is a simple procedure with a failure rate of only 7.3% caused by tearing and agitation. Use of CIC-T for epidemiological surveys of VAD was suggested.

In our study, VAD was more prevalent in girls than boys. This could be related to cultural factors biasing the distribution of food in families. In a study in the South Pacific, VAD was more common among males due to the occurrence of evident clinical infection [*23*]. Another possible reason could be differences in the amounts of carotinoid materials received by males and females. Of course, the amount of vitamin A supplementation in these 2 groups plays a distinct role. In a Thai study, however, the deficiency was higher among boys than girls [*24*].

In the present study, VAD was more common in children under 3 years of age, perhaps a result of irregular use of multivitamin and vitamin A drops during the

breastfeeding period. In this age range, diarrhoea and respiratory infections are also more common. In the Reddy and Reddy study, the deficiency was more common among children of lower ages [*4*], whereas in the Schaumburg et al. study, VAD was seen more commonly in older children [*23*]. This difference could be related to the different methods used for evaluation. Schaumburg assessed the deficiency using xerophthalmia and nyctalopia (night blindness), a method not easily used in younger children; results therefore had a false positive bias. This method is more effective in children of higher ages.

In this study, no difference was seen between rural and urban children. The reason could be a similarity in the populations of the 2 areas and the proximity between the cities and the suburbs and the progress in the suburbs.

Conclusion

The rate of sub-clinical VAD among children aged 2–5 years in this study is classified as moderate [*17*]. It is, therefore, very important to implement programmes enhancing the distribution of medical supplements and the fortification of foods, and to emphasize the significance of educational programmes.

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