Vitamin A status in wheezing Saudi children

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ABSTRACT

الأهداف: تحليل مستويات فيتامين أ لدى الأطفال المصابين بالصفير، ودراسة مدى ارتباط مستويات هذا الفيتامين في مصل الدم بدرجة شدة الصفير.

الطريقة: أُجريت هذه الدراسة الاستطلاعية المستعرضة في جناح الأطفال بمستشفى أحد، المدينة المنورة، المملكة العربية السعودية، وامتدت خلال الفترة من يناير 2009م إلى أغسطس 2010. شملت الدراسة 400 طفلاً سعودياً مصاباً بالصفير وتتراوح أعمارهم ما بين 3–36 شهراً. لقد تم تقسيم المرضى اعتماداً على المسار السريري للمرض إلى 93 طفلاً مصاباً بالصفير المستمر، و307 طفلاً مصاباً بالصفير الحاد، فيما تم تقسيم درجة شدة المرض إلى كلاً من: خفيفة، ومتوسطة، وشديدة.

النتائج: أشارت الدراسة إلى أن نتائج تحليل مصل الدم في مجموعة الأطفال المصابين بالصفير المستمر كانت كالتالي: 9.7% من المرضى كان لديهم نقص في مستويات فيتامين أ، و20.4% منهم كان لديه مستويات حدية من فيتامين أ، فيما كانت النتائج في مجموعة الأطفال المصابين بالصفير الحاد: 1.6% من المرضى كان لديهم نقص في مستويات فيتامين أ، و 8.1% كان لديه مستويات حدية من فيتامين أ. كشفت النتائج بأن مستويات الفيتامين الناقصة والحدية كانت أعلى بكثير في المجموعة المستمرة الصفير مقارنةً بالمجموعة الحادة الصفير ($p<0.05$)، ولقد كان هناك علاقة بين مستويات فيتامين أ ودرجة شدة الصفير، حيث ظهرت المستويات الناقصة من الفيتامين في المجموعات التي تعاني من درجة شديدة من الصفير.

خاتمة: أثبتت الدراسة مدى انتشار مستويات فيتامين أ الناقصة والحدية بين الأطفال السعوديين المصابين بالصفير. ولقد كان هناك علاقة واضحة بين مستويات تركيز الفيتامين من جهة ودرجة شدة الصفير ومساره السريري من جهة أخرى.

Objectives: To examine the serum retinol levels of wheezing children, and to investigate the relation as an biochemical indicator of vitamin A status in wheezing children between serum levels of retinol and severity of wheezing.

Method: A prospective cohort study including 400 wheezing Saudi children aged 3-36 months, who were treated in the pediatrics ward of Ohoud Public Hospital in Al-Madinah, Kingdom of Saudi Arabia between January 2009 and August 2010. Patients were subdivided according to the course of wheeze into 93 patients with persistent wheeze, and 307 patients with acute wheeze. Severity of wheeze was graded mild, moderate, and severe.

Results: Among the persistent wheezing patients 9.7% had deficient, and 20.4% had marginal serum vitamin A concentration, while in the acute wheezing patients, 1.6% had deficient, and 8.1% had marginal serum vitamin A concentrations. Results revealed that the prevalence of deficient and marginal vitamin A concentration is higher in the persistent than in the acute wheezing group ($p<0.05$). Serum vitamin A was related to wheezing severity. Deficient serum vitamin A was found only in severe wheezing groups.

Conclusion: Wheezing Saudi infants had prevalent deficiency and marginal deficiency of vitamin A. Serum vitamin A concentrations were related to the wheezing severity and course.

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Research has demonstrated that in addition to affecting the visual cycle, vitamin A deficiency (VAD) is directly linked to reproduction, fetal development, the immune system, and to regulation of cell proliferation and differentiation.[1] The VAD interferes with immunity and with the respiratory epithelium differentiation

and maintenance. It may lead to squamous metaplasia with subsequent loss of defence mechanisms against microbial invasion and to the development of obstructive phenomena caused by bronchial hyper-responsiveness.[2] The majority of nationally representative large-scale surveys related to VAD were conducted primarily among preschool-age children; this is mainly because this age group is the most commonly recognized to be at risk of this nutritional deficiency and its adverse health consequences.[3] An updated estimate for the global prevalence of VAD in children appears in the 2002 West report,[4] which indicated that worldwide, approximately 21% of all children have serum retinol concentrations <0.70 µmol/l. South-East Asian and African Regions had the highest prevalence rates and the largest number of affected children.[4] The WHO reported that VAD was of public health significance in 60 countries, and was likely to be a problem in an additional 13 countries.[5] The current estimates reflect the time period between 1995 and 2005, and indicate that 45 countries have vitamin A deficiency of public health significance based on the prevalence of night blindness and 122 countries have vitamin A deficiency of public health significance based on the prevalence of biochemical vitamin A deficiency (serum retinol concentration <0.70 µmol/l), in preschool-age children.[5] Serum retinol is usually assessed by high-performance liquid chromatography (HPLC) or spectrophotometry.[6] Although spectrophotometry is much simpler and less costly, it is also much less accurate; therefore, HPLC analysis is preferred. Although many other biochemical indicators of vitamin A status can be assessed,[7] serum retinol is the preferred indicator for population level assessment of VAD because many laboratories can analyze it, and it is the best-established biochemical indicator of vitamin A status.[8] The ability of immune cells to fight infection could be reduced by VAD, with subsequent increase in the risk of respiratory and digestive diseases in children.[2] One of the common symptoms and signs of VAD is infant wheezing.[3] Cough and breathing difficulty may occur in association with VAD, and are a common cause for seeking medical consultation by the parents.[9] Many conditions such as wheezy bronchitis, infantile asthma, viral induced wheezing, and bronchiolitis may be presented by wheezing. Other causes of wheezing include viral infection, immature development of the lung, and other uncertain factors. The development, maintenance, differentiation, and regeneration of lung epithelial cells are all influenced by vitamin A, which may play a central role in the development of airway diseases.[10] The VAD has been reported to increase exacerbation of asthma as a result of increased responsiveness of the respiratory tract.[2]

Human airway smooth muscle cell migration in response to platelet-derived growth factor, and platelet-derived growth factor that induced actin reorganization associated with migration can be inhibited by all-trans retinoic acid. Promotion of bronchial hyperactivity in rats by altering muscarinic M2 receptor function is one of the reported consequences of VAD.[11-13] The inhibition of smooth muscle cells migration and actin reorganization which are histological changes accompanying human air ways hypersensitivity were inhibited by all trans-retinoic acid derivatives supports their role of VAD in the pathophysiology of wheeze.[4]

There are a limited number of studies regarding vitamin A status in the Kingdom of Saudi Arabia. The current study was carried out to examine the serum retinol level in wheezing children, and to investigate the relation between serum levels of retinol and severity and course of wheezing.

Methods. *Patients.* This prospective cohort study included 400 Saudi infants and children with wheezing, aged between 3 and 36 months, who had been admitted as inpatients to the pediatrics ward of Ohoud Public Hospital in Al-Madinah, Kingdom of Saudi Arabia between January 2009 and August 2010. Infants and children with chronic diseases, such as kidney, liver, heart, lung, and blood diseases, and acquired immunodeficiency syndrome, were not included in the study. Neither were those who received vitamin A in drug form before hospital admission, and those who needed transfusion of blood, or blood products. The clinical score adapted from Zheng et al[14] was used to evaluate infants and toddlers on admission, and a score of 4 or more on a scale of 0-12 was required for eligibility as a study participant. A score of 0 was given for respiratory rate (breaths/min) <30, no wheezing, no retraction, and normal general condition. A score of one was given for respiratory rate (breaths/min) of 30-45, wheezing on terminal expiration or only with stethoscope, and intercostal retraction only. A score of 2 was given for respiratory rate (breaths/min) 46-60, wheezing during entire expiration or audible on expiration without stethoscope, and tracheosternal retraction. A score of 3 was given for respiratory rate (breaths/min) >60, wheezing during inspiration and expiration without stethoscope; severe retraction with nasal flow; and general condition irritable/lethargic, poor feeding.

Patients who scored 0-4.9 points were considered to have mild wheeze, those who scored 5-8.99 points were considered to have moderate wheeze, and patients who scored 9-12 points were considered to have severe wheeze. Eighty-four patients suffered from severe wheezing (oxygen saturation <94% in room air or significant respiratory distress with retraction, nasal

flaring, or requiring admission to intensive care), and 316 mild to moderate wheezing. Persistent wheezing (wheezing lasted for 4 or more weeks) presented in 93 patients and acute wheezing in 307 patients. All clinical exams were assessed by the same authors (pediatricians). Duration of hospitalization was calculated as the time between the admission of the infant and actual discharge time.

Biochemical measurement of serum retinol. Five ml of blood was drawn by venipuncture, in light-protected tubes, centrifuged (1,600 Xg, 10 minutes, 16°C) and serum was separated and stored in the dark at -20°C. Biochemical analysis for the determination of serum retinol levels was conducted by HPLC using a method that has previously described.[15] The recommendations of the WHO and expert committee reports was used to assess the prevalence of VAD. Serum retinol concentrations are classified as normal ≥0.70 µmol/L, marginal 0.35-0.70 µmol/L, and deficient <0.35 µmol/L.[16]

Ethical consideration. The Ethics and Human Research Committees of Ohoud Hospital approved the study. This study was conducted within the frame of the collaboration program between the University and the Ministry of Health. Informative written consent was obtained from parents of all children. Brief counselling regarding vitamin A deficiency, together with clarification of the aim and method (regarding blood sampling) were given.

Statistical analysis. The Statistical Package For Social Sciences 13 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Data were expressed as means±SD and percentage. To compare serum retinol, in the categorized form, with the clinical and epidemiological variables, we used the chi-square test. A $p<0.05$ was considered statistically significant. The 95% confidence interval (CI) was also calculated.

Results. The age of the children ranged from 3-36 months, with a mean of 10.9±6.9 months. One hundred eighty-four (46%) boys, 216 (54%) girls were enrolled in the study. The mean serum retinol concentration of the whole cohort was 0.97±0.52 µmol/L. Serum retinol concentrations were normal (≥0.70 µmol/L) in 342 (85.5%) patients, and marginal concentrations 0.35-0.70 µmol/L were found in 44 patients (11%), while 14 (3.5%) patients had serum retinol concentrations <0.35 µmol/L. Thus, the prevalence of serum retinol concentrations <0.70 µmol/L was 14.5%.

No statistical significance was found in mean serum retinol concentrations ($p=0.723$) or in prevalence of marginal and deficient levels of vitamin A ($p=0.917$) among different age groups.

There was a close relation between serum retinol concentrations and severity and course of wheezing. In the 93 patients with persistent wheezing, 65 (69.9%) had normal serum retinol concentration (≥0.70 µmol/L), while serum retinol concentrations of 0.35-0.70 µmol/L were found in 19 (20.4%) and <0.35 µmol/L were found in 9 (9.7%) patients. Among the 307 patients with acute wheezing, 277 (90.2%) had normal serum vitamin A concentrations, 25 (8.1%) had marginal, and 5 (1.6%) had deficient serum vitamin A concentrations (**Figure 1**).

Among the patients with persistent wheezing, 24 patients (25.8%) were diagnosed as having severe wheezing, 42 (45.2%) having moderate wheezing, and 25 patients (25%) having mild wheezing. In the acute wheeze group, 41 patients (13.4%) were diagnosed with severe wheeze, 114 patients (37.1%) with moderate

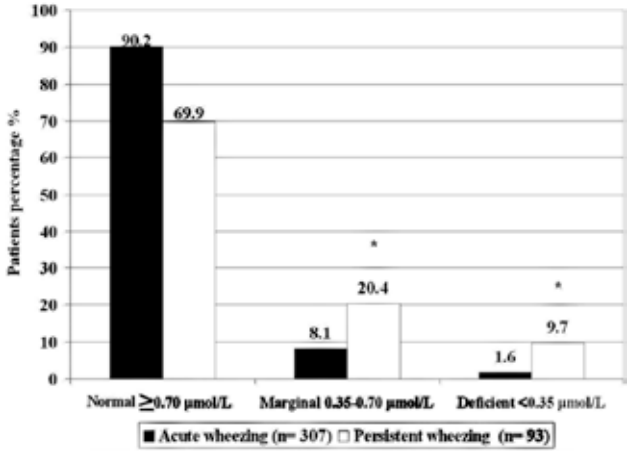

Figure 1 - A comparison of serum retinal levels among Saudi patients aged 3-36 months with acute and persistent wheeze. *$p=0.0427$

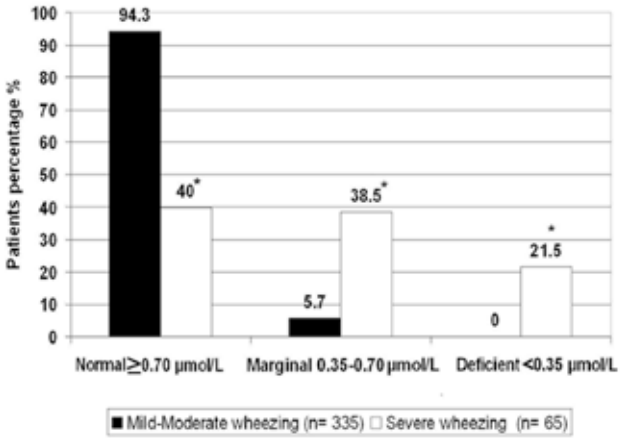

Figure 2 - A comparison of serum retinal levels among Saudi patients aged 3-36 months with mild-moderate and severe wheeze. *$p=0.0399$

wheeze, and 152 (49.5%) with mild wheeze. Normal serum retinol concentration (≥0.70) was found in 26 (40%) of the 65 patients with severe wheeze, and 316 (94.3%) of the 335 patients with mild to moderate wheeze.

Marginal serum retinol concentrations of (0.35-0.70 µmol/L) was found in 25 (38.5%) of the 65 patients with severe wheeze. while in the 335 mild to moderate wheezing patients, only 19 patients (5.7%) had serum retinol concentrations of (0.35-0.70 µmol/L). Thus, marginal levels of vitamin A were significantly higher in the severe wheezing group than in the mild to moderate wheezing group (p=0.039). Deficient serum retinol concentrations (<0.35 µmol/L) were found in 14 (21.5%) of the 65 patients with severe wheezing, while not found in any of 335 patients with mild to moderate wheezing (**Figure 2**).

Discussion. Severe VAD is rare in most populations; however, preschool children with marginal levels of vitamin A still can show adverse health effects together with increased mortality and morbidity rates even at subclinical concentrations.[17] Values lower than 0.7 µmol/l was recommended by the WHO as a cut-off point for the identification of retinol deficiency. However, marginal involvement of liver stores in the absence of classic manifestations of VAD still can be seen at levels below 1.05 µmol/l. However, immune system and tissue repair disorders, which affect mainly the respiratory epithelium, can still occur.[2, 17]

Al-Saleh et al[18] in a cross-sectional survey to measure retinol in 513 Saudi children 3-16 years old attending the Primary Health Care Units in Al-Kharj district due to various mild health problems reported that mean serum vitamin A level was 1.26 ±0.47 µmol/L. None of the children in this study had a severe all-trans-retinol deficiency (<10 microg/dl) and the percentage of children with marginal deficiency <20 microg/dl was 5.5. Our results showed that the prevalence of marginal concentrations of vitamin A (0.35-0.70 µmol/L) was as high as 11% (44/400), and the prevalence of <0.35 µmol/L serum concentrations of vitamin A was 3.5% (14 of 400) in patients with wheezing, which was higher than that in normal children studied by Al-Saleh et al.[18] Our findings also indicate that mean serum vitamin A level (0.98 ± 0.52 µmol/L) was even lower when patients suffered from wheezing. Preschool children were the target population for most national wide scale studies as they are at greater risk for VAD than infants.[4] The findings of the current study could be of special importance as it showed the frequency of low serum retinol levels among a younger age group, since the median age of patients was 18 months.

The VAD remains an important problem in the developing world. In the age group from 0-4 years approximately 0.25 billion children suffer from nutritional diseases of vitamin A in the world annually.[4] An inverse relation between the prevalence of VAD and age was reported by Hatun et al[19] who stated that, with increased age of children the prevalence of VAD decreased while the severity of deficiency increased as the age decreased. However, our results did not show the same correlation between serum vitamin A levels and the age of wheezing infants. Our study revealed that the course of wheezing was closely related to the serum vitamin A concentrations. We also found that the vitamin A concentrations (<0.35µmol/L) were only found in patients with severe wheezing while not found in the non-severe group, indicating that the level of vitamin A might be associated with the severity of wheezing. No definitive criteria exist for determining with certainty whether or not critical wheezing is causally related to severe VAD or whether severe VAD causes critical wheezing.[17] We agree with cited authors[14] who supposed that maintenance of the integrity of tracheobronchial epithelium in patients with persistent wheezing required more vitamin A than in patients with acute wheezing. While the intake of vitamin A during the course of disease decreases, the level of retinol binding protein decreases during the inflammation period. Significant decrease in serum vitamin A in patients with persistent wheezing could be attributed to the free oxygen radicals as a result of inflammation accompanied with increased consumption of antioxidant. Low vitamin A status may result in greater bacterial adherence and colonization and, therefore, may increase the risk of bacterial infection and prolong the disease course.[14] The renovation ability of the immune system and respiratory tract epithelium might be damaged in a serum vitamin A level of 1.05 µmol/L or less.[11,20] Low circulating vitamin A levels are associated with an increased risk of epithelial damage in the eye. Impaired gut integrity is common in malnutrition. Damage to the integrity of epithelia and mucosal barriers facilitates translocation of microorganisms and contributes to the increased severity of infections interaction between these 2 factors may lead to prolongation of wheezing with subsequent decrease in vitamin A levels.[14]

One of the limitations of this study was the absence of a control group. Therefore, the model proposed here does not allow determining whether the deficient and marginal serum levels observed in 14.5% of the children resulted from the depletion caused by the infectious process or other causes of wheezing, or whether they already existed and were then predisposed to wheezing.

In short, the current study shows that there is a high prevalence of marginal and deficient concentrations of

vitamin A (<0.70 µmol/L) in wheezing patients in the Kingdom of Saudi Arabia, which does not vary with ages. The degree of VAD was related to the course and severity of wheezing. Future studies to test the effect of administration of therapeutic levels of vitamin A to the high-risk age groups on the prevalence and severity of wheezing are highly recommended.

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Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.