ABSTRACT
Background: Plasma retinol concentrations are depressed by infection but are commonly used to assess vitamin A status. Objective: We measured 2 acute phase proteins, α₁-antichymotrypsin (ACT) and α₁-acid glycoprotein (AGP), to determine whether they could be used to assist in interpreting vitamin A status. Design: In 1997, a 2-stage cluster-sampling procedure was used to select 3074 apparently healthy, 6–60-mo-old children from rural and urban areas of North West Frontier Province, Pakistan. Plasma retinol, ACT, AGP, and ferritin measurements and anthropometric measurements were obtained for 2519 children. Results: Median plasma retinol, ACT, AGP, and ferritin concentrations were 0.86 µmol/L, 0.39 g/L, 1.14 g/L, and 5.5 µg/L, respectively. There were no significant (P > 0.05) differences in retinol, ACT, or AGP by sex or age. Some 797 children (32%) had retinol concentrations <0.7 µmol/L and 87 (4%) had retinol concentrations <0.35 µmol/L; 274 children (11%) had elevated ACT (>0.6 g/L) and 1141 (45%) had elevated AGP (>1.2 g/L). Retinol concentration correlated with ACT (r = 0.35; P < 0.001), but stepwise multiple regression indicated that these 3 variables made a minimal although quantifiable contribution to the variance of retinol (ACT, r² = 0.02; all 3 variables, r² = 0.03). Conclusions: The transient depression in plasma retinol produced by subclinical infection increased the number of at-risk children by 10% (76 of 797) and 56% (49 of 87) for plasma retinol concentrations <0.7 and <0.35 µmol/L, respectively. In addition, dietary inadequacy may be responsible for retinol concentrations being 16% lower in Pakistani children than in children in the United Kingdom, where dietary vitamin A is adequate. Am J Clin Nutr 2000;72:1164–9.

KEY WORDS Acute phase proteins, α₁-antichymotrypsin, α₁-acid glycoprotein, ferritin, vitamin A status, plasma retinol, children, Pakistan, United Kingdom

INTRODUCTION
It is estimated that there are 254 million preschool children in the world who are at risk of vitamin A deficiency; 50% of these children are from Southeast Asia (1). In addition, vitamin A deficiency worldwide may be responsible for 1–2.5 million deaths of young children annually and may cause blindness in >500000 children (2, 3). These estimates of vitamin A status are based predominantly on dietary or biochemical data, particularly plasma retinol, but both are difficult to interpret because the bioavailability of provitamin A carotenoids is in doubt (4) and plasma retinol concentrations are depressed by concurrent disease (5, 6). The interpretation of other nutrient concentrations is also made more difficult by disease (7, 8), but it has been suggested that acute phase proteins might be used to correct biochemical markers of vitamin A (9) and iron status (10).

The acute phase response is the body’s immediate reaction to infection and inflammation, and changes in the synthesis of acute phase proteins are to maintain body homeostasis and avert tissue damage. C-reactive protein (CRP) is probably the most commonly used and sensitive acute phase protein for monitoring infection and inflammatory activity and is particularly useful in assessing bacterial and connective tissue diseases (11, 12). However, CRP concentration may not remain abnormal long enough to detect patients in early convalescence (12) and thus is unlikely to be useful in nutritional studies of apparently healthy subjects. Other acute phase proteins remain elevated for longer than does CRP and may therefore be more useful for detecting children in whom infection has subsided but whose retinol concentrations may still be reduced. α₁-Antichymotrypsin (ACT; 11) and α₁-acid glycoprotein (AGP; 11, 13) are 2 such proteins. The response time for ACT is similar to that for CRP (ie, 6–10 h), whereas the response time for AGP is 24 h. These proteins remain elevated for longer than CRP does because AGP may be needed postinfection for immunomodulatory effects and ACT for the synthesis of new tissue (11). Reports from Bangladesh (14), the Gambia (15), and central Africa (16) suggest that these proteins may be useful indicators of subclinical, chronic, or recent ill health.

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Because poor vitamin A status and infections coexist in children of less developed countries, it is difficult to establish whether low plasma retinol concentrations are due to nutritional or to pathologic causes. The differentiation is important for health professionals, policymakers, and program managers as they devise more cost-effective strategies to reduce vitamin A deficiency. In the study reported in this article, AGP and ACT measurements were used to assess subclinical infection in the population and the association between subclinical infection and plasma retinol. Plasma ferritin is also influenced by infection and the concentrations are included in the analysis of the vitamin A data. The relation of plasma ferritin to iron status will be reported separately. A short report on this study was presented at the XIX International Vitamin A Consultative Group Meeting (17).

SUBJECTS AND METHODS

A study was carried out to assess the vitamin A, iron, and zinc status of 6–60-mo-old children in North West Frontier Province (NWFP), Pakistan. Ethical approval for the study was obtained from the Health Department of NWFP. A 2-stage cluster-sampling procedure was followed to select 3074 apparently healthy children from 2193 households in both the urban and rural communities of NWFP. However, in this article only the relation between vitamin A status and acute phase proteins, for which 2519 children had complete biochemical and anthropometric data, is examined.

The households in the selected clusters were visited by the nutrition officers and health workers of the survey teams to identify children aged 6–60 mo. Children were excluded if they were malnourished [<60% of weight-for-age according to the National Center for Health Statistics reference standards (18)]; if they had symptoms of disease, such as runny nose, fever, or diarrhea; or if they had acute or chronic infections such as pneumonia, typhoid, or malaria. Such symptoms and infections were assessed both by history from the caregiver and by observation during the clinical assessment and interview. Informed consent was obtained from each child’s parents. Confirmation of a child’s age was made with the mother with the help of a local-events calendar followed by discussion of the child’s feeding practices and frequency of food consumption, and completion of demographic and socioeconomic questionnaires. Weight and height measurements of the children were taken according to the recommended procedures of the World Health Organization (WHO; 19) and were followed by an eye examination for clinical signs of vitamin A deficiency performed by ophthalmic technicians. The WHO (20) clinical classification of vitamin A deficiency was used to identify children with clinical signs of vitamin A deficiency and the results were recorded on the questionnaire.

A 5-mL blood sample was collected from each child in 4 different disposable EDTA-containing evacuated tubes (Becton Dickinson, Franklin Lakes, NJ) with use of disposable single-use needles (Sherwood & Co, Sussex, United Kingdom). Hemoglobin concentration was determined spectrophotometrically by using a Hemocue device (21, 22) in the field; the accuracy and precision of the method were checked by analyzing a pooled plasma sample (<span data-bbox="84 140 89 145">n</span> = 150) with each batch and by using a human serum standard reference material (SRM 968b) from the National Institute of Standards and Technology (Gaithersburg, MD). The mean (±SD) plasma retinol concentrations from 5 determinations of reference materials with low, medium, and high concentrations were 1.03 ± 0.04, 1.79 ± 0.05, and 3.12 ± 0.16 μmol/L compared with certified concentrations of 1.04 ± 0.05, 1.80 ± 0.06, and 3.12 ± 0.31 μmol/L, respectively.

Plasma ferritin was determined with an Immunulite Automated Analyser (24) by a chemiluminescent enzyme immunometric assay at Aga Khan University, Karachi. The accuracy and precision of the method were checked by analyzing pooled plasma samples and controls (low, medium, and high). ACT and AGP were measured on a Cobas Fara analyzer (Roche Products, Welyn, United Kingdom) with use of immunoturbidimetric techniques (25) at the Northern Ireland Centre for Diet and Health, University of Ulster, Northern Ireland. The accuracy of the method was monitored in each batch by including control samples of low and high concentrations. Interbatch precision with use of these controls was <8%.

The prevalences of micronutrient deficiencies (ie, vitamin A and iron) were assessed according to the recommendations of WHO and expert committee reports. Children with plasma retinol concentrations <0.35 μmol/L (<10 μg/dL) and <0.70 μmol/L (<20 μg/dL) were characterized as having vitamin A deficient or having low vitamin A status, respectively (26), and children with plasma ferritin concentrations <12 μg/L were characterized as iron deficient (27).

The EPI-INFO (28) and SAS software programs (29) were used to prepare descriptive statistics, clean the data, and check for skewness. Plasma retinol, ferritin, ACT, and AGP data were skewed; therefore, nonparametric statistics were used for comparative analyses at a 5% level of significance, and median and ranges were computed. Analysis of the differences in plasma retinol between the groups with different acute phase protein statuses was done by using analysis of variance (ANOVA) followed by a Scheffe test on both log-transformed and arithmetic data. Differences between the groups were identical by either method of analysis. Medians and ranges are reported for consistency. Stepwise multiple regression analysis was performed by using log-transformed data to determine the variance contributed by different variables to plasma retinol and to determine Pearson product-moment correlation coefficients (30).

RESULTS

The median plasma retinol concentration was 0.86 μmol/L and there was no significant difference between boys and girls (Table 1). The prevalence of low vitamin A concentration (<0.7 μmol/L) was 32% in both boys and girls. However, the girls had a significantly higher median plasma ferritin concentration (6.1 μg/L) than did the boys (5.0 μg/L) and the prevalence of iron deficiency (ferritin < 12 μg/L) was 66% in girls and 71% in boys (NS). The median ACT and AGP concentrations were 0.39 and 1.14 g/L, respectively, and there were no significant differences between boys and girls. When elevated ACT
(>0.6 g/L) and AGP (>1.2 g/L) concentrations were used as markers of infection, 11% and 45%, respectively, of the children were identified as having elevated concentrations. The median z scores for weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ) of the children were −1.68, −1.57, and −0.89, respectively. The boys had significantly lower (poorer) z scores than did the girls (Table 1).

Median plasma retinol, ferritin, ACT, and AGP concentrations in children aged <24 mo and ≥24 mo were not significantly different. Similarly, there were no significant differences in the prevalence of children with low plasma retinol or markers of infection between these 2 age groups. However, both the WAZ and WHZ of the children aged <24 mo were significantly lower than those of the children aged ≥24 mo, but the HAZ of the younger group was significantly higher than that of the older group (Table 2). There were no significant differences in z scores between children with normal or elevated acute phase proteins (Table 3).

Information about age, retinol, and ferritin in the children classified according to whether they had normal or elevated ACT or AGP concentrations is also shown in Table 3. Plasma retinol was significantly higher in children with normal ACT and AGP concentrations. Furthermore, ferritin was significantly lower in the children with normal ACT, but the difference was not significant in those with normal AGP.

The relation between plasma retinol and elevated ACT and AGP concentrations is explored further in Table 4. Children with a current subclinical infection, ie, elevated ACT (with or without elevated AGP) had lower retinol concentrations than did children with elevated ACT only or no elevated acute phase proteins (P < 0.001; ANOVA). In addition, the prevalence of low retinol concentrations (<0.7 μmol/L) was significantly higher in the children with elevated ACT (with or without elevated AGP) than in the children with elevated AGP only or without elevated concentrations.

The strong association (r = 0.74) between ACT and AGP (Table 5) indicated that both are measuring the same thing, namely infection. Furthermore, correlation coefficients between logarithms of plasma retinol, ACT, and AGP showed that plasma retinol was significantly associated with both ACT and AGP. Stepwise multiple regression analysis showed that ACT explained most of the variance (r² = 0.02) in plasma retinol compared with AGP and ferritin; however, the total variance that could be accounted for by the 3 independent variables (ACT, AGP, and ferritin) was small (r² = 0.03) (Table 6). The correlation coefficients between logarithms of plasma ferritin, ACT, and AGP showed that plasma ferritin was slightly more strongly associated with ACT than with AGP. These weak associations indicated that the acute phase proteins have only minimal, but nevertheless quantifiable, effects on plasma retinol and that ferritin is probably more strongly associated with iron status than with infection.

**DISCUSSION**

Infection in children aged <5 y is common in Pakistan and it is estimated that diarrhea and acute respiratory infection cause 500000 deaths/y (31). Poor health and nutritional status of women and unhealthy living environments are the leading causes of early childhood morbidity and mortality (31). The children selected in this study were apparently healthy, yet 32% had low plasma retinol concentrations (<0.7 μmol/L) and 4% were biochemically vitamin A deficient (<0.35 μmol/L). The observed prevalence rates of vitamin A deficiency were similar to those found in previous surveys (32, 33) conducted in different areas of Pakistan, where 13–47% of the children had low plasma retinol (<0.7 μmol/L) and 2% were vitamin A deficient (<0.35 μmol/L).

Low plasma retinol may be partly responsible for increased childhood morbidity due to impaired immune function. However, it is important to know whether low plasma retinol is due to infection,
and thus is transient, or to dietary deficiency, and therefore is potentially more permanent. Elevated ACT and AGP are both used as markers of infection (11). In this study, elevated ACT identified 247 children (11%) and elevated AGP identified 1141 children (45%) who showed evidence of subclinical infection. After the onset of infection, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure, ACT, CRP, AGP, and serum amyloid A (SAA) all behave similarly, with an initial rise at the time of exposure.

\[ \text{ACT, } \alpha_1\text{-antichymotrypsin; AGP, } \alpha_1\text{-acid glycoprotein.} \]

\[ ^1 \text{Significantly different from children with ACT } \leq 0.6 \text{ g/L, P } < 0.05. \]

\[ ^2 \text{Median; range in parentheses.} \]

\[ ^3 \text{Significantly different from children with AGP } \leq 1.2 \text{ g/L, P } < 0.05. \]

\[ ^4 n/\% \text{ percentage in parentheses.} \]

\[ ^5 \text{Values within the column with different superscript letters are significantly different, } P < 0.05 \text{ (ANOVA and Scheffe’s test).} \]

\[ ^6 \text{Hypothetical state of subclinical infection based on presence or absence of elevated ACT (>0.6 g/L) and AGP (>1.2 g/L).} \]

\[ ^7 \text{Significant difference between groups, } P < 0.01 \text{ (chi-square test).} \]

\[ ^8 \text{Hypothetical state of subclinical infection based on presence or absence of elevated ACT (>0.6 g/L) and AGP (>1.2 g/L).} \]

\[ ^9 \text{Significant difference between groups, } P < 0.01 \text{ (chi-square test).} \]
elevated AGP alone than in those with no elevated acute phase protein concentrations \((P < 0.05)\). In addition, there was both a higher proportion of low retinol concentrations and lower median retinol concentrations in most of the children with elevated ACT (groups 1 and 2, Table 4), suggesting that elevated ACT is associated with that stage of the acute phase response when plasma retinol is more depressed than when only AGP is elevated.

The information in Table 4 was used to calculate the effect of infection on plasma retinol in the Pakistani children. The difference in median retinol concentrations between groups 2 and 3, ie, those with elevated ACT and AGP (0.73) and those with elevated AGP only (0.83), was 0.10 \(\mu\)mol/L, and there is a further difference of 0.07 \(\mu\)mol/L between those in groups 3 and 4 (Table 4). Thus, by rounding off these differences to 0.2 and 0.1 \(\mu\)mol/L, the current criteria used to assess threshold plasma retinol concentrations for low and deficient vitamin A status (ie, 0.7 and 0.6, or 0.5 \(\mu\)mol/L, as appropriate) that can be attributed to a dietary deficiency of vitamin A is reduced from 797 to 721 (10%, or 76 of 797) and the proportion of those with deficient plasma retinol is reduced from 87 to 38 (56%, or 49 of 87). That is, although poor diet appears to be the main contributor to low plasma retinol (28%, or 721 of 2519), infection is responsible in more than one-half of children at high risk of vitamin A deficiency. However, it is important to realize that, irrespective of the cause of the depressed plasma retinol (ie, infection or poor diet), a low plasma retinol concentration plays a major role in determining functional vitamin A status.

Plasma retinol is tightly controlled by homeostatic mechanisms. However, it may be possible to assess the influence of dietary vitamin A deficiency on plasma retinol in Pakistani children by comparing the data with results from the British Preschool Survey, in which vitamin A deficiency was not likely to be present (36). Only the acute phase protein ACT was measured, but because the children were apparently healthy, most of them, like those in the British Preschool Survey, would probably also have had an elevated AGP concentration. Mean (±SD) plasma retinol concentrations in British children with normal \((n = 624)\) and elevated \((n = 129)\) ACT concentrations were 1.04 ± 0.28 and 0.91 ± 0.28 \(\mu\)mol/L \((P < 0.001)\), respectively. Thus, there were similar differences in plasma retinol between those with normal and those with abnormal ACT values in Pakistani \((0.17 \mu\)mol/L) and British \((0.13 \mu\)mol/L) children. Furthermore, plasma retinol in Pakistani children was lower than that in British children by 13% and 19% (\(\bar{x}: 16\%\)) in those with normal and elevated ACT, respectively, and this may represent the effects of dietary vitamin A insufficiency in Pakistan. However, the difference in dietary intake of vitamin A between children in the 2 countries may be far larger than 16% because plasma retinol is under homeostatic control.

The adjustments to plasma retinol cutoff values suggested above are probably appropriate only for an apparently healthy group of children. In children who are clinically sick, the depression of serum retinol appears to be much greater. Hospitalized children with shigellosis were recently reported to have mean (±SD) serum retinol concentrations of 0.36 ± 0.22 \(\mu\)mol/L on admission and 1.15 ± 0.5 \(\mu\)mol/L on discharge (37). The children did not receive any vitamin A supplements during treatment but were, of course, fed, and the food eaten may have contributed to the discharge plasma retinol concentration. However, during the clinical phase of disease, children may lose a considerable amount of vitamin A in urine. Urinary losses of vitamin A are associated with fever and the severity of the infection (38). The depressed plasma retinol concentration on admission represents the depression due to disease plus the added drain of urinary losses. Children who are apparently healthy do not have an elevated temperature or loss of vitamin A in the urine.

The transient decrease of plasma retinol in response to infection and its return to normal concentrations on recovery was reported by several researchers (6, 9, 35, 39). The decrease was ascribed mainly to the acute inflammatory response that results in decreased synthesis of retinol binding protein and transthyretin and increased excretion of urinary retinol, independent of the body’s vitamin A stores (37). The results obtained for the Pakistani children show a relatively weak correlation between retinol and both ACT \((r = –0.14)\) and AGP \((r = –0.14)\), whereas those reported by others—for Ghanaian children, for example (9)—were stronger for both AGP \((r = –0.35)\) and SAA \((r = –0.20)\). Undoubtedly, differences in environmental factors, sickness, and measurement method will all contribute to these differences and will have to be properly standardized if acute phase proteins are to be used to correct plasma retinol and improve its usefulness as a marker of vitamin A status.

The results of this study suggest that subclinical infection is widely prevalent in apparently healthy preschool-age Pakistani children. A large proportion of the children also showed evidence of low vitamin A status, but when the data were adjusted to take into account the depression of plasma retinol caused by the presence of subclinical infection or dietary deficiency, infection had its greatest effect on children with biochemically deficient retinol concentrations (<0.35 \(\mu\)mol/L), whereas poor diet may have been responsible for a 16% (13–19%) deficit in overall plasma retinol concentrations. It is important to be able to correctly quantify the extent of vitamin A deficiency in less

### Table 5

<table>
<thead>
<tr>
<th>Retinol</th>
<th>ACT</th>
<th>AGP</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.141</td>
<td>-0.138</td>
<td>-0.09</td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
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<td></td>
</tr>
</tbody>
</table>

1 Log values were used for the correlation analysis. ACT, α\(_1\)-antichymotrypsin; AGP, α\(_1\) acid glycoprotein. P < 0.0001 for all correlations.

### Table 6

Stepwise multiple regression to predict plasma retinol in the 2519 children

<table>
<thead>
<tr>
<th>Variable</th>
<th>β ± SE</th>
<th>Partial (R^2)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.23988 ± 0.0598</td>
<td>—</td>
<td>0.0001</td>
</tr>
<tr>
<td>ACT</td>
<td>-0.09026 ± 0.0343</td>
<td>0.0198</td>
<td>0.0001</td>
</tr>
<tr>
<td>AGP</td>
<td>-0.06958 ± 0.0263</td>
<td>0.0028</td>
<td>0.0076</td>
</tr>
<tr>
<td>Ferritin</td>
<td>-0.02977 ± 0.0072</td>
<td>0.0061</td>
<td>0.0001</td>
</tr>
<tr>
<td>Height-for-age</td>
<td>0.01871 ± 0.0088</td>
<td>0.0014</td>
<td>0.0347</td>
</tr>
</tbody>
</table>

1 Log values were used for the analysis. The model included the following independent variables: age, weight-for-age \(z\) score, weight-for-height \(z\) score, height-for-age \(z\) score, ACT, AGP, and ferritin. ACT, α\(_1\)-antichymotrypsin; AGP, α\(_1\) acid glycoprotein.
developed countries to correctly target intervention strategies. This study provides basic information to allied health professionals, planners, and policymakers and, we hope, will encourage them to find better ways of combating both infection and vitamin A deficiency, which go side by side in less developed countries such as Pakistan.

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