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Evaluation of plasma retinol-binding protein as a surrogate measure for plasma retinol concentrations

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Plasma retinol-binding protein (RBP) concentrations have been suggested as surrogate indicators for plasma retinol concentrations in the assessment of vitamin A status in less technologically developed settings. Plasma RBP was measured by radial immunodiffusion and plasma retinol by high performance liquid chromatography in a cross-sectional study of 900 pregnant women at the Queen Elizabeth Central Hospital, Blantyre, Malawi. The Spearman correlation coefficient between plasma RBP and retinol concentrations was 0.95 ($p<0.0001$). By linear regression, 0.70 μmol l^{-1} retinol was equivalent to 21.1 mg l^{-1} RBP. With these cut-off points for defining vitamin A deficiency, there was high concordance between categorical descriptions of deficiency using retinol and RBP by chi-square analysis ($p<0.001$). Measurement of plasma RBP by radial immunodiffusion is simple, inexpensive, and does not require expensive instrumentation. Plasma RBP concentrations measured by radial immunodiffusion are highly correlated with plasma retinol and can be used as a simple surrogate measure for vitamin A concentrations in large field studies.

Key words: Retinol; retinol-binding protein; vitamin A deficiency

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INTRODUCTION

Vitamin A deficiency is a major cause of morbidity and mortality worldwide [1], and methods are needed for the simple and rapid assessment of vitamin A deficiency in field studies. In the evaluation of vitamin A deficiency in populations, one commonly used method of assessment is to measure serum or plasma retinol concentrations using high performance liquid chromatography [2]. Measurement of retinol concentrations by high performance liquid chromatography, however, requires expensive instrumentation and may not be suitable for fieldwork. In settings with limited resources and technical support, alternatives may be sought for the assessment of vitamin A deficiency.

It has been proposed that serum or plasma retinol-binding protein (RBP) can be used as a surrogate indicator for serum or plasma retinol concentrations [3]. RBP is a low molecular weight protein (21 kD) which is synthesized by the liver and occurs in serum or plasma in a complex with transthyretin (61–73 kD) and retinol [4]. Because RBP occurs in a 1:1 molar complex with retinol, the concentrations of RBP alone may reflect the concentrations of retinol in plasma. The affinity of RBP for transthyretin decreases as retinol is taken up by target tissues, and apo-RBP is freely filtered in the glomerulus [4]. High correlation has been noted between plasma concentrations of RBP and plasma retinol [5], but factors which may affect the complex binding to transthyretin and retinol include the acute phase response [6], protein energy malnutrition [7], liver disease [8], and chronic renal failure [9]. We examined the hypothesis that plasma RBP concentrations, as measured by radial immunodiffusion, can be used as a surrogate indicator for plasma retinol concentrations in a large field study.

MATERIALS AND METHODS

Subjects

The study population consisted of pregnant women from 18 to 28 weeks' gestation who were seen and enrolled in the antenatal clinic of the Queen Elizabeth Central Hospital in Blantyre, Malawi, from November 1995 through December 1996. The Queen Elizabeth Central Hospital is the main hospital for Blantyre, a city of approximately 300,000 inhabitants. The design was a cross-sectional study, and the study of RBP and retinol concentrations was conducted within the context of a larger study of micronutrient status in pregnant women with and without HIV infection. Written, informed consent was obtained from all study participants. The study protocol was approved by four different independent ethical review committees: the Johns Hopkins School of Medicine, Baltimore, Maryland, USA; the Malawi Health Sciences Research Committee, Government of Malawi; the National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; the Ministry of Health and Population, Government of Malawi. Final approval was given by the Office for Protection from Research Risk, National Institutes of Health, Bethesda, Maryland, USA.

During the period of enrollment, 3209 of 3949 (81.2%) of women from 18 to 28 weeks of gestation in the antenatal clinic requested HIV testing, and of the women who were tested, 985 (30.6%) were HIV-positive. 697 of 985 HIV-infected women enrolled in the study. Simple random sampling was used to enroll 203 HIV-negative women from the 2224 HIV-negative women identified by HIV testing. In total, 900 women were enrolled in the study, and the mean age (SD) of the participants was 23.8 (4.9) years. At enrollment, the mean body mass index (Quetelet) was 22.6 (2.4).

Laboratory methods

A blood sample was drawn by venipuncture using vacutainer tubes and ethylene-diamine-tetra-acetate as an anti-coagulant. Blood was shielded from bright light, plasma was separated by centrifugation, and an aliquot of plasma was quickly frozen at −70°C until analysis of retinol concentrations by high performance liquid chromatography [10], and analysis of RBP concentrations by radial immunodiffusion (Nanorid, The Binding Site, Birmingham, UK). Patients were tested for the presence of HIV-1 antibody using enzyme-linked immunosorbent assay (Wellcozyme, Wellcome Diagnostics, Dartford, Kent, UK, and Genetic Systems EIA, Seattle, Washington, USA). Both enzyme-linked immunosorbent assays were required to be positive for a woman to be considered HIV-1-positive. Immunoblotting (Bio-Rad Laboratories, Hercules, California, USA) was used to confirm HIV-1 status in women with equivocal HIV-1 testing.

Radial immunodiffusion assays were run with plasma which had been diluted 20-fold with 7% bovine serum albumin diluent provided by the manufacturer. 10 μL of diluted sample was applied to wells of each plate; plates were tightly closed, resealed in foil pouches to minimize evaporation, and placed on a level bench at room temperature (about 22–24°C) for 96 h at conditions of equilibrium. Ring diameters were read using a jeweller's eyepiece, bright side lighting, and a dark background, and results were recorded to the nearest

0.1 mm. Any samples which were below range at 20-fold dilution were re-run at 10-fold dilution as above. The concentration of RBP was calculated using a calibration curve established by use of known calibration standards provided by the manufacturer.

Plasma retinol concentrations were measured by high performance liquid chromatography in 900 women. Pooled human plasma standards were prepared from plasma obtained from healthy adults, shielded from light, and stored in aliquots at −70°C. Mean plasma retinol of a pooled human plasma standard (n=28) was 2.88 μmol l^{-1}. The within-assay and between-assay coefficients of variation for plasma retinol of a pooled human plasma standard were 4.2% and 4.7%, respectively. RBP was measured by radial immunodiffusion in 872 women. Mean RBP for the pooled human plasma standard (n=75) was 57.2 mg l^{-1}; the within-assay and between-assay coefficients of variation were 2.5% and 3.5%, respectively. It was not possible to measure RBP in 28 women because of a limited sample volume. A plasma retinol concentration of <0.7 μmol l^{-1} was considered consistent with vitamin A deficiency [13]. The mean for RBP using this RBP radial immunodiffusion kit for sera from 55 healthy adult British blood donors was 46.2 mg l^{-1}, and 5th to 95th percentile range was 32.8–60.4 mg l^{-1} according to the manufacturer's instructions (Nanorid, The Binding Site, Birmingham, UK).

Statistical analysis

Mean and standard deviations were calculated for continuous variables. Spearman correlation was used to examine the relationship between plasma vitamin A and RBP concentrations. Chi-squared tests were used to compare categorical variables between groups.

RESULTS

Fig. 1 shows the relationship between retinol and RBP concentrations in 872 plasma samples from pregnant women in the 18th to 28th week of gestation. The regression equation ($y=\alpha+\beta x$) for retinol concentration versus RBP concentration was retinol $=-1.97+1.04$ (RBP), where retinol was expressed in μmol l^{-1}, and RBP in mg l^{-1}. The Spearman correlation

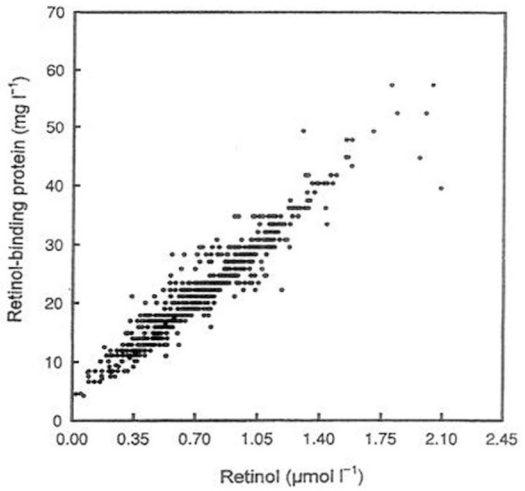

FIG. 1. Relationship between retinol and retinol-binding protein concentrations in 872 plasma samples from pregnant women in the 18th to 28th week of gestation. Spearman correlation coefficient for the two variables $r_c=0.95$, $p<0.0001$.

between plasma retinol concentrations and plasma RBP concentrations was 0.95 ($p<0.0001$). The relationship of plasma retinol concentration to RBP concentrations did not differ by HIV status of the mothers. The Spearman correlation between plasma retinol concentration and plasma RBP concentration was 0.95 ($p<0.0001$) among 672 HIV-seropositive women and 0.93 ($p<0.0001$) among 200 HIV-seronegative women. According to the regression equation, a plasma retinol concentration of 0.70 μmol/L was equivalent to 21.1 mg l^{-1} of RBP. Using these cut-offs, plasma RBP had 87.6% sensitivity (340/388) and 94.6% specificity (340/388) in predicting vitamin A deficiency as defined by plasma retinol concentrations (Table I).

DISCUSSION

This study suggests that plasma RBP measurements by radial immunodiffusion are a useful surrogate indicator for plasma retinol concentrations, showing high correlation as a continuous measure and good concordance as a categorical measure. Radial immunodiffusion is easy, simple, and requires commercially prepared radial immunodiffusion plates, a micropipettor, and a jewelers' eyepiece with a

TABLE I. Vitamin A deficiency defined by retinol and retinol-binding protein cut-off points.

	Plasma retinol ≤ 0.70 μmol l^{-1}	Plasma retinol >0.70 μmol l^{-1}	
Plasma retinol-binding protein ≤ 21.1 mg l^{-1}	$n=340$	$n=26$	$n=366$
Plasma retinol-binding protein >21.1 mg l^{-1}	$n=48$	$n=458$	$n=506$
	$n=388$	$n=484$	$n=872$

$p<0.001$.

micrometer. One laboratory technician can easily prepare about 100 samples in one day. Radial immunodiffusion does not require more expensive instrumentation, such as a microplate reader or instrumentation for high performance liquid chromatography.

The correlation between plasma RBP and retinol concentrations has also been described between 0.62 and 0.93 in other studies using fluorometric techniques for measurement of RBP [7, 11, 12]. Data from the present study also corroborate a high correlation described between serum retinol and serum RBP concentrations measured by radioimmunoassay among 239 preschool children in the Marshall Islands [3]. The concentrations of plasma retinol and RBP were lower than the normal range described for healthy adults, which is greater than 1.05 μmol/L retinol [13], and 30–60 mg l^{-1} RBP for adults [14], and these findings are consistent with lower vitamin A and carotenoid status of pregnant women in sub-Saharan Africa [15].

Plasma RBP has the same limitations as plasma retinol in the assessment of vitamin A deficiency. Plasma RBP, like plasma retinol, may not reflect hepatic stores of retinol, especially in the presence of an acute phase response during which both plasma retinol and RBP decrease. The acute phase response may contribute to decreased hepatic synthesis of RBP, increased utilization of retinol by peripheral tissues, and abnormal urinary losses of retinol [4, 5, 16], and low plasma retinol concentrations and elevated acute phase response proteins have been noted in women with clinical vitamin A deficiency [6]. Other factors which may limit the diagnostic potential of RBP as an indicator of vitamin A status include protein energy malnutrition [7], liver disease [8], and chronic renal failure [9]. In this study (Fig. 1), the regression line at zero retinol concentrations did not intersect zero for plasma RBP, and the concentration of plasma RBP at this theoretical zero plasma retinol level is thought to represent the concentration of apo-RBP [3].

Plasma retinol concentrations are considered to be useful for the assessment of vitamin A status in populations [2], and it seems that plasma RBP measurements will have similar usefulness for large field studies is less technologically developed settings.

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