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Conjunctival Impression Cytology with Transfer (CIC-T) detects Hypovitaminosis A in Nigerian Children

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Summary

Akinyinka OO, Falade AG, Akanni AO Akang EEU. Conjunctival Impression Cytology with Transfer (CIC-T) detects Hypovitaminosis A in Nigerian Children. *Nigerian Journal of Paediatrics* **2000: 27:15.** Vitamin A deficiency is an important public health problem in children under 5 years of age living in the tropics. We have previously demonstrated that Conjunctival Impression Cytology with transfer (CIC-T) is a simple procedure, which is adequately sensitive and specific as a screening tool for epidemiological surveys of vitamin A status. The present study was designed to evaluate the utility of CIC-T and serum retinal in malnourished children with kwashiorkor and children with chronic cholestasis. The study population comprised 15 cases of kwashiorkor, 5 cases of chronic cholestasis, and 20 healthy well-nourished controls, all aged less than 3 years. The mean serum retinol in the well-nourished children of 37.55 ± 18.32mg/dl was significantly higher than the 17.2 ± 11.53mg/dl in the kwashiorkor group (p=0.001). The serum retinol in children suffering from chronic cholestasis had an intermediate value of 23.59 ± 11.21mg/dl, which was similar to the levels in children with kwashiorkor (p=0.15) but significantly lower than that of the controls (p=0.03). This study demonstrates that a normal CIC-T smear is likely to indicate a serum retinol of >20mg/dl (Odds Ratio = 0.07, 95% confidence Interval = 0.01-0.44; p=0.002). This study also confirms that CIC-T smear classification of normal and abnormal is the most predictive of serum retinal status. The simplicity, specificity, high positive predictive value and the acceptably low failure rate of CIC-T should make this test useful in predicting vitamin A status of children in this community. The nearly universal hypovitaminosis A in kwashiorkor in this study also confirms the need for vitamin A therapy in all children suffering from kwashiorkor.

Introduction

VITAMIN A deficiency is a public health problem in developing countries with prevalence rates in children <5 years varying between 6 and 16 percent.[1-4] It has been estimated that 35 percent of children with chronic cholestasis[5] are vitamin A deficient and that malnutrition[3,6] is a risk factor for vitamin A deficiency. These deficiency states may be secondary to inadequate intake and/or impaired absorption.[3,5,6] While the established methods of determining vitamin A status have been associated with varying degrees of disadvantages,[7,8] conjunctival impression cytology with transfer has been shown to be a simple, sensitive, highly specific and minimally

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invasive technique.[4 9 10] Vitamin A is important for epithelial cellular differentiation and the deficiency state results progressively in enlargement and squamous metaplasia of conjunctival goblet epithelial cells.[4 9 10] These epithelial cellular changes occur relatively early, and are the basis of the cytological detection and categorisation of the degree of deficiency on evaluation of CIC-T smears[9 10] The present study was designed to compare the CIC-T findings and serum retinol in children with cholestasis and kwashiorkor.

Patients and Method

Fifteen children suffering from kwashiorkor, 5 children suffering from chronic cholestasis and twenty healthy well-nourished children, all aged less than 3 years were enrolled into the study. The healthy well-nourished children were enrolled from the well baby clinic while those suffering from kwashiorkor and chronic cholestasis were recruited from the children's wards of University College Hospital, Ibadan, Nigeria. Patients were diagnosed as suffering from kwashiorkor based on the Wellcome classification[11] using the 50th centile of the National Centre for Health Statistics weight-for-age chart. This chart compares favourably with the Janes local standard.[12] Children were classified as suffering from chronic cholestasis when the conjugated bilirubin exceeded 20 percent of the total serum bilirubin.[13] The chronic cholestasis patients comprised 3 cases of extrahepatic biliary atresia, one case of Alagille syndrome (the subject of a separate case report)[14] and one case of neonatal hepatitis.

Conjunctival impressions were obtained and processed as we have earlier described in detail elsewhere.[4] Briefly, conjunctival impressions obtained by light application of precut strips of cellulose acetate paper (HAWP 36480: Millipore Corporation, Bedford, Mass, USA) were applied to the temporal bulbar conjunctiva of each eye. The sheet of harvested cells was immediately transferred to pre-labelled glass slides, and were then fixed in 95 percent ethanol, dried and immersed in staining solution of one volume of carbol-fuschin and two volumes of 0.2 percent Alcian-green 2GX in 5 percent acetic acid. The slides were then washed under running water, dried and mounted under cover slips using Canada balsam. The imprints were examined under light microscope and classified, according to the method of Carlier *et al*;[15] as normal (N), borderline normal (+), borderline abnormal (M-), and deficient (D). The slides were read blind without prior knowledge of serum retinol which were determined using the modified spectrophotometric ultraviolet inactivation method of Bessey *et al*[16] using N-hexane in place of a xylene-kerosine mixture. Smears were excluded if they failed to meet the minimal criteria for smear adequacy outlined by International Centre for Preventive Ophthalmology (ICEPO).[4 17] Non-readable slides were excluded from all statistical calculations.

Statistical analysis was done by Epi-Info version 6 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA).[18] Differences between variables of sample populations were determined by Student's '*t*' test. Specificity, sensitivity, negative and positive predictive values of CIC-T were determined by 2 x 2 contingency tables and levels of significance at the 95 percent Confidence Interval (CI) was taken at $P<0.05$.

Results

There were 20 healthy well nourished controls with a mean age of 24.10 ± 10.89 months

which compares with 20.38 ±7.75 months in kwashiorkor patients (p>0.05). However, the mean age of children suffering from cholestasis of 7.00 ± 2.92 months was significantly lower than in healthy children (p<0.05) and kwashiorkor children (p<0.05). The mean serum retinol of control subjects (37.55 ± 18.32mg/dl) was significantly higher than 17.21 ± 11.53mg/dl in kwashiorkor (p=0.0003). Similarly the mean serum retinol in the healthy controls was also higher than the 23.59 ± 11.21mg/dl in children suffering from chronic cholestasis (p=0.03). However, no statistically significant difference was observed between the mean serum retinal levels in children suffering from chronic cholestasis and those children with the diagnosis of kwashiorkor (p=0.15).

Of the 20 healthy children evaluated, 16 had normal serum retinol (>20mg/dl) and 4 had low serum retinol (20mg/dl), while in the same 20 healthy children 15 had normal CIC-T smears (N) and five were abnormal (M+, M- and D). Similarly of the 15 cases of kwashiorkor, 14 had low serum retinol (<20mg/dl) and 13 had abnormal CIC-T (M+, M- and D) smears while among the five cases of chronic cholestasis, three patients each had low serum retinol and abnormal CIC-T smears (M+, M- and D). In order to determine the utility value of CIC-T, all the slides and serum retinol of the children studied were taken together. Nineteen of 40 slides comprising all the children studied were classified as normal (N) while eight were classified as deficient (D) and the remaining 13 were classified as marginal (M+ and M-). Classifying the smears as normal CIC-T (N) and abnormal (M+, M-, D), this smear classification (N versus M+, M-, D), is more likely to predict normal serum retinol >20mg/dl (OR=0.07: 95% CI: 0.01-0.44; p=0.002) than the classification utilising deficient smear classification (D) against non-deficient smear (N, M+, M-) classification, (D versus N, M+, M-) which is less likely to predict serum retinal >20mg/d; (OR=0.17: 95% CI: 0.00-2.05: p=0.16). The power of CIC-T to predict normal serum retinol (>20mg/dl) is dependent on the CIC-T classification used. The CIC-T classification of Normal (N) versus abnormal (M+, M- and D) was the most predictive and robust classification for detection of normal serum retinol as it had a sensitivity of 76.2 percent and a positive predictive value of 84.2 percent (Table).

Table

Diagnostic Value of CIC-T at Serum Retinol Threshold of <20m/dl

CIC-T Classification	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)
Abnormal (D,M+,M-) vs Normal (N)	76.2 (52.4-90.0)	84.2 (59.5-95.8)	84.2 (59.5-95.8)	76.2 (52.4-90.9)
Positive (D,M+) vs Negative (N<M+)	81.3 (53.7-95.0)	75.0 (52.9-89.4)	68.4 (43.5-86.4)	85.7 (62.6-96.2)
Deficient (D) vs Non-Deficient (M+M-,N)	70.7 (35.4-91.9)	60.0 (40.7-76.8)	36.8 (17.2-61.4)	85.7 (62.6-96.2)

PPV: Positive predictive value
NPV: Negative predictive value
Figures in parentheses represent 95 percent Confidence Intervals

Discussion

The present study confirms the results of many others that CIC-T is a useful tool in the evaluation of vitamin A status of children.[4 9 10] However, the utility value of CIC-T is highly dependent on CIC-T smear classification.[9 15 19] Results by many workers[4 9 10] and confirmed in this study, show that CIC-T smear classification into normal (N) and abnormal (M+, M-, D) is the most robust and specific enough to predict normal serum retinol (>20mg/dl) and abnormal serum retinol (<20mg/dl) with a specificity of 84.2 percent and positive predictive value of 84.2 percent. Based on this smear classification of normal and abnormal, only 13 percent of children suffering from kwashiorkor had normal vitamin A status which compares favourably with 20 percent determined by serum retinol estimation in the same group of children, though our results are lower than the 35 percent by serum retinol estimation demonstrated by Carlier *et al.*[3] Expectedly,[3] the serum retinol in children suffering from chronic cholestasis was similar to the serum retinol in children with the diagnosis of kwashiorkor.

Abnormal CIC-T and low serum retinol (<20mg/dl) may be due to inadequate comsumption of vitamin A rich food probably due to poverty and ignorance[5] and increased anti-oxidant consumption[20] in kwashiorkor. The abnormal CIC-T and low serum retinol (<20mg/dl) in chronic cholestasis may be secondary to inadequate absorption of fat-soluble vitamin A[5] due to inadequate bile flow into the gut either from significant neonatal hepatitis or biliary atresia where there is no bile flow into the gut.

This study has demonstrated the utility value of conjunctival impression cytology technique as a simple, safe and a comparably reliable screening tool for the detection of hypovitaminosis A in Nigerian children. Also, the minimal failure rate of five percent makes CIC-T an enviable tool for assessment of vitamin A status in Nigerian children. In addition the high prevalence of low serum retinal in kwashiorkor in this study confirms the need for vitamin A therapy in all children suffering from kwashiorkor.

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