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Effect of childhood malnutrition on salivary flow and pH

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Abstract

Introduction—While protein-energy malnutrition may have multiple effects on oral tissues and subsequent disease development, reports of the effect of malnutrition on the human salivary glands are sparse.

Methods—A retrospective cohort study of the effect of early childhood protein-energy malnutrition (EC-PEM) and adolescent nutritional status on salivary flow and pH was conducted with rural Haitian children, ages 11–19 years (n=1,017). Malnutrition strata exposure cohorts were based on 1988–96 weight-for-age records which covered the birth through 5-year old period for all subjects. Then, data on *current* anthropometrical defined nutritional status categories, stimulated and unstimulated salivary flow rates, and salivary pH were collected for the same subjects then 11–19 years old during field examinations in the summer of 2005. Multivariate analysis of variance (MANOVA) was used for the analyses.

Results—Stimulated and unstimulated salivary flow rates were reduced at statistically significant levels in subjects who had experienced severe malnutrition in their early childhood or who had continuing nutrition stress which resulted in delayed growth, as measured at ages 11–19 years. Salivary pH demonstrated little clinically meaningful variability between malnourished and non-malnourished groups.

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Conclusion—This study is the first to report of a continuing effect on diminished salivary gland function into adolescence as a result of early childhood malnutrition (EC-PEM) and suggests that exocrine glandular systems may be compromised for extended periods following EC-PEM, which may have important implications for the body's systemic antimicrobial defenses.

Keywords

protein-energy malnutrition; saliva; permanent teeth; Haiti

Introduction

Protein-energy malnutrition (PEM) appears to have multiple effects on the oral tissues and subsequent oral disease development (1,2). However, reports of the effect of malnutrition on human salivary glands are sparse. Animal studies have reported decreased salivary flow, salivary proteins (total protein, lysozyme, lactoperoxidase and immunoglobulins) and glandular weight (3–8). The few human studies of PEM and salivary function taken collectively report an association between PEM and decreased flow, buffering capacity and the composition of saliva, though these studies were limited to cross-sectional data (9–13). A reduced rate of salivary secretion and of IgA in both saliva and tears was reported in 71 one-to-two year olds in 1977 (9). A dose-response pattern with PEM was found for overall protein levels, amylase, lysozyme, and immunoglobulins in 94 one-to-ten year olds (10). Another report found Gambian children (n=155, 5–14-years old) had significantly lower salivary IgA than British children (n=57, 9–10-year olds) with the investigators proposing, though not ascertaining, that malnutrition in the African subjects was the factor responsible for the difference (11). Two studies by Johansson and coworkers on a sample of 68 Indian children ages 8–12 years old reported salivary hypofunction in the children having PEM compared to children not experiencing malnutrition (12,13). These studies report that children with moderate-to-severe PEM had a reduced salivary secretion rate, reduced buffering capacity, lower calcium, lower protein secretion in stimulated saliva, and reduced agglutinating defense factors in unstimulated saliva, while not finding unstimulated salivary flow affected.

This paper reports the findings of the first study to assess the continuing effect into the second decade of life of early (i.e., from birth to age 5 years) childhood protein-energy malnutrition (EC-PEM) on the salivary flow and pH in a longitudinal study of those same children at ages 11–19.

Methods

Overview

This was a retrospective cohort design which studied the effect of both past early childhood protein-energy malnutrition (EC-PEM) and current adolescent nutritional status on salivary flow and pH. Malnutrition strata exposure cohorts were based on 1988–96 weight-forage records which covered the birth through 5-years old period for all subjects. The weight-forage for each subject was established from a computerized database of the Haitian Health Foundation (HHF), a non-governmental organization providing primary health care in the Jeremie region of Haiti. These data were converted to z-scores based on the National Center for Health Statistics (NCHS) 1978 data that is used as a Centers of Disease Control and Prevention (CDC) (14), and World Health Organization (WHO) reference database (15–17). Based upon these z-scores, three malnutrition levels of the early childhood period were created for this study. Additionally, using height and weight data collected on the same subjects, 11–19 year olds during examinations in 2005, *current* anthropometrically defined nutritional status categories were established. The study sample included 1,017 rural Haitian children, age 11–19 years old for which complete EC-PEM data was available. Stimulated and unstimulated

salivary flow rates and pH were assessed using the protocol from the University of Malmö (Sweden) Department of Cariology for saliva secretion rate (18), and the Dentobuff® Strip System (19), respectively. This study was approved by the New York University Medical School Institutional Review Board.

Study sample

Inclusion criteria required that subjects were enrolled in the Haitian Health Foundation (HHF) and that their HHF records had 1) date of birth, and 2) two weights with date of weighting for at least three of the first five years of life. The study's sampling frame included 3,163 potential subjects for whom EC-PEM data as defined by the inclusion criteria were available with a final target of at least 1,000 subjects aged 11–19 years in 2005.

Recruitment

Two recruiters visited each study village between November 2004 and April 2005. Mothers of eligible children were invited to participate and gave their consent at that time. To ensure the independence of each study subject for analysis, recruitment was limited to only one child per household, with the oldest eligible and available child in any given household being enrolled in the study. A total of 1,183 eligible 11–19 year olds were enrolled in the study and only 10 mothers refused (0.3%) to participate. Examinations were conducted on 90.2% of the enrolled subjects ($n = 1,058$) and this report is based on the 1,017 children with complete data (96% of the examined subjects, or 86.0% of the originally recruited subjects).

Categorization of the EC-PEM groups (exposure groups)

All subjects in this study were categorized into one of three groups: severe EC-PEM, questionable EC-PEM, and normal (no evidence of EC-PEM). This categorization was based on the z-scores for the weight-for-age data of the subjects during their first five years of life for the period of 1988–96 as recorded in the HHF computerized database. These data had been used in a 10-year program evaluation study for USAID in 1998 and were found to be 95% accurate (20).

All EC-PEM definitions used a z-score based upon the weight-for-age for each child on a given visit, which had been normalized using the National Center for Health Statistics (NCHS) data (15–17). A z-score of 0.00 indicates a weight-for-age equal to the median for a child of the same gender in the NCHS database. A z-score less than 0.00 indicates a weight-for-age that is the indicated number of standard deviations below the median for a child of the same gender and age in the NCHS database, while a z-score of more than 0.00 indicates a weight-for-age that is above the median for a child. The NCHS database was selected for normalization to allow international comparisons as recommended by the WHO. For the birth through 5-years old age period, subjects were hierarchically classified by weight-for-age as severe (any z-scores ≤ -2), questionable (any z-scores > -2 and ≤ -1), and normal (all z-scores > -1). Height-for-age data were not available due to cultural concerns, i.e., measuring an infant's height is associated with measuring for a coffin size in rural Haiti.

Weight and height measurements obtained during the 2005 field examinations were used to determine whether there had been continued chronic malnutrition, as well as more recent acute malnutrition. For the 2005 field examinations, staff were trained and calibrated in the height and weight measurements using a standard Detecto Balance Beam Scale and the study scales themselves were re-calibrated daily upon arrival in a study village as a routine part of the field operation. Age and gender standardized z-scores, again based on the NCHS database, were computed and used to calculate height-for-age (HAz) and body mass index-for-age scores (BMIz). These were then used to classify the 11–19 year old subjects as *currently* (2005): 1) stunted (HAz ≤ -2), 2) wasted (BMIz ≤ -2), 3) stunted and wasted (HAz and BMIz ≤ -2) and

4) normal (all z-scores >-2). For these calculations on the 11–19 year olds, BMIz rather than height-for-weight was used to determine wasting, as it is the preferred measure (14,17).

Salivary flow and pH (the outcome measures)

Salivary flow was determined using the protocol of the University of Malmö (Sweden) Department of Cariology for both unstimulated and stimulated saliva secretion rates (18). The subjects took the test at least one hour after having eaten or smoked; drinking water was allowed in this time period. In order to test the unstimulated saliva flow rate, the subject was instructed to sit in an upright position and to incline his head forward so the produced saliva could be collected in the floor of the mouth and then flow over the lower lip into a measuring cup using a funnel for a 15 minute period. For stimulated saliva collection, subjects were instructed to chew a piece of paraffin until it softened; the saliva produced during this time was then swallowed before the collection was started. The timer was then started for 3 minutes during which the chewing resumed with saliva being spat out at short intervals into a measuring cup. The total amount of saliva collected, excluding the foam that was formed during the collection process, was recorded.

The results were expressed in milliliters per minute. The references values from the University of Malmö protocol for the categorization of the unstimulated saliva flow rates are: normal (more than 0.25 ml/mn), low (0.1–0.25 ml/mn) and very low (less than 0.1 ml/mn), while the references values for stimulated saliva flow rates are: normal (more than 1.0 ml/mn), low (0.7–1.0 ml/mn) and very low (less than 0.7 ml/mn).

Saliva pH measurement was used as a field proxy for salivary buffering capacity. The determination of pH for the unstimulated and stimulated saliva was assessed using pH test strips following the Dentobuff® Strip System instructions (19). After the saliva was collected for both stimulated and unstimulated flow rates, the test strip was dipped into the mixed saliva and the timer was set for 5 minutes to allow the strip to chemically react completely. After the 5 minute period the test strip was compared to the reference standard color chart to assess the pH level in 0.5 pH increments. The subjects pH scores were then categorized into high, medium and low levels as described for the Dentobuff® Strip System(19,21)

Field Training and Calibration

Preceding the beginning of the field examinations, four dentists were trained and calibrated in the applied protocols for saliva flow and pH measurements to at least a 90% correlation. Four different dentists were trained and calibrated to WHO diagnostic standards for dental caries examinations to at least 90% agreement and a Kappa score of at least 0.75 to the referent examiner for decay, missing (due to caries), and filled tooth surfaces (22).

Field operations

Field data collection was conducted between May and August 2005 in the subjects' villages on the western tip of Haiti's southern peninsula in the Grande Anse Department in the region around Jeremie. Following the intra-oral clinical examinations and questionnaire administration, groups composed of 6–8 subjects were formed with each individual in the group being tested for unstimulated and stimulated salivary flow rates as well for salivary pH levels. All data were recorded on no-carbon-required, multiple copy data entry forms. The examiners were blinded as to EC-PEM status.

Data management

All data were double entered into an EpiInfo 2000 database and confirmed against the original datasheets. These data were then converted to a SPSS dataset and cleaned for illogical and out-of-range values by the use of frequency counts and graphing.

Statistical analysis

Descriptive statistics were produced. To allow for the intra-individual correlation of the salivary flow rate measurements, MANOVA (multivariate analysis of variance) was utilized for the analyses. Separate MANOVA analyses were conducted for the following two primary independent variables: 1) early childhood malnutrition (EC-PEM) status, 2) *current* (2005) anthropometric classifications of stunting, wasting, stunted and wasting, and normal. EC-PEM and current anthropometric classifications were then dichotomized based on multiple comparison testing. All models were adjusted for gender and age. MANOVA was then utilized to test the dichotomized variables. Lastly, the final model was adjusted for DMFS and dmfs (D(d) decayed, M(m) missing due to caries, F(f) filled surfaces) for the permanent and primary teeth, respectively. Two-sided p-values ≤ 0.05 were considered statistically significant.

Results

Descriptive statistics for each of the three EC-PEM categories for the 1,017 children, based on the data from the 2005 field examinations, are presented in Table 1. The mean age of the subjects was 13.88 (s.d. 1.73) years and slightly more males, (54%) than females were examined. Small downward trends were observed for both stimulated ($p=0.042$) and unstimulated ($p=0.045$) flow rates across the three malnutrition categories with the strongest flow rates observed in those with no EC-PEM. When pH were categorized as previously described (21), only 3 subjects did not record a “high” pH level. Given the lack of clinically meaningful pH variability, this outcome was dropped from further analysis.

The descriptive results for each of the *current* (2005) four malnutrition categories (normal, stunted, wasted, and stunted-and-wasted) are found in Table 2. Overall, the salivary outcomes were similar for the stunted and the stunted-and-wasted groups, and similar for the wasted and normal groups. Comparatively, the former two groups had higher salivary flow values than did the latter two groups..

Initial MANOVA analyses were conducted with the two salivary flow rate measures as outcomes first using the three EC-PEM categories, and then, separately, using the current 2005 four anthropometric categories, while controlling for age and gender. The EC-PEM model demonstrated no statistically significant difference between the questionable malnutrition category and normal. For the subsequent analyses EC-PEM was modeled as a dichotomized variable, malnutrition (any early childhood z-scores ≤ -2) or no malnutrition (all early childhood z-scores > -2), i.e., this latter category now including the former two categories of questionable and no malnutrition. As the MANOVA analysis using the four current 2005 anthropometric categories demonstrated no statistically significant difference between the wasted and the normal groups, subsequent analyses used a new dichotomized variable, which had a ‘no stunting’ group (comprised of the previous normal and wasted groups) and a ‘any stunting’ group (comprised of the previous stunted and stunted-and-wasted’ groups)..

Three MANOVA models were constructed with the primary independent variable as: 1) early childhood malnutrition (EC-PEM) status (any early childhood z-scores ≤ -2), 2) *current* (2005) anthropometric classifications of stunting (any height-for-age z-score ≤ -2), and 3) the EC-PEM and stunting variables included concurrently. All models included age in years and gender. The overall multivariate models significance (Pillai’s trace values) for the three final

models examined (EC-PEM, any stunting, and EC PEM and-stunting) are presented in Table 3. EC-PEM, by itself, was statistically significant, but not when the “any stunting” variable was included in the model. ‘Any stunting’ was significant in the models that excluded and included the EC-PEM variable. Details of the individual variable parameter estimates, i.e., beta coefficients are presented for each of the two salivary outcomes with each of the three models discussed and presented in tabular form in Table 4 (salivary flow rates).

Unstimulated salivary flow

The two analyses having EC-PEM only or ‘any stunting’ as the primary variable of interest demonstrated statistical significance for each variable. The EC-PEM predicted mean estimate was -0.03 ml/minute less saliva flow for the children having early childhood malnutrition, while the ‘any stunting’ group had a predicted -0.04 ml/minute unstimulated salivary flow (Table 4). For the third analysis that included both EC-PEM and ‘any stunting’ the decreased salivary flow rate was slightly higher for both variables, but EC-PEM did not reach statistical significance. However, the change in both the EC-PEM and the stunting beta coefficients were 45% and 10%, respectively, which suggests a possible confounding between those two malnutrition variables, and a combined 0.055 ml/minute. This 0.055 ml/minute represents an average decrease of 20% from the upper level for the defined low flow (0.25 ml/minute)

Stimulated salivary flow

The analyses for stimulated salivary flow demonstrated the same pattern of results for the EC-PEM and ‘any stunting’ groups as was observed in the unstimulated salivary flow. In the analyses exclusively modeling EC-PEM or ‘any stunting’ the observed statistically significant decreased stimulated salivary flows were -0.09 and -0.11 ml/minute, respectively. These represent a 10% decrease from the upper level of a low stimulated flow rate of 1.0 ml/minute. The analysis including both variables demonstrated a diminished effect for each variable individually. Again, beta coefficient changes of 34% and 18% suggests a confounding effect by EC-PEM and ‘any stunting’ status.

In summary, salivary flow rates (both stimulated and unstimulated) were reduced in subjects who had experienced malnutrition in the early childhood and/or who had continuing chronic nutrition stress resulting in delayed growth (‘any stunting’). The pH when categorized into high, medium and low levels showed no clinically meaningful variability between malnutrition groups.

Discussion

The longitudinal study findings reported here, that salivary flow for stimulated and unstimulated saliva was decreased in malnourished children is generally consistent with the limited earlier reports on animal and human studies. Importantly, this is the first study to provide evidence that a malnutrition episode in early childhood appears to have a continuing effect on salivary function into the adolescent years, i.e., that both severe EC-PEM and continuing nutritional stress as evidenced by stunting were associated with the salivary hypofunction found in these 11–19 year old Haitian children. The possible confounding effect observed on the beta coefficients of EC-PEM and current stunting when those variables were included and excluded from the models suggest that the most conservative interpretation of these findings is that chronic post-natal malnutrition beginning in the early childhood, correlates with decreased salivary flow into the adolescent years, i.e., from birth through age 19 years.

Two explanations could account for this observed continuing effect of chronic post-natal malnutrition (EC-PEM) throughout childhood: 1) the 11–19 year olds who were categorized

as stunted via the 2005 examinations may well have been continuously malnourished beyond the age of five years old (our upper age limit for measuring EC-PEM), as our conservative interpretation of the data proposes; or, alternatively, 2) the 0–5 year olds who experienced EC-PEM may have been pre-programmed during the post-natal period, through various molecular mechanisms associated with EC-PEM for stunting, i.e., unable to experience a timely growth catch-up, and that the effect on the salivary glands was similarly pre-programmed. Observations in our study provide supporting evidence for both explanations (i.e., that the odds of being stunted had a four-fold increase (odds ratio = 4.05) if the child experienced severe EC-PEM, and the Spearman's correlation coefficient was 0.42 between EC-PEM status and height-for-age); the former explanation remains speculative due to the investigators' lack of growth data between ages 0–5 in 1996 and ages 11–19 in 2005. Given the data available within our study design, it is beyond the scope of the report to determine which of these two possible explanations is the more likely, much less true.

We note that mean DMFS scores Presented in Table 1 showed a statistically significant decrease across the spectrum from normal through severe EC-PEM ($p=0.02$). No difference in primary dentition caries, as measured by dmfs scores, was found.

Strengths of this study include the cohort study design, a large sample size, longitudinal data, and trained and calibrated examiners. There are several limitations. The early childhood weights, i.e., those from the HHH database when the children were younger than 5 years old were collected by a standardized procedure designed for clinical rather than scientific measurements. Thus these measurements and possible data entry errors may have introduced some EC-PEM misclassification; however, any such misclassification would be random and result in a tendency toward the null in the analyses. The lack of anthropometric measures between 1996 and 2005 results in our inability to determine which of two proposed alternative explanations of the observed 'continued malnutrition effect' into the second decade of life is more likely.

However, as we have noted, these data do support the statement that early childhood malnutrition is associated with salivary hypofunction (and stunting). Further, these data support the statement that this effect is related to chronic, rather than acute severe nutritional insufficiency as evidenced by the fact that current wasting was not associated with the saliva flow rates. We conclude that childhood chronic malnutrition beginning in the early childhood and extending into adolescence is correlated both with decreased stimulated and unstimulated salivary flow.

This study is the first with longitudinal data on a large cohort of subjects with early childhood anthropometric measures to assess later effects of malnutrition in the second decade of life. Future studies on the relationship of malnutrition and salivary function should be conducted to confirm or refute the conclusions drawn here and extend the investigation to examine specific salivary constituents. While, the relatively small absolute differences in salivary findings observed between the malnutrition groups may not appear to be large enough to result in differences in dental caries rates, the findings reported here do clearly suggest that exocrine glandular systems in humans may be compromised for extended periods following EC-PEM, and this finding may have important implications for the body's systemic antimicrobial defenses, well beyond the local effect in the oral cavity and on oral diseases.

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Table 1

Descriptive statistics of early Childhood Protein-Energy malnutrition (EC-PEM) among Haitian children ages 11 through 19 years old in 2005

	Malnutrition			
	No Malnutrition	Questionable Malnutrition	Severe Malnutrition	Total
N	176	375	466	1017
Age (years) (s.d.)	13.81 (1.83)	14.00 (1.74)	13.81 (1.67)	13.88 (1.73)
Saliva [*]				
Saliva (ml) stimulated (mean) (s.d.)	1.05 (0.60)	1.02 (0.63)	0.93 (0.58)	0.99 (0.61)
Saliva (ml) unstimulated (mean) (s.d.)	0.40 (0.21)	0.38 (0.20)	0.36 (0.19)	0.38 (0.20)
Gender (n,%)				
Female	87 (18.6%)	164 (35.1%)	217 (46.3%)	468 (100%)
Male	89 (16.2%)	211 (38.4%)	249 (45.4%)	549 (100%)
DMFS Score (s.d.) for Permanent Teeth	2.84 (6.10)	1.95 (4.65)	1.43 (3.46)	1.87 (4.48)
dmfs Score for Primary Teeth	0.19 (1.05)	0.13 (0.92)	0.19 (0.97)	0.17 (0.96)

* pH stimulated & pH unstimulated saliva: All categorized as high (pH>5.6), except for 3 subjects with medium pH scores (4.6–5.5)

Descriptive statistics of anthropometric measurements among Haitian children ages 11 through 19 years old in 2005

Table 2

	Stunting and Wasting			
	Normal	Stunted	Wasted	Total
N	408	422	51	1017
Age (years) (s.d.)	14.10 (1.93)	13.57 (1.48)	13.80 (1.79)	13.88 (1.73)
Saliva				
Saliva (ml) stimulated (mean) (s.d.)	1.04 (0.62)	0.94 (0.61)	1.09 (0.53)	0.99 (0.61)
Saliva (ml) unstimulated (mean) (s.d.)	0.40 (0.21)	0.35 (0.19)	0.39 (0.17)	0.37 (0.19)
Gender (n,%)				
Female	229 (48.9%)	174 (37.2%)	24 (5.1%)	468 (100%)
Male	179 (32.6%)	248 (45.2%)	27 (5.2%)	549 (100%)
DMFS Score (s.d.) for Permanent Teeth	2.63 (5.37)	1.23 (3.79)	1.84 (4.25)	1.87 (4.48)
dmfs Score for Primary Teeth	0.18 (1.02)	0.11 (0.64)	0.14 (0.75)	0.17 (0.96)

* pH stimulated & pH unstimulated saliva: All categorized as high (pH>5.6), except for 3 subjects with medium pH scores (4.6–5.5)

Table 3

Multivariate Analysis Pillai's Trace statistics for three malnutrition models with Unstimulated and stimulated salivary flows as the dependent variables

Model	Dependant Variables	F	P-value
Malnutrition			
	Gender	2.821	0.06
	Age	0.479	0.619
	EC-PEM (Malnutrition) *	3.738	0.024
Stunting			
	Gender	1.536	0.216
	Age	0.9	0.407
	Stunting **	6.786	0.001
Stunting/Malnutrition			
	Gender	1.919	0.148
	Age	.686	0.504
	Stunting **	4.804	0.008
	EC-PEM (Malnutrition) *	1.254	0.286

* dichotomous variable: referent category=no malnutrition (all of first five years of life with a z-score >-2)

** dichotomous variable: referent category = no stunting (Height-for-age z-score >-2 (2005))

Table 4
Salivary flow rate (ml/minute) parameter estimates (coefficients) and P-values for three malnutrition models (MANOVA)

Dependant Variables	Model	Parameter	coefficient	S.E.	p-value
Unstimulated Saliva Flow	EC-PEM	Intercept	0.390	0.05	
		age (months)	-0.003	0.01	0.402
		Gender [*]	-0.028	0.01	0.026
		EC-PEM [†]	-0.029	0.01	0.02
	Stunting	Intercept	0.401	0.05	
		age (months)	0.001	0.01	0.274
		Gender [*]	0.090	0.01	0.11
		Stunting (2005) [‡]	-0.044	0.01	<0.009
	EC-PEM & Stunting	Intercept	0.394	0.05	
		age (months)	-0.004	0.01	0.272
		Gender [*]	-0.021	0.01	0.093
		EC-PEM [†]	-0.016	0.01	0.223
		Stunting (2005) [‡]	-0.039	0.01	0.004
Stimulated Saliva Flow	EC-PEM	Intercept	0.924	0.16	
		age (months)	0.001	0.01	0.943
		Gender [*]	-0.010	0.04	0.784
		EC-PEM [†]	-0.090	0.04	0.019
	Stunting	Intercept	0.960	0.16	
		age (years)	-0.001	0.01	0.898
		Gender [*]	0.009	0.04	0.813
		Stunting (2005) [‡]	-0.114	0.04	0.004
	EC-PEM & Stunting	Intercept	0.936	0.16	
		Age (months)	-0.001	0.01	0.892
		Gender [*]	0.005	0.04	0.894
		EC-PEM [†]	-0.059	0.04	0.147
		Stunting (2005) [‡]	-0.094	0.04	0.023

* referent category = female

[†] dichotomous variable: referent category=no malnutrition (all of first five years of life with a z-score >-2)

[‡] dichotomous variable: referent category = no stunting (Height-for-age z-score >-2 (2005))