Inflammatory cytokine profile and circulating cortisol levels in malnourished children with necrotizing ulcerative gingivitis

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ABSTRACT. Necrotizing ulcerative gingivitis (NUG), a periodontal disease traditionally associated with stressful lifestyles in young adults in developed countries, is very prevalent in socioeconomically deprived Nigerian children. Random incident cases (153) of NUG, along with their neighborhood village counterparts of comparable age and without NUG, as control, were recruited for this study. Anthropometric evaluation revealed widespread malnutrition and poor health in both groups of children, with more severe stunting in NUG cases. The poor nutritional status of the village children, with and without NUG, was also confirmed by markedly reduced levels of circulating micronutrients. Compared with the neighborhood children, NUG victims showed significant ($p < 0.05$ or < 0.001) increases in serum levels of interleukin (IL)-8 (+ 233%), IL-18 (+ 30%), IL-6 (+ 190%), IL-1β (+ 341%), IL-10 (+ 186%), with a small decrease in interferon (IFN)-γ (-19%) and nonsignificant increases in soluble tumor necrosis factor (TNF) receptors (sTNFR-p55, p75). Associated with NUG was a significant, 38% ($p < 0.05$) increase in plasma cortisol above the already high levels observed in the neighborhood village children, as well as some micronutrient deficiencies. The findings suggest that NUG is associated with dysregulated cytokine production, with a complex interplay of elevated levels of pro- and anti-inflammatory mediators. Such changes may serve as the common link between the seemingly unrelated risk conditions (*e.g.* stressful life styles, smoking, microbial infections, diabetes, malnutrition, alcoholism) traditionally implicated in the genesis of NUG, and all known to promote an increase in the blood level of cortisol, as well as a Th_1 to Th_2 cytokine shift.

Keywords: necrotizing gingivitis, poverty, malnutrition, cytokines, noma

Necrotizing ulcerative gingivitis (NUG), a unique form of periodontal disease, is characterized by ulceration involving one or more interdental papillae, pain, and some non-pathognomonic secondary signs such as fetid breath, bad taste, fever and lymphoadenopathy [1, 2]. Loss of periodontal fiber attachment and alveolar bone is infrequent except after multiple recurrences of the disease [3], an observation validated by a recent report [4]. NUG used to be prevalent in association with stressful life styles in young adults in the technically developed countries, but is now rarely seen in these countries except in individuals with severely compromised immunity such as patients with HIV-infection/AIDS and those receiving organ transplants [2]. In marked contrast, this disease is still very prevalent in the developing countries, particularly in sub-Saharan Africa where NUG predominantly affects socioeconomically deprived children, and has a peak incidence at the age of 2-5 years [1, 5, 6]. Relatively recent published reports indicate an escalating increase in the incidence of NUG in Africa [7, 8], and this trend is largely unrelated to the current AIDS pandemic in the continent [6-8]. The clinical importance of NUG in African children lies in the observation that if untreated, some cases may evolve into the life-threatening, orofacial gangrene known as noma [1, 2, 9]. NUG-like lesions have also been occasionally reported among the earliest diagnostic oral signs of HIV infection [10]. Increasing evidence of some association between periodontal lesions including NUG and other diseases [10, 11] has placed heightened emphasis on the potential systemic implications of the former.

NUG is an infectious disease, but its aetiology and pathogenesis are poorly understood [3]. The consensus of opinion is that NUG results from ill-defined interactions between malnutrition, compromised antioxidant status, poor oral hygiene, stress, smoking, endocrine dysfunctions, immune defects, and infections by specific bacteria and viruses [1, 2, 5, 7, 9]. There is increasing evidence that host factors, more so than the number and virulence of oral microorganisms, determine the expression of periodontal diseases, including NUG [12-14]. We had, in a previous study [7], examined why NUG, usually reported in young adults in developed countries, is in Africa almost exclusively seen in socioeconomically deprived malnourished children. Human cytomegalovirus (HCMV), Epstein-Barr virus (EBV)-1, and possibly other Herpes viruses have been suggested to contribute to onset and/or progression of

NUG in malnourished Nigerian children [7]. Since NUG is an infectious disease, we hypothesized that it may significantly contribute to the overall inflammatory burden of the malnourished child through excessive or inappropriate response to stimulation by the causative oral microorganisms. Cytokines released from activated immune and non-immune cells are endogenous inflammatory and immunomodulatory proteins that mediate the pathophysiology of inflammatory diseases. We therefore studied the serum levels of several pro- and anti-inflammatory/regulatory cytokines in rural, socioeconomically deprived Nigerian children with incident NUG, and their neighborhood counterparts of comparable age but without the disease. Excluded from the study were adequately nourished children of educated, elite Nigerians since NUG is very rare in this group [1, 5, 7]. Also evaluated in the study groups were anthropometric parameters and circulating levels of some micronutrients as indices of the status of nutrition and general health, as well as the serum level of free cortisol as a biochemical index of stress.

PATIENTS AND METHODS

Ethical considerations

This study was part of a major research project on noma, and was approved by the Institutional Review Board (IRB) of the University of Maryland, School of Medicine, and the Ministry of Health in Sokoto State, Nigeria. The research project was classified as high risk by the IRB. Informed consent was obtained from the children's parents or legal guardians, usually in the presence of a neutral, primary healthcare worker. In all cases, the child's dissent prevailed over parental permission. Children who refused enrollment into the study were referred to the Sokoto State Hospitals and the Noma Children Hospital based in Sokoto city where they received free treatment for their urgent oral health problems.

Site of study

Sokoto State, located in the northwest corner of Nigeria at the border with the Republic of Niger, was the site of this study. Details about the demography of the State have been reported [8]. Indigenes of the rural communities in the twenty three Local Government Areas of the State generally resided in over-crowded, poorly ventilated mud huts with thatched bamboo roofs and dirt floors [15]. They often shared the residential facilities in close proximity with their domestic animals. Drinking water was usually obtained from contaminated shallow wells. Facilities for safe disposal of human and animal fecal wastes were inadequate.

The principal health problems in the communities were malnutrition, particularly micronutrient deficiencies, and infections such as malaria, measles, tuberculosis, and diarrhea [8, 15]. The prevalence of low birth weight in such rural Nigerian villages is about 20% [16], and this is attributed mainly to fetal growth retardation rather than to prematurity [17]. Infant mortality rate, as reported by the Health Ministry, was estimated to be about 114/1,000 live births, and mortality rate among children less than five years was as high as 300/1,000 live births in some communities. Exclusive breast feeding in the first three months of life was extremely rare. Supplementary foods given to the infants included locally obtained, unpasteurized cow's milk, glucose water, herbal tea, and various indigenous cereal-based diets prepared under less than adequate hygienic conditions. At the time of the study, immunization coverage against measles and the other prevalent childhood diseases was very low.

PATIENTS

This investigation was essentially a cross-sectional and group-matching study. Over a period of five years (1997-2002), our field team, consisting of dentists, pediatricians, public health nurses, and other health personnel, actively searched, in the rural communities of Sokoto State for children, ages one to eight years, with NUG. Using a map of the 23 local Government Areas (LGAs) in the State, we identified 14 LGAs whose indigenes accounted for many of the noma cases seen at the outpatients clinics of the State Hospitals. These 14 LGAs were also readily accessible by modern means of transportation. Five LGAs were randomly selected from the list of 14 LGAs. A total of 2,558 children were screened for NUG, and of these, 247 cases were identified. Criteria for diagnosis of NUG were bleeding gums with irreversible destruction of the interdental papillae and pain in many cases. As observed in an earlier study [1], severe pain was not a universal feature of NUG in malnourished Nigerian children. In another study of acute NUG in western Nigerian children, only 64% of 160 cases reported pain [6]. Similarly, out of 28 children with NUG seen in Columbia, South America, pain was absent in 10 cases [18]. In some individuals, the lesion was covered by a pseudomembrane and there was marked *fetor oris*.

Since we were interested in only untreated incident/fresh NUG and not relatively long standing (prevalent cases), the latter were excluded from the study and referred to the Dental Clinics for the necessary treatment. This selection bias reduced the number available for study to 153 cases. Identification of incident cases was largely predicated on the history provided by the mothers. Additional exclusion criteria included congenital abnormalities, concurrent presence of other overt diseases/infections such as malaria, measles and oral herpes, clinical suggestion of HIV-infection/AIDS as demonstrated by presence of characteristic oral lesions such as pseudomembranous candidiasis, and subsequent serological evidence of HIV-infection. Also excluded were individuals reporting usage of traditional medications in the previous month, and those whose ages could not be ascertained with any reasonable degree of certainty. The age of each child was determined from birth records where available, but mainly from interviews with mothers/legal guardians, using a validated local calendar of important political/ceremonial/cultural events occurring in recent months/years as a guide. The calendar was developed following a focus group discussion with parents. In some instances, the reported timing of dental eruption in Nigerian village children was used as an additional guide [19]. Only children who were 0-8yr of age were enrolled.

Since NUG is a socioeconomic disease very rarely seen in well-fed children of urban-based, elite Nigerians [1, 5-7], neighborhood village children without NUG but within a

comparable age range (0-8 yr) and gender distribution as the NUG group, served as the control. Group matching of the NUG victims and the NUG-free children was by socioeconomic status and age, and thus presented us with an opportunity to evaluate any unique features peculiar to each group. We anticipated, for example, that if the occurrence of NUG was related to growth retardation, a common feature of socioeconomic deprivation in children, the prevalence and severity of growth failure should be greater in the NUG children than in the control. The control children without NUG (256 in number) were randomly selected from the 2,311 children left over after identification of the original 247 cases of NUG. More than 60 per cent of the selected control children refused to participate in the study. Additional control children (52) were subsequently selected in a random fashion from the neighborhood individuals attending the primary health care center for routine immunizations. The control children were subjected to the same exclusion criteria as the NUG victims. The total numbers of control and NUG children finally enrolled in the study were 107 and 97 respectively.

Anthropometry

Body weight was measured to the nearest 50 g. Children less than 3 years of age were weighed using a balance-beam scale, and those more than 3 years, on a standing-beam scale. Length/height was measured to the nearest 1 mm. For children under 3 years of age, height was measured as supine length, and for those more than 3 years, standing height was determined using a stadiometer attached to a wall. Assessment of nutrition and health status was carried out using low weight for height, low height for age and low weight for age as indices of wasting, failure to grow/stunting, and body mass relative to chronological age respectively [8, 20]. Height-for-age (HAZ), weight-for-height (WHZ) and weight-for-age (WAZ) Z-scores [standard deviation (SD) scores] were calculated using the EPI 2000 program from the Centers for Disease Control and Prevention, Atlanta, GA., USA This software program uses the National Center for Health Statistics (NCHS) references values [21]. The Z-score system expresses the anthropometric value as a number of standard deviations below or above the reference mean or median. In this study, we chose the Z-score cut-off points of -2.0 SD, -3.0 SD and -4.0 SD of the reference median to reflect moderate, severe, and critical malnutrition/poor health status respectively.

Biochemical studies

Venous blood from each subject was collected into plain and heparinized tubes between 8:00 am and 10:00 am. Because of the high-risk nature of the study, no more than 5 mL of blood were collected from each child, and only at one encounter. This was a major constraint on the number of biochemical assays carried out on each child's sample. Care was taken to protect the blood samples from undue exposure to light, heat, and air. The tubes were wrapped in aluminum foil. The samples were centrifuged (2,000 x g for 10 min) within 30 minutes after collection. Hemolyzed samples were discarded. The separated plasma and serum samples were divided into aliquots for storage at -70°C and subsequent analysis. The aliquot for cortisol assay was stored in a siliconized tube. The aliquot for the ascorbate assay was stabilized with an equal volume of freshly prepared 10% metaphosphoric acid before storage. The aliquot for the zinc assay was stored in zinc-free plastic tubes. The assays were carried out within one to three months of blood collection.

Assays

Serum cytokine levels were measured in duplicate using ELISA/EASIA (enzyme amplified sensitivity immunoassay) kits (Biosource International, Inc, Camarillo, California, USA). The instructions from the manufacturer of the kits were strictly followed. Plates were read at 450 nm using a Packard SPECTRACOUNT™ plate reader and I SMART 2.0 software. Standards, as well as positive and negative controls, were run with each plate. Other details were as previously reported [22].

Determination of cortisol level in plasma was carried out with the "Gamma Coat" ^{125}I-Radioimmunoassay Kit from Incstar Corporation, Stillwater, MN, USA. The kit contained test tubes coated with rabbit anti-cortisol serum, ^{125}I-labeled cortisol in phosphate-buffered saline, ANS (8-anilino-1-napthalene sulfonic acid) with 0.02 M sodium azide preservative, cortisol-free processed human serum as the blank, phosphate-buffered saline, and cortisol standards in processed human serum. The reference ranges for plasma cortisol levels were 193-690 nmol/L (mornings) and 55-248 nmol/L (evenings).

Plasma levels of retinol, vitamin C, zinc, and albumin were analyzed as previously reported [8]. For interpretation of vitamin A status, plasma retinol concentrations lower than 0.35 μmol/L were considered deficient, and levels ranging from 0.35 to 1.05 μmol/L were considered low to marginal [23]. Plasma vitamin C concentrations less than 11 μmol/L signified frank deficiency, values between 11 and 23 μmol/L suggested marginal or moderate risk of developing clinical deficiency signs, and values more than 23 μmol/L were considered normal [24]. Plasma zinc levels less than 10.8 μmol/L were suggestive of deficiency state. For plasma albumin, values lower than 34 g/L were considered low [8]. It must be underscored that blood circulating levels of micronutrients are affected by infections, in addition to dietary intake [8, 22].

Statistical analysis

The data are expressed as means ± standard deviation (SD). Statistical analyses were carried out using SPSS 11.5 for windows (SPSS Inc., Chicago, ILL, USA). Differences were considered statistically significant when $p < 0.05$.

The age distribution of children in each study group was not normal. Therefore, comparisons of the ages between groups were performed using the non-parametric Kruskal-Wallis test and the Dunn's multiple comparison test. For the anthropometric data, calculated Z scores in each group were tested for normality using the Kolmogorov and Smirnov test, and their means compared using a one-way ANOVA and Tukey-Kramer multiple comparison test.

The Z scores for each group were also classified as $Z \leq 2.0$, 3.0, and 4.0 SD and the numbers of Z scores in each subgroup transformed into a percentage of the total. Comparison of percentages between the groups was done by

Fisher's exact test using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA (www.graphpad.com).

Cytokine and cortisol levels in the various groups were in a skewed distribution. The values were therefore transformed logarithmically prior to analysis. Comparison of the serum cytokine levels between groups was by analysis of variance (ANOVA), with Tukey-Kramer multiple comparison test. For the purposes of this study, the cytokines were classified as pro-inflammatory (IFN-γ, IL-1β, IL-6, IL-8, IL-12, IL-18) and anti-inflammatory/regulatory (IL-10). Also studied were the TNF receptors sTNFR-p55 and sTNFR-p75. It must be stated that many poorly understood factors affect blood cytokine concentrations [26], and that a given cytokine may behave as pro-or anti-inflammatory, depending on its amount, nature of activating signals and other factors [27]. Plasma levels of the micronutrients and cortisol were compared by the unpaired *t* test.

RESULTS

Figure 1 is a case of necrotizing ulcerative gingivitis (NUG) involving the deciduous, mandibular incisors in a 3.5 year-old village child with extremely poor oral hygiene status as demonstrated by accumulation of plaque and food debris on the teeth. This child had a plasma total ascorbic acid level of 5.4 μmol/L, which was suggestive of severe cellular depletion of this vitamin [24]. The marginal gingiva in both the maxilla and the mandible was swollen *(figure 1)*, an observation consistent with poor oral hygiene and vitamin C deficiency [1, 28].

For analysis of the anthropometric data, each study group was divided into two, non-overlapping age groups (0-4.9 yr; 5-8 yr). Only enrolled individuals whose ages could be accurately verified, were included in the anthropometric study. Preliminary analysis of the data revealed no significant gender differences. Findings in males and females were therefore combined in *tables 1* and *2*.

Table 1 summarizes the results in children 0 to 4.9 years of age. The findings demonstrated evidence of widespread malnutrition and poor health status in both study groups. Stunting (HAZ ≤ 2.0SD) and weight loss (WAZ ≤ 2.0SD) were observed in 35 and 44% respectively of the village children without NUG. Comparative values for the children with NUG were 49 and 42% respectively. Mean HAZ, WAZ and WHZ scores were not significantly different between the two groups in the 0-4.9 yr age range, but the prevalence of severe stunting (HAZ ≤ 3.0SD) was more marked in the NUG patients. Thirty-two percent of the NUG victims were severely stunted compared to 13% ($p = 0.002$) in the village children without NUG. Findings in the older age group (5-8 yr) demonstrated increased prominence ($p < 0.001$) in the prevalence and severity of stunting and weight loss in the NUG group relative to the control children *(table 2)*. The prevalence of stunting and body weight loss decreased with increase in age in the neighborhood village children, while the opposite was the case in children with NUG (compare *tables 1* and *2*).

Plasma levels of the micronutrient antioxidants and albumin in the village children, with and without NUG are shown in *table 3*. Most of the village children without NUG had low plasma concentrations of albumin, retinol, ascorbic acid, zinc, and albumin compared to published values in well-fed children [23-25], including "elite" Nigerian children [8]. NUG was associated with a significant reduction ($p < 0.05$) in plasma levels of retinol and ascorbic acid compared with the group without NUG *(table 3)*. It should be mentioned that plasma levels of the measured antioxidant micronutrients did not accurately reflect nutritional status, and could be due, in part, to infections [8, 29].

Mean plasma cortisol concentration in the neighborhood village children without NUG was 695.7 ± 233.7 (nmol/L ± 1SD), a value very close to the upper limits of the normal reference morning range (193-690 nmol/L)

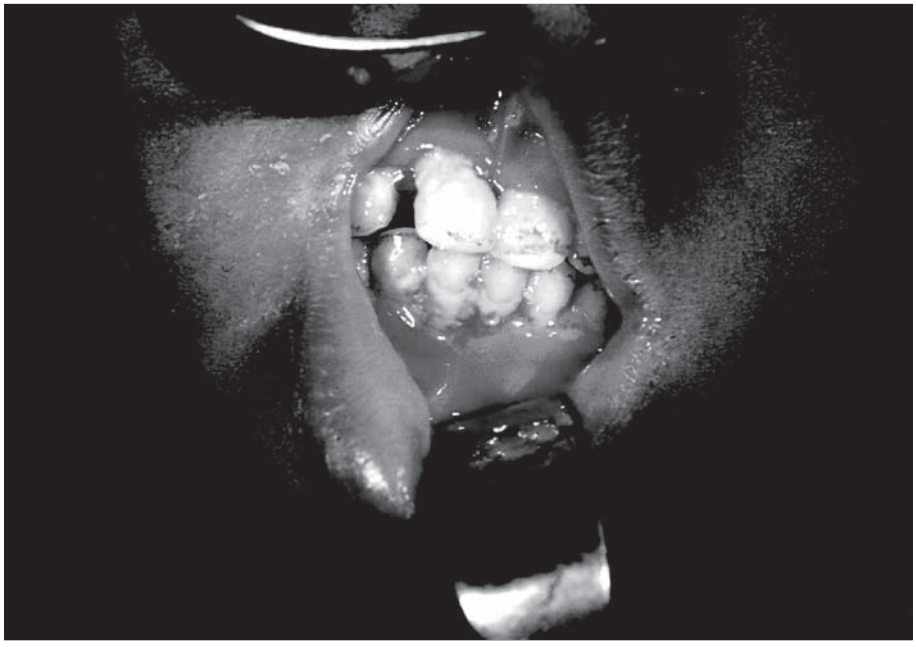

Figure 1

Necrotizing ulcerative gingivitis involving the deciduous, mandibular incisors in a 3.5 year-old village child with very poor oral hygiene status. The child had a total plasma ascorbic acid level of 5.4 μmol/L, which was indicative of a severe deficiency of this micronutrient.

Table 1
Anthropometric data for children, 0-4.9 years

Item	Neighborhood village children (n = 63)	NUG victims (n = 57)	p value
Age, yr (mean ± 1 SD)	3.81 ± 1.60	4.25 ± 0.99	
Range	1.17 – 4.95	1.50 – 4.90	
HAZ (mean ± 1 SD)	-1.29 ± 1.97	-1.78 ± 2.43	
Range	-5.58 – 7.37	-7.89 – 6.03	
% ≤ 2.0SD	35	49	p = 0.002
% ≤ 3.0SD	13	32	
% ≤ 4.0SD	5	13	
WAZ (mean ± 1 SD)	-1.78 ± 1.50	-1.87 ± 1.31	
Range	-5.64 – 2.32	-5.27 – 0.88	
% ≤ 2.0SD	44	42	
% ≤ 3.0SD	14	13	
% ≤ 4.0SD	8	4	
WHZ (mean ± 1 SD)	-1.21 ± 1.68	-1.09 ± 1.42	
Range	-6.07 – 2.59	-3.97 – 2.05	
% ≤ 2.0SD	27	25	
% ≤ 3.0SD	8	15	
% ≤ 4.0SD	5	N/A	

N/A: none.

Table 2
Anthropometric data for children, 5-8 years old

Item	Neighborhood village children (n = 44)	NUG victims (n = 40)	p value
Age, yr (mean ± 1 SD)	6.63 ± 0.77	5.95 ± 0.31	
Range	5.50 – 8.00	5.50 – 7.00	
HAZ (mean ± 1 SD)	-0.87 ± 1.24	-2.34 ± 1.65	p < 0.001
Range	-3.18 – 1.22	-7.23 – 0.80	
% ≤ 2.0SD	21	64	p < 0.001
% ≤ 3.0SD	3	36	p < 0.001
% ≤ 4.0SD	N/A	5	
WAZ (mean ± 1 SD)	-1.37 ± 1.03	-2.29 ± 1.02	
Range	-3.58 – 0.31	-4.24 – 0.11	
% ≤ 2.0SD	35	73	p < 0.001
% ≤ 3.0SD	3	23	p < 0.001
% ≤ 4.0SD	N/A	5	
WHZ (mean ± 1 SD)	-1.24 ± 1.07	-1.30 ± 1.22	
Range	-3.20 – 1.55	-3.62 – 1.37	
% ≤ 2.0SD	30	32	
% ≤ 3.0SD	N/A	9	
% ≤ 4.0SD	N/A	N/A	

N/A: none.

Table 3
Plasma levels of micronutrients in the study groups [a]

Item	Village control (n = 18)	NUG group (n = 22)
Age, yr	3.66 ± 0.97	3.37 ± 0.69
Retinol (µMol/L)	1.31 ± 0.55±	0.92 ± 0.37±
Ascorbic acid (µMol/L)	10.92 ± 2.11±	8.80 ± 1.50±
Zinc (µMol/L)	11.88 ± 2.66	9.82 ± 3.44
Albumin (g/L)	31.66 ± 4.55	30.22 ± 3.87

± Significantly different ($p < 0.05$).
[a] Data are expressed as mean ± SD; data on "elite" control children already reported [8].

Table 4
Plasma concentrations of cortisol in the study population

	Neighborhood village children (n = 29)	NUG group (n = 58)	p value
Mean (nmol/L) ± 1SD	695.1 ± 233.7	957.9 ± 385.1	P < 0.05
Median	725.3	943.0	
Range[a]	301.3 – 1136.2	365.0 – 1999.8	

[a] Normal reference range for mornings (193 – 690 nmol/L).

(table 4). Necrotizing ulcerative gingivitis produced a 38% increase (957.9 ± 385.1) which was statistically significant ($p < 0.05$).

Table 5 summarizes the serum concentrations of various cytokines assayed in the children. For many of the children studied, very limited quantities of samples were available for analysis. In effect, not all the cytokines studied could be measured in each individual sample. Within each study group, there was marked individual variability in the levels of the various cytokines. Nonetheless, certain trends were observed. Most prominently observed in the NUG group relative to their neighborhood village counterparts without NUG, were significant increases in serum levels of IL-8 ($p < 0.001$) and IL-10 ($p < 0.001$). Also significantly increased in NUG patients were levels of IL-18 ($p = 0.04$), IL-6 ($p = 0.05$), and IL-1β ($p = 0.02$), while IFNγ and IL-12 showed small, nonsignificant reductions. There were also nonsignificant elevations in the levels of sTNFR-p55 and sTNFR-p75. Overall, NUG, in comparison to the control group, was associated with a much higher elevation in serum levels of the so-called pro-inflammatory cytokines relative to the changes in the anti-inflammatory/regulatory cytokines *(table 5)*.

DISCUSSION

Stunting in children is a cumulative process and the 35% prevalence of HAZ-score less than -2.0 SD observed in the neighborhood village children without NUG (*table 1*), was consistent with reports in malnourished children of comparable age in other impoverished, African rural communities [20, 30]. In an earlier study in northwest Nigeria, a 50.4% prevalence of stunting was reported in children 48-59 months of age [31]. Growth faltering, particularly linear growth retardation in infants in rural African communities becomes noticeable at about 3-4 months postnatally, with the introduction of contaminated, indigenous weaning foods since exclusive breast feeding in the first three months of life is extremely rare [32]. Malnutrition is

Table 5
Serum cytokine levels in children

Cytokine (pg/mL)	Neighborhood village children	NUG victims	p value
IL-18	1096.3 ± 416.5	1,423.3 ± 738.2	p = 0.04
	[386.4 – 1,873.4]	[2,557.3 – 4,196.2]	
	N = 28	N = 31	
IL-6	134.7 ± 315.7	392.3 ± 269.3	p = 0.05
	[3.15 – 1,755]	[16.4 – 1,158]	
	N = 42	N = 37	
IL-8	127.3 ± 238.9	423.2 ± 285.5	p < 0.001
	[4.6 – 950.8]	[16.9 – 885.5]	
	N = 41	N = 31	
IL-1β	17.1 ± 28.8	74.9 ± 138.3	p = 0.02
	[1.8 – 119.4]	[4.5 – 482.8]	
	N = 17	N = 17	
IL-10	12.8 ± 14.6	36.6 ± 64.4	p < 0.001
	[1.7 – 74.2]	[3.32 – 400]	
	N = 47	N = 40	
IL-12	354.3 ± 229.7	279.9 ± 83.5	NS
	[162.7 – 1,248.2]	[125.3 – 459.3]	
	N = 30	N = 33	
IFN-γ	9.6 ± 4.1	7.8 ± 8.0	NS
	[4.7 – 15.1]	[1.9 – 38.8]	
	N = 19	N = 24	
sTNFR-p55	2,730 ± 970	3,080 ± 1,650	NS
	[970 – 5,410]	[1,710 – 10,900]	
	N = 23	N = 27	
STNFR-p75	15,420 ± 8,180	19,290 ± 12,180	NS
	[6,600 – 45,930]	[8,870 – 71,970]	
	N = 24	N = 27	

Data are expressed as means ± SD.
[ ]: Range; N: number of samples; NS: not significantly different.

believed to account for no more than 40% of the variance in the occurrence of linear growth retardation (LGR), which is attributed mainly to the continuous burden of immunostimulation by environmental antigens [33, 34]. In fact, stunting in a child from a developing country has been likened to impaired growth of poultry and livestock reared under unsanitary conditions [35]. In both age groups examined in this study, anthropometric *(tables 1* and *2)* and biochemical data *(table 3)* suggested that malnutrition and poor health were highly prevalent in the study population, and that they were more severe and prolonged in the children with NUG than in those without the disease since height/length for age should not change very rapidly with an illness like NUG [36].

In comparison to the village children without NUG, children with this oral disease demonstrated markedly increased serum levels of several cytokines *(table 5)*. Our findings were consistent with published reports of significantly elevated ($p < 0.01$) plasma concentrations of interleukin (IL)-6, C-reactive protein (CRP), and the soluble receptors of tumor necrosis factor-α (sTNFR-p55; sTNFR-p75) in malnourished African children with compromised antioxidant status, compared to healthy control children [37]. The same study [37] showed that in both the control and malnourished children, infections elicited further elevation in levels of the inflammatory mediators. It must be mentioned that in socioeconomically deprived African communities, multiple infections are the rule rather than the exception. For example, malaria and intestinal parasitisms often coexist in children in our study sites [15, 38, 39]. Nonetheless, all the children enrolled in this study were free of other overt clinical infections. Additionally, our recently concluded study in the same communities indicated that even mild malaria had more profound effects on serum cytokine levels than NUG (Enwonwu *et al.*, unpublished findings). This view is supported by a published report on impoverished children with malaria in Mali, West Africa [40].

Our data did not necessarily imply that NUG was the cause of the observed cytokine changes. The findings did however suggest that like other periodontal diseases [41, 42], NUG was associated with an intensified, systemic inflammatory burden in malnourished African children. Epithelial cells, including oral mucosal cells in contact with microbes, secrete many inflammatory mediators to alert various cell types and also attract neutrophils [43]. Interleukin 18 for example, is constitutively expressed by oral epithelial cells [44], and is suggested to play some role in oral mucosal inflammation [45]. Future studies will examine the relationship between circulating levels of these cytokines and their expression/concentration at the oral tissue sites of production in both NUG patients, and in underprivileged village children potentially at risk for NUG. Cavaillon [27] has reviewed the complexity of interactions between cytokines during inflammation, emphasizing the difficulties inherent in their simple classification into pro- and anti-inflammatory cytokines. The multifunctional cytokine IL-18, is for example, involved in both tissue destruction and compensatory reactions including reduction of chondrocyte proliferation, inhibition of osteoclast formation, induction of several cytokines (*e.g.* TNF-α, IL-1β, IL-8, IL-4), activation of matrix metalloproteinases and abrogation of oral tolerance [46, 47]. Along with TNF-α and IL-6, IL-18 plays a role in pathological bone loss [33], which may be relevant to the loss of periodontal fibre attachment to bone reported in some cases of recurrent NUG [2, 4]. The significantly increased serum IL-6 level in NUG *(table 5)*, along with elevated serum cortisol *(table 4)*, could promote hepatic, acute-phase protein response [48]. IL-10 possesses both anti-inflammatory and immunosuppressive properties [27].

The Th_1 to Th_2 cytokine shift observed in NUG children *(table 5)* was consistent with the marked elevation in serum level of cortisol *(table 4)* [49]. An increased circulating level of cortisol is a common finding in malnourished children [50], particularly in those with infections [51]. Elevated serum levels of cortisol result in increased levels of the hormone in saliva and the gingival crevicular fluid, and is associated with a significant risk for periodontal inflammation [52, 53]. The effects of glucocorticoids include inhibition of synthesis, release and/or efficacy of cytokines and other mediators that influence immune and inflammatory reactions [54]. NUG has long been considered by many [1, 5, 9] to be an important precursor for orofacial gangrene (noma). It is possible that the Th_1 to Th_2 switch in NUG impairs the host's protective mechanism against the putative microorganism(s) involved in the causation of noma. A good analogy is the observation of depressed host defense against tuberculosis by HIV-infection or malaria [55].

About three decades ago, evidence was presented that impoverished, malnourished, Nigerian children, relative to their elite ethnic counterparts, suffered more severe periodontal diseases than could be attributed solely to poor oral hygiene and other local factors [1, 56]. Since then, several human [57] and experimental animal studies [58] have confirmed that periodontal inflammation is more related to circulating levels of inflammatory mediators than to oral bacterial load. In addition to malnutrition, several recognized risk indicators for NUG include infection by viruses (*e.g.* HIV, *Herpes viridae*, measles), psychosocial and physical stress, smoking, and alcohol abuse [1, 2, 7, 53, 59]. Our earlier studies [1, 7] have demonstrated a prominent role for viruses in the causation of NUG, and have also offered some explanation for why this is a socioeconomic disease of children in the developing countries, contrary to its occurrence mainly in stressed young adults in the developed world. Features shared in common by all the reported risk indicators are increased blood circulating levels of the stress hormones, particularly the glucocorticoids and catecholamines [49, 51, 53], as well as a marked shift towards a Th_2 cytokine pattern with increased production/release of the pro-inflammatory and regulatory cytokines [49, 53, 60-62]. Perhaps, accurate knowledge about the complex interactions between elevated circulating levels of the glucocorticoids and the inflammatory mediators in malnutrition will explain the high prevalence of NUG in socioeconomically deprived African children.

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