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Plasma cytokine levels and human papillomavirus infection at the cervix in rural Nigerian women

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ABSTRACT

Introduction: We conducted a study to test the hypothesis that systemic dysregulation of Th1/Th2 cytokine levels was associated with detection of carcinogenic or overall human papillomavirus (HPV) at the cervix among 964 women residing in a rural village in Nigeria.
Methods: Levels in plasma were measured for 19 cytokines, including Th1-like cytokines IL-2, IL-12 (p40), TNF-a, IFN-g; Th2-like cytokines IL-4, IL-5, IL-6, IL-10, IL-13; innate/inflammation cytokines IL-1a, IL-1b, IL-8, eotaxin, MCP-1, MIP-1a, and IL-7; and cell development cytokines G-CSF, VEGF, and IL-17. Analysis was restricted to 5 cytokines, TNF-α (Th1), IL-8 (Th2), eotaxin and MCP-1 (innate/inflammation), and G-CSF (cell development), whose levels were detected in 80% or more of the samples measured as well as had a coefficient of variation of <30%.
Results: Strong correlations were noted between levels of eotaxin and TNF-α ($r = 0.75$), IL-8 and MCP-1 ($r = 0.60$), eotaxin and G-CSF ($r = 0.44$), and G-CSF and IFN-γ ($r = 0.43$). Detection of carcinogenic or non-carcinogenic HPV DNA was unrelated to cytokine levels, except for levels of eotaxin and TNF-α, which were inversely correlated, albeit weakly, with detection of any carcinogenic HPV ($P = 0.048$ and $P = 0.067$, respectively). In analyses stratified by age group, levels of eotaxin were inversely correlated with detection of any HPV DNA ($P = 0.026$) and carcinogenic HPV ($P = 0.042$) in older, but not younger, women.
Conclusions: Our results do not support the hypothesis of association between systemic cytokine dysregulation and detection of HPV at the cervix in Nigerian women, but subgroup analyses raise questions about inverse associations between eotaxin and TNF-α in older women.

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1. Introduction

Squamous carcinoma of the cervix (SCC) is the first or second leading cancer and cause of cancer death in many African countries [1], where some of the world's highest incidence rates are observed [2]. This high incidence and mortality impact is due to high prevalence of human papillomaviruses (HPV) [3,4], specifically of several of the 13 HPV genotypes considered carcinogenic [3], combined with lack of basic cervical cancer screening and early treatment services. In the West, the age-specific prevalence of HPV infection falls with age as the corresponding probabilities of new sexual partners and of encountering new HPV infections decrease [5,6]. However, in contrast to patterns observed in women in developed countries [5–7], HPV prevalence does not decrease with age in women in most sub-Saharan. HPV prevalence appears to persist at high levels among older African women [5,7,8]. For example, HPV prevalence was 14.7% among 1282 women from households selected at random in a rural village in Ile-Ife in Nigeria [9].

The reasons for HPV persistence in women in sub-Saharan Africa are not well understood, but they include a high rate of acquisition of new infections (incidence) and low rate of clearance of acquired infections (duration). The risk factors for prevalent HPV infection include early age at first pregnancy, more than 2 lifetime sex partners, and oral contraceptive use [10]. Surprisingly, being in a multiple wife household, which may be a surrogate for HPV transmission in a married setting, was marginally associated with detection of carcinogenic HPV in older women [10]. While sexual contact is a necessary driver of acquisition of new HPV infections, acquisition of effective immune response to HPV proteins is

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necessary for clearance and protection against new HPV infections [11,12]. One study, which noted a geographical correlation between the distribution of high grade of cervical cancer and malaria endemicity in Uganda, hypothesized that relative immunosuppression from lifelong malaria infection may contribute [13]. Plausibly, co-infection with malaria or stool parasites [14] may modulate T helper (TH) 1/2 immunity [15], and, through that mechanism, influence persistence of cervical HPV prevalence at the cervix or progression to cancer. We therefore conducted a study to evaluate the hypothesis that HPV prevalence at the cervix, particularly among older women, in Nigeria may be associated with self-reported malaria or plasma levels of inflammatory cytokines.

2. Materials and methods

2.1. Study population

Detailed methods of the study have been reported elsewhere [9,10]. Briefly, 2100 women residing in 439 households selected at random in a village in Ondo State in Nigeria were invited to participate in an HPV/SCC screening survey. Multiple-wife households were over-sampled to study intra-familial HPV transmission in that setting. In total, 1420 of 2091 eligible women (not pregnant, without a hysterectomy, 15+ years of age, lived in the house for more than 3 months) consented for cervical screening using acetic acid and visual inspection of the cervix. Cervical cell samples were taken and placed in liquid media for cytology. Venous blood samples were taken in EDTA tubes, separated within 2 h of collection, and stored at −80 °C until shipped to the National Cancer Institute Repository. Women answered an interviewer-administered questionnaire about established risk factors for HPV, cervical cancer and a history of malaria in the past 24 months. Exposure to other endemic parasites was not evaluated.

2.2. HPV testing

HPV DNA testing was performed in the U.S. using polymerase chain reaction (PCR) MY09-MY11 primers to detect HPV genotypes [16]. Samples were considered adequate when positive for β-globin. HPV status was scored categorized as positive when at least one of ~42 HPV genotypes was detected, otherwise as negative. Results were further categorized as "definitely or possibly carcinogenic HPV positive" when at least one of the 13 carcinogenic types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 [9] were detected; otherwise as negative.

2.3. Cytokine testing

Levels were measured for 19 cytokines/chemokines on a 96-well plate format using a custom Milliplex bead assay (Millipore, Billerica, MA) according to the manufacturer's protocol [17]. The cytokines included were as follows: (a) IL-2, IL-12 (p40), TNF-α, and IFN-g (Th1-like cytokines); (b) IL-4, IL-5, IL-10, and IL-13 (Th2-like cytokines); (c) IL-1a, IL-1b, IL-6, IL-8, Eotaxin, MCP-1, MIP-1a, and IL-7 (innate/inflammation cytokines); and (d) G-CSF, VEGF (growth factors), and (e) IL-17 (Th2–17 cytokine). All tests were performed in duplicate and the mean results used for analysis. The assay readings from the BioPlex 100 instrument (v6.0) were expressed as mean fluorescent index (MFI). MFI values were extrapolated to cytokine levels based on a standard fitting curve derived from results of six serial dilutions of a recombinant cytokine standard in assay diluent. An additional standard point was added to extend the lower level of the standard curve to 0.64 pg/ml. Four replicate samples (2 within- and 2 between-batches from a woman whose cervical sample was HPV positive and a woman whose cervical sample was HPV negative) were included to assure assay reliability and guard against assay drift.

2.4. Statistical analysis

Statistical analyses were performed using STATA (version 11, STATA Corp., College Station, Texas, USA). The MFI results for cytokines were log-transformed to normalize the distribution of results. Reliability of cytokine assays was evaluated by calculating within- or between-plate coefficients of variations (CVs) by dividing the assay standard deviation by the assay mean of replicate samples. Only assays with CVs less than 30% and detected in more than 80% of samples passed and were included in analyses. Assays with CVs equal or higher than 30% were considered to have failed and were excluded in analyses. Spearman's correlation coefficient was calculated for each pair of cytokines to identify cytokines with strongly correlated levels. Analysis of continuous values was performed using the Student's *t*-test. Differences in the proportional distribution of tertile levels for cytokine levels according to self-reported malaria or HVP DNA detection at the cervix were evaluated using Chi-squared tests. Tertiles for each cytokine were calculated based on cutoff points obtained among the HPV negative women. The possible effect of age group on associations between cytokines and self-reported malaria or HPV persistence was evaluated in separate analyses for women aged 15–34 years and 35+ years. These age groups were selected *a priori* based on consideration that

Table 1
Cytokine results, including mean values and within and inter-batch coefficient of variation in rural women from Nigeria.

Cytokine	All subjects	Number (%)		Detected		
	All subjects	# Not detected[a]	# detected[a]	Mean§	SD§	C.V. (*n*/*n*)±
Th-1						
TNF-α	964	3 (0.3)	961	2.11	0.65	12/9 (62/62)
IFN-γ	964	485 (50)	479	3.05	1.44	32/34 (28/35)
Th-2						
IL-10	964	496 (51)	468	2.42	1.25	27/26 (33/31)
Innate/inflammatory						
IL-8	964	9 (1.4)	955	3.12	1.34	19/16 (62/62)
Eotaxin	964	16 (1.7)	948	4.00	0.64	15/12 (59/61)
MCP1	964	1 (0.1)	963	5.31	0.55	13/9 (62/62)
MIP1a	964	590 (61)	374	4.08	1.54	22/23 (25/30)
Growth factors						
G-CSF	964	173 (18)	791	3.53	0.79	15/25 (52/51)
VEGF	964	351 (45)	613	5.12	0.88	18/17 (37/39)

[a] Detection based on lower limit of detection for the assay (lower than the lowest standard deviation, see methods: §Mean and standard deviation (SD) based on log-transformed values; ± within/between batch C.V., (*n*/*n*) number in parentheses indicate the number of samples tested in duplicate.