Research Article

Cancer Epidemiology, Biomarkers & Prevention

Circulating Biomarkers of Iron Storage and Clearance of Incident Human Papillomavirus Infection

Erin M. Siegel[1], Nitin Patel[2], Beibei Lu[1], Ji-Hyun Lee[1], Alan G. Nyitray[1], Xi Huang[3], Luisa L. Villa[4], Eduardo L. Franco[5], and Anna R. Giuliano[1]

Abstract

Background: Iron is an essential mineral for both cellular and pathogen survival and is essential for viral replication. In turn, iron metabolism has been shown to be altered by several viral infections. However, little is known about the association between iron status and human papillomavirus (HPV) natural history. We hypothesize iron to be an HPV cofactor that is associated with longer duration of infection.

Methods: Ferritin and soluble transferrin receptor (sTfR) were measured in baseline serum samples from 327 women enrolled in the Ludwig–McGill cohort. Incident HPV clearance rates (any-type, oncogenic HPV, nononcogenic HPV, and HPV-16) over a 3 year time period were estimated from Cox proportional hazard models accounting for correlations between multiple infections.

Results: Women with ferritin levels above the median were less likely to clear incident oncogenic HPV [adjusted hazard ratio (AHR), 0.73; 95% confidence interval (CI), 0.55–0.96] and HPV-16 infections (AHR, 0.29; 95% CI, 0.11–0.73). Using physiologic cutoff points, women with enriched iron stores (>120 μg/L) were less likely to clear incident oncogenic HPV infections than those with low levels of iron (<20 μg/L; AHR, 0.34; 95% CI, 0.15–0.81).

Conclusion: This study observed that women with the highest ferritin levels were less likely to clear incident oncogenic and HPV-16 infections than women with low ferritin. Rising iron stores may decrease probability of clearing new HPV infection, possibly by promoting viral activity and contributing to oxidative DNA damage.

Impact: This novel study suggests that elevated iron stores may put women at risk for persistent HPV infection, an early event in cervical carcinogenesis. Further examination of the association between iron status and HPV natural history is warranted. *Cancer Epidemiol Biomarkers Prev; 21(5); 859–65. ©2012 AACR.*

Introduction

Iron is an essential mineral that is required for oxygen transfer as well as cellular metabolism. Iron can exist in several oxidation states, a property that supports electron transfer for ATP generation as well as promotion of reactive oxygen species (ROS). Iron status has been examined as a contributor to carcinogenesis (1, 2) and associated with increased risk of cancer in several epidemiologic studies (1, 2). Several studies have documented that elevated iron promotes cancer cell proliferation and causes oxidative DNA damage through its interaction with oxygen and hydrogen peroxide (3). ROS produced by elevated iron has been shown to directly alter the viral activity of several cancer-causing viruses, including human papillomavirus (HPV), such as by directly influencing HPV transcriptional activity (4).

Epidemiologic studies have reported an association between longer duration of HPV infections and factors that induce ROS, including smoking, coinfection with *Chlamydia trachamatis* and reduced antioxidant intake (5–7). Thus, we hypothesize that iron may be an HPV cofactor that is associated with longer duration of infection and HPV-associated carcinogenesis. To date, the association between iron status and early events in cervical carcinogenesis, such as the inability to clear HPV infections, has not been investigated. Serum ferritin, an iron storage protein, and soluble transferrin receptor (sTfR), a cellular iron transport protein, have been shown to be reliable markers of iron status (8). In addition, they can be used to calculate the sTfR-ferritin index (molar ratio of sTfR per ferritin, sTfR-F), which is a robust biomarker for determining iron deficiency (9, 10). Nested within the Ludwig–McGill Cohort, we examined the association between biomarkers of iron status (ferritin, sTfR, and sTfR-F index) and clearance of incident HPV infection

Authors' Affiliations: [1]Cancer Epidemiology Program, Division of Population Sciences, Moffitt Cancer Center; [2]James A. Haley Veterans' Hospital, HSR&D/RR&D Research Center of Excellence, Tampa, Florida; [3]Departments of Medicine and Environmental Medicine, NYU Cancer Institute and School of Medicine, New York; [4]Ludwig Institute for Cancer Research, São Paulo, Brazil; and [5]Departments of Oncology and of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada

Corresponding Author: Erin M. Siegel, Cancer Epidemiology Program, Division of Population Sciences, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612. Phone: 813-745-6533; Fax: 813-745-6507; E-mail: Erin.Siegel@moffitt.org

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among 327 women contributing 494 infections (249 oncogenic, 245 nononcogenic, and 64 HPV-16 infections).

Materials and Methods

Study sample

The current analysis included women participating in the Ludwig–McGill cohort study, an HPV natural history study of 2,528 low-income women living in São Paulo, Brazil, recruited between 1993 and 1997. Study design, clinical sampling, and HPV testing for the Ludwig–McGill cohort study have been previously detailed (11). All HPV analyses were conducted using standard PCR of the L1 gene with PG MY09/11 consensus primers and the β-globin gene as an internal control as previously reported (11, 12). Study visits were every 4 months in the first year and twice yearly thereafter for up to 5 years. All participants signed an approved informed consent before entering the study. The study was approved by the Institutional Review Board at each participating institutions. Women with normal cytology who were enrolled during the first 2 years of the Ludwig–McGill study that had an incident HPV infection detected within 3 years of enrollment and baseline serum available were included (N = 327).

Serum iron marker testing

Nonfasting blood samples were processed for serum and stored at −20°C. Ferritin (μg/L) and sTfR (μg/L) were measured in the baseline serum specimen as previously described (9). The sTfR-F index was calculated using the following formula (in μg/L): sTfR/log(ferritin) (9, 10). sTfR and sTfR-F index are inversely associated with iron level, thus a high sTfR-F index reflects lower iron status. The percent coefficient of variability was 9% for ferritin and 4% for sTfR (9). Serum levels of ferritin and sTfR are relatively stable over time and a single measurement from a subject reflects long-term average level (10).

Statistical analysis

Time-to-clearance of HPV infection was defined as the time between the first positive HPV DNA test and the subsequent first negative test (clearance event). Clearance time was censored at the woman's last visit if she did not clear the infection within 3 years of follow-up or at the first of 2 consecutive visits with missing HPV results. All clearance events were determined on a type-specific basis and then grouped as any HPV, oncogenic infections (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) or nononcogenic infections (6, 11, 26, 32, 34, 40, 42, 44, 53, 54, 55, 57, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89; ref 13). Analyses were conducted on the basis of individual HPV infections; therefore, women infected with multiple HPV types contributed multiple outcome events (37%).

Iron status biomarkers were evaluated as continuous measures (log transformed for the skewed data) and dichotomized at the median. Because women in the cohort were mostly young premenopausal, we have used levels of ferritin <20 μg/L as iron deficient, 20 to 120 μg/L as iron adequate, and >120 μg/L as iron rich (14). Differences in ferritin, sTfR, and sTfR-F index by baseline characteristics were tested by the Wilcoxon rank-sum or Kruskal–Wallis test. Median clearance time was estimated using the Kaplan–Meier method and evaluated using the log-rank test. We examined associations of baseline iron status with type-specific clearance of incident HPV infections during the first 3 years of follow-up using Cox proportional hazard models, with a robust sandwich estimator to take into account within-subject correlations (15). Covariates in the final model were selected using backward selection based on models run individually for any type, oncogenic, nononcogenic, and HPV-16. Variables that were significant at 0.10 level were then adjusted for in the final models, including age, age at menarche, smoking status, education, number of lifetime sexual partners, oral contraceptive use, alcohol, age at first intercourse, and number of pregnancies. The proportional hazard assumption for each Cox model was met, as determined by the Kolmogorov-type Supremum test. All statistical tests were 2-sided and considered as statistically significant at the level of 0.05. All analyses were conducted with SAS (SAS9.2., SAS Institute).

Results and Discussion

Iron biomarker levels are presented by demographic and risk-factor characteristics (Table 1). The median levels of ferritin, sTfR, and sTfR-F index were 26.6 μg/L (range, 1.0–391.5 μg/L), 2.0 (range, 0.09–8.17 μg/L), and 1.08 (range, 0.72–6.21), respectively. Among this population of premenopausal Brazilian women, median ferritin differed across age categories (≤20, 21–30, 31–40, and ≥40 years of age; $P = 0.05$). These data were similar to that previously reported among premenopausal women in the United States (16) and a wide age range of women in Brazil (18–81 years; ref. 17). Median sTfR and sTfR-index were significantly lower (reflecting higher iron status) among white women ($P = 0.003$ and $P = 0.004$), smokers ($P = 0.04$ and $P = 0.09$), and alcohol drinkers ($P = 0.04$ and $P = 0.005$), respectively. These observations of lower median sTfR levels among smokers and alcohol drinkers were consistent with a study by Pynaert and colleagues (18), which reported that never smokers has significantly higher sTfR levels than current smokers (1.12 vs. 1.05 μg/L; ref. 18). Finally, iron levels were elevated, as measured by lower median sTfR-F index, with increasing duration of oral contraceptive use ($P = 0.01$), with the largest difference between women taking oral contraceptive for more than 6 years. Casabellata and colleagues reported similar finding however the duration of OC use in that study was 3 months (19).

Table 2 presents the median time-to-clearance of type-specific incident HPV infections and the adjusted hazard ratios (AHR) of type-specific HPV clearance by iron status. Median duration of HPV infections did not significantly differ by iron status (Table 2). However, women

Table 1. Demographic characteristics among women who tested positive for any HPV type (N = 327)

	N (%)	Ferritin, μg/L Median (min–max)	sTfR, μg/L Median (min–max)	sTfR-F index Median (min–max)
Age,[a] y				
≤20	36 (11.0)	26.6 (14.2–391.5)	2.1 (1.3–5.0)	1.2 (0.6–2.8)
21–30	135 (41.3)	30.5 (1.0–350.0)	1.9 (0.1–4.8)	1.0 (0.1–3.2)
31–40	102 (31.2)	26.4 (12.9–302.2)	2.0 (1.1–8.2)	1.1 (0.4–6.2)
>40	54 (16.5)	22.0 (11.5–315.9)	2.0 (1.0–7.5)	1.0 (0.6–4.6)
Race/ethnicity[b,c]				
White	192 (58.9)	26.4 (1.0–391.5)	1.9 (0.1–8.2)	1.0 (0.1–6.2)
Non-white	134 (41.1)	27.5 (14.3–350.0)	2.1 (1.1–7.1)	1.2 (0.4–4.3)
Marital status				
Common law	113 (34.6)	29.3 (13.1–338.7)	2.0 (1.2–7.1)	1.1 (0.6–4.3)
Divorced	28 (8.6)	22.1 (11.5–184.4)	1.9 (1.0–7.5)	1.0 (0.6–4.6)
Married	109 (33.3)	25.1 (1.0–302.2)	2.0 (0.1–8.2)	1.0 (0.1–6.2)
Single	70 (21.4)	31.7 (10.9–391.5)	2.1 (1.0–4.5)	1.2 (0.5–2.9)
Widow	7 (2.1)	21.3 (15.5–46.5)	1.8 (1.4–2.1)	0.9 (0.6–1.3)
Education				
<Elementary	63 (19.3)	27.6 (12.9–315.9)	2.2 (1.1–5.8)	1.2 (0.4–3.1)
Elementary	181 (55.5)	26.4 (1.0–391.5)	2.0 (0.1–7.5)	1.0 (0.1–4.6)
<High school	44 (13.5)	36.7 (10.9–338.7)	1.9 (1.0–4.5)	1.0 (0.6–2.9)
≥High school	38 (11.7)	24.5 (13.2–209.1)	1.8 (1.2–8.2)	1.0 (0.5–6.2)
Monthly income, U.S. $				
<250	75 (23.7)	26.5 (11.54–350.0)	2.0 (1.0–7.1)	1.1 (0.4–4.3)
250–450	83 (26.2)	26.6 (1.0–240.1)	1.9 (0.1–7.5)	1.1 (0.1–4.6)
451–724	71 (22.4)	27.9 (13.1–230.2)	2.0 (1.2–5.2)	1.1 (0.6–4.5)
≥725	88 (27.8)	28.8 (10.9–391.5)	2.0 (1.0–5.4)	1.1 (0.6–3.2)
Smoking status[b]				
Never	161 (49.4)	27.7 (1.0–391.5)	2.1 (0.1–8.2)	1.1 (0.1–6.2)
Former	55 (16.9)	28.4 (13.1–315.9)	1.9 (1.1–5.0)	1.0 (0.4–3.1)
Current	110 (33.7)	26.0 (12.9–350.0)	1.9 (1.2–7.5)	1.0 (0.5–4.6)
Alcohol use[b,c]				
Yes	237 (72.5)	26.6 (1.0–391.5)	2.0 (0.1–7.1)	1.0 (0.1–4.3)
No	90 (27.5)	27.5 (11.5–315.9)	2.1 (1.0–8.2)	1.2 (0.5–6.2)
Oral contraceptive use[c]				
Never	61 (18.7)	26.5 (10.9–391.5)	2.0 (1.0–7.5)	1.2 (0.5–4.6)
<6 y	185 (56.8)	29.4 (11.5–247.2)	2.1 (1.0–8.2)	1.1 (0.6–6.2)
≥6 y	80 (24.5)	22.1 (1.0–338.7)	1.9 (0.1–5.2)	0.9 (0.1–4.5)
Total no. of pregnancies				
0–1	67 (20.7)	26.3 (10.9–391.5)	2.0 (1.0–4.5)	1.1 (0.6–2.9)
2–3	133 (41.2)	28.9 (1.0–240.1)	1.9 (0.1–8.2)	1.1 (0.1–6.2)
4–6	94 (29.1)	25.7 (11.5–315.9)	2.0 (1.0–5.4)	1.0 (0.4–3.1)
7+	29 (9.0)	33.8 (15.2–104.4)	2.2 (1.4–7.1)	1.2 (0.6–4.3)
Age at first intercourse				
≤15	101 (31.0)	27.2 (1.0–391.5)	2.1 (0.1–5.4)	1.1 (0.1–3.2)
16–17	88 (27.0)	26.4 (11.5–338.7)	1.9 (1.0–7.1)	0.9 (0.6–4.3)
18–19	73 (22.4)	28.0 (10.9–184.4)	2.0 (1.0–5.0)	1.1 (0.6–3.8)
≥20	64 (19.6)	26.7 (15.5–350.0)	2.0 (1.1–8.2)	1.2 (0.4–6.2)
Lifetime no. of sexual partners[b,c]				
0–1	112 (34.4)	25.9 (1.0–338.7)	2.0 (0.1–8.2)	1.1 (0.1–6.2)
2–3	125 (38.3)	29.1 (10.9–391.5)	2.1 (1.0–7.1)	1.2 (0.6–4.3)
≥4	89 (27.3)	25.5 (11.5–209.1)	1.8 (1.0–5.0)	1.0 (0.5–3.8)

(Continued on the following page)

Table 1. Demographic characteristics among women who tested positive for any HPV type (N = 327) (Cont'd)

	N (%)	Ferritin, μg/L Median (min–max)	sTfR, μg/L Median (min–max)	sTfR-F index Median (min–max)
Total no. of sexual partners in the last five years				
0–1	208 (63.8)	28.5 (1.0–338.7)	2.0 (0.1–8.2)	1.1 (0.1–6.2)
≥2	118 (36.2)	26.0 (10.9–391.5)	2.0 (1.0–5.4)	1.1 (0.6–3.2)
Total no. of sexual partners during the last year				
0–1	291 (89.8)	27.5 (1.0–391.5)	2.0 (0.1–8.2)	1.1 (0.1–6.2)
≥2	33 (10.2)	27.0 (13.2–350.0)	2.1 (1.2–4.7)	1.1 (0.7–2.6)
Age at menarche, y				
0–11	74 (22.6)	26.3 (13.1–209.1)	2.0 (1.2–8.2)	1.1 (0.6–6.2)
12–19	253 (77.4)	27.5 (1.0–391.5)	2.0 (0.1–7.5)	1.1 (0.1–4.6)
Condom use				
Always	14 (4.3)	33.4 (18.8–153.9)	1.8 (1.3–3.6)	1.0 (0.6–2.8)
Never/occasionally	313 (95.7)	26.6 (1.0–391.5)	2.0 (0.1–8.2)	1.1 (0.1–6.2)

NOTE: Kruskal–Wallis test was used to compare means.
[a]Ferritin vary significantly.
[b]sTfR vary significantly.
[c]sTfR per ferritin vary significantly.

with ferritin levels above the median were less likely to clear an incident oncogenic HPV infection (AHR, 0.73; 95% confidence interval (CI), 0.55–0.96). Using physiologic cutoff points, women with enriched iron stores (>120 μg/L) were less likely to clear incident any type HPV (AHR, 0.61; 95% CI, 0.38–0.98; Fig. 1A) or oncogenic HPV infections (AHR, 0.34; 95% CI, 0.15–0.81; Fig. 1B) than those with low levels of iron (<20 μg/L). There was no significant association between ferritin at adequate or enriched levels and clearance of incident nononcogenic HPV infections (Fig. 1C). A total of 57 incident HPV-16 infections were detected during the first 3 years of follow-up. Overall, the median duration of an HPV-16 infection was 6.9 months (95% CI, 6.0–12.1) and 9.6 months (95% CI, 6.0–12.2) for women with ferritin below or above the median (26.6 μg/L; $P = 0.86$). Women with elevated ferritin were less likely to clear HPV-16 infections (AHR, 0.29; 95% CI, 0.11–0.73). There was no significant difference in HPV-16 clearance by sTfR level or sTfR-F index.

Overall, we found that women with ferritin levels above the median were less likely to clear an incident oncogenic and HPV-16 infection. Our findings are consistent with our hypothesis that rising iron stores may increase risk of persistent HPV infection (reduced clearance) by promoting viral activity and contributing to oxidative DNA damage. Iron is a growth nutrient for humans and is required for DNA replication (2); however, it is also essential for pathogens to survive inside hosts and shown essential for some viral replication (20). Iron metabolism has been shown to be altered by several viral infections, including HIV and cytomegalovirus (20); however, relatively little is known about how HPV uses cellular iron. *In vitro*, elevated iron concentrations promoted cell growth of HPV-16 SiHa cells, increased E6/E7 expression (21), and treatment with iron chelators induced growth arrest and apoptosis (22). Furthermore, HPV is dependent on iron sensitive host transcription factors, such as NF-κB (23), for viral gene expression. Thus, elevated iron stores, (e.g., elevated ferritin), may promote viral activity and persistence by increasing the activity of cellular transcription. As viruses require iron for replication and transcription, it is biologically plausible that rising iron stores may increase risk for persistent HPV infection by promoting viral activity.

Iron also contributes to oxidative DNA damage which is an additional mechanism by which elevated iron stores may be associated with decreased clearance. Because of its ability to interact with oxygen and hydrogen peroxide, iron is an active metal species responsible for generating ROS through Fenton, Haber-Weiss, or iron autooxidation reactions (3). ROS contribute to carcinogenesis by oxidizing cellular proteins and DNA that could result in (i) lethal mutations, (ii) downregulation of host immunity, and/or (iii) altering cellular activity by activating AP-1 and NF-κB (transcription factors), cell proliferation, and apoptosis (24, 25). Thus, it is biologically plausible that excess iron stores leads to ROS which can promote HPV viral replication and transcriptional activity (expression of HPV-16 E6 and E7 proteins), as well as cell proliferation and apoptosis; all pivotal events in cervical carcinogenesis.

HPV clearance was not associated with sTfR or sTfR-F index. Serum concentrations of sTfR are relatively stable and not influenced by infection or inflammation, unlike ferritin levels (8). While sTfR is a good biomarker for iron deficiency (9), it may not be the most ideal biomarker

Table 2. Incident type specific median clearance time (in months) and risk of clearance by biomarkers of iron status

	Any HPV[a]			Oncogenic HPV		
	N	Median (95% CI)[b]	AHR (95% CI)[c]	N	Median (95% CI)	AHR (95% CI)
Ferritin,[d] μg/L						
Continuous	453	—	0.94 (0.82–1.06)	226	—	0.89 (0.73–1.10)
Dichotomize						
<26.6	228	6.67 (6.01–8.54)	Ref	116	8.05 (6.08–11.73)	Ref
≥26.6	233	7.89 (6.01–9.89)	0.88 (0.72–1.07)	116	9.79 (6.28–11.99)	0.73 (0.55–0.96)
sTfR,[d] μg/L						
Continuous	453	—	1.17 (0.94–1.45)	226	—	1.14 (0.86–1.51)
Dichotomize						
<1.97	232	6.54 (6.01–8.54)	Ref	117	7.95 (6.08–11.73)	Ref
≥1.97	229	7.95 (6.01–11.01)	0.94 (0.77–1.16)	115	10.81 (6.54–11.96)	0.80 (0.58–1.10)
sTfR-F index[d]						
Continuous	453	—	1.12 (0.92–1.36)	226	—	1.07 (0.79–1.44)
Dichotomize						
<1.08	229	7.36 (6.01–9.79)	Ref	117	8.05 (6.08–11.70)	Ref
≥1.08	232	6.77 (6.01–10.81)	1.01 (0.82–1.25)	115	11.01 (6.44–11.96)	0.83 (0.60–1.16)
	Non-oncogenic HPV			**HPV-16**		
	N	Median (95% CI)	AHR (95% CI)	N	Median (95% CI)	AHR (95% CI)
Ferritin,[d] μg/L						
Continuous	227	—	0.96 (0.80–1.14)	57	—	0.60 (0.26–1.41)
Dichotomize						
<26.6	112	6.01 (5.88–8.05)	Ref	28	6.90 (5.95–12.01)	Ref
≥26.6	117	6.01 (5.68–8.05)	0.99 (0.76–1.29)	29	9.63 (6.01–12.19)	0.29 (0.11–0.73)
sTfR,[d] μg/L						
Continuous	227	—	1.14 (0.81–1.60)	57	—	0.45 (0.12–1.71)
Dichotomize						
<1.97	115	6.01 (5.85–8.05)	Ref	33	7.95 (6.01–12.52)	Ref
≥1.97	114	6.01 (5.62–9.03)	1.00 (0.75–1.33)	24	6.70 (5.91–11.99)	0.54 (0.19–1.52)
sTfR-F index[d]						
Continuous	227	—	1.14 (0.86–1.51)	57	—	0.56 (0.22–1.48)
Dichotomize						
<1.08	112	6.05 (5.85–9.63)	Ref	32	7.06 (5.95–12.19)	Ref
≥1.08	117	6.01 (5.68–7.92)	1.14 (0.86–1.52)	25	6.77 (6.01–12.02)	0.65 (0.33–1.29)

Abbreviation: Ref, reference group.

[a]Infections, not women, were used as the unit of analyses for each outcome.

[b]Log-rank test for differences in median time to clearance were not significant for all comparisons.

[c]All Cox models were adjusted for age, condom use, education, monthly income, menarche, lifetime number of sexual partners, oral contraceptive use, race, and smoking status.

[d]Continuous measures of iron status were log transformed.

when investigating the association between iron and viral infection. It is unclear why ferritin was the only iron marker that was associated with longer duration of HPV infection.

As with any observational study, there are strengths and limitations that need to be considered when interpreting the findings. In view of this being a cohort of mostly young women, we used a comprehensive approach in evaluating the associations between iron biomarkers and HPV clearance by examining iron across the range of values (continuous measures), dichotomized at the median, and clinically defined cutoff points as deficient, adequate, and relatively enriched iron status (14). Furthermore, we analyzed only incident HPV outcomes (any type infection, oncogenic, nononcogenic, and HPV-16 infections). This

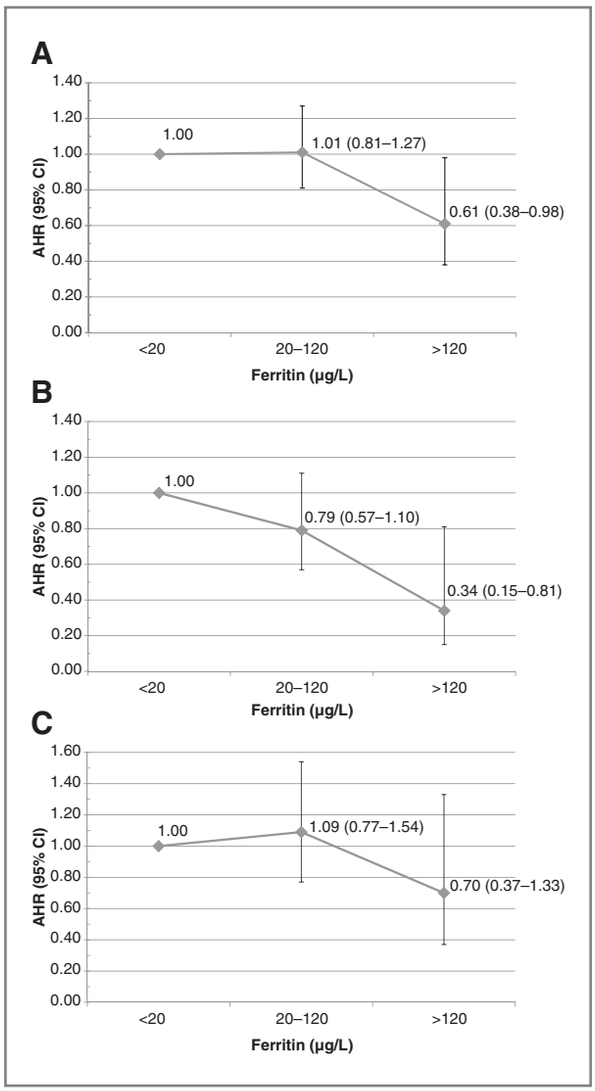

Figure 1. Hazard of clearing incident HPV infection by ferritin level. Ferritin levels were categorized using physiologic cutoff points with <20 μg/L being the reference value: any-type HPV clearance (A); oncogenic HPV clearance (B); and nononcogenic HPV clearance (C). Cox models were adjusted for age, condom use, education, monthly income, menarche, lifetime number of sexual partners, oral contraceptive use, race, and smoking status.

study was nested within the Ludwig–McGill cohort study, which had a relatively large sample size, providing sufficient power to adequately test our *a priori* hypothesis. As in any observational study, there is a possibility our finding were due to chance. Similar to other biologic markers, the iron biomarker values may not reflect the absolute value due to possible loss during specimen processing, storage and/or extraction; however, this loss would be similar across all samples and not differ by HPV status. Therefore, the associations observed within this study should be valid with potentially a lower magnitude of the associations due to methodologic errors. The study population was primarily premenopausal, with only 2.8% of women reported as postmenopausal at enrollment and was adequately reflected with the lower ferritin levels observed among premenopausal women.

In conclusion, we observed that women in the highest category of ferritin levels were less likely to clear incident oncogenic and HPV-16 infections than women with the low levels of ferritin. This association was strongest among women with enriched iron stores (>120 μg/L). Rising iron stores may increase risk of persistent HPV infection (reduced clearance) by promoting viral replication and transcription as well as contributing to oxidative DNA damage. Further examination of the association between iron status and HPV natural history is warranted.

Disclosure of Potential Conflicts of Interest

A.G. Nyitray, E.L. Franco, and A.R. Giuliano have commercial research grants for Merck Sharp & Dohme Corporation. L.L. Villa and A.R. Giuliano have honoraria from Speakers Bureau and are consultant/advisory board members for Merck Sharp & Dohme Corporation. E.L. Franco is an occasional consultant or advisory board member to companies that produce HPV vaccines (Merck and GSK) or cervical screening diagnostic assays (Roche, Qiagen, Gen-Probe, BD). No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: E.M. Siegel, B. Lu, X. Huang, L.L. Villa, E.L. Franco, A.R. Giuliano.
Development of methodology: E.M. Siegel, N. Patel, X. Huang, A.R. Giuliano.
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B. Lu, X. Huang, L.L. Villa, E.L. Franco.
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.M. Siegel, N. Patel, B. Lu, J.-H. Lee, A.G. Nyitray, L.L. Villa, E.L. Franco, A.R. Giuliano.
Writing, review, and/or revision of the manuscript: E.M. Siegel, N. Patel, B. Lu, J.-H. Lee, A.G. Nyitray, X. Huang, L.L. Villa, E.L. Franco, A.R. Giuliano.
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E.M. Siegel, N. Patel, B. Lu, E.L. Franco.
Study supervision: E.M. Siegel, L.L. Villa, E.L. Franco, A.R. Giuliano.

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Erin M. Siegel, Nitin Patel, Beibei Lu, et al.

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