Serum Levels of Insulin-like Growth Factor I and Risk of Squamous Intraepithelial Lesions of the Cervix[1]

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ABSTRACT

Squamous intraepithelial lesions (SILs) are areas of precancerous growth in the cervix that can be indicative of future cervical cancer. Insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) have been implicated in cancer development. Recent studies have demonstrated that elevated plasma IGF-I levels are associated with increased risk of prostate, lung, colon, and breast cancers. In this case-control study, we analyzed the relationship between serum levels of IGF-I and IGFBP-3, and SILs of the cervix. The case patients were comprised of 267 women treated at The University of Texas M. D. Anderson Cancer Center Colposcopy Clinic in Houston, Texas for abnormal Pap smears. The clinic serves minority and economically disadvantaged women referred from the County Health Department clinics of Harris County, Texas. The control subjects were 238 healthy women receiving family planning and screening services at two Harris County Health Department clinics. Case patients with either high-grade or low-grade SILs had significantly higher serum levels of IGF-I, IGFBP-3, and molar ratios of IGF-I:IGFBP-3 than the control subjects did. IGF-I levels in the highest quartile were associated with significantly higher risk of SILs compared with the lowest quartile, independent of IGFBP-3 levels. The odds ratio for the fourth quartile of IGF-I level, relative to the first quartile, was 8.54 (95% confidence interval, 4.15–17.60; $P < 0.0001$) after adjustment for age, ethnicity, smoking status, and IGFBP-3 level. There was a dose-response relationship between risk of SILs and the level of IGF-I: as the level of IGF-I increased, so did the risk of SILs. In addition, the serum level of IGFBP-3 was significantly higher in case patients than in control subjects. However, after adjustment for IGF-I, no relationship was evident between IGFBP-3 level and risk of SILs. Serum levels of IGF-I may be a useful biomarker for assessing risk of SIL development.

INTRODUCTION

In the United States, it is estimated that approximately 12,200 new cases of cervical cancer and 4,100 deaths will occur in 2003 (1). Progression of the disease is relatively slow, allowing for more opportunities for early detection and intervention. Precancerous conditions of the cervix, such as SILs,[3] can be detected months or even years before the development of cervical cancer (2). Detection of key factors correlated with the development of SILs could reveal predictive risk factors and thereby help identify high-risk subgroups of women.

IGFs are peptide hormones that play a vital role in the control of cell proliferation, differentiation, and apoptosis in many cell types (3–7). IGF-I has powerful mitogenic effects on both normal and cancer cells (3–4). IGF-I interacts with its cell membrane receptor (IGF-IR) to influence various cellular activities (8). IGF-I, by binding to its receptor, influences cell transit from the G_1 to S phase by stimulating the mitogen-activated protein kinase signal transduction pathway, which increases the production of cyclin D1 (4, 9). IGFs also contribute to suppression of apoptosis and increased cell growth by enhancing the production of Bcl proteins and inhibiting the production of Bax proteins (6, 10).

There is considerable individual variability in the circulating levels of IGF-I. Recent prospective and retrospective studies have demonstrated that elevated plasma IGF-I levels are associated with increased risk of various cancers (11–20). In a prospective study of prostate cancer risk, Chan *et al.* (11) reported a relative risk of 4.3 for men in the highest quartile of IGF-I level compared with the lowest quartile. Wolk *et al.* (12) also found an elevated risk of prostate cancer in individuals with elevated serum IGF-I. In a retrospective case-control analysis, we found that lung cancer patients had higher plasma levels of IGF-I than healthy control subjects did (13, 14), but the finding was not repeated in a case-control analysis nested in the CARET (β-Carotene and Retinol Efficacy Trial) study (21). Increased risk of breast cancer has been correlated with elevated circulating IGF-I levels in premenopausal women (15).

IGFBPs play a role in controlling the interaction between IGFs and IGF-IR by binding to IGFs and thereby blocking their binding to IGF-IR (4). In some situations, the binding of IGFBPs to IGFs can protect the IGFs from degradation, thereby

[3] The abbreviations used are: SIL, squamous intraepithelial lesion; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; IGF-IR, IGF-I receptor; HPV, human papilloma virus; OR, odds ratio; CI, confidence interval; CIS, Carcinoma-in-situ; CIN, cervical intraepithelial neoplasia.

increasing IGF activity (22–24). Because of their role in the regulation of IGFs, IGFBPs have also been investigated as possible predictors of cancer risk in prostate, lung, colorectal, and breast cancers (11–20). Of the IGFBPs, IGFBP-3 has been the most intensively studied because it is the main IGFBP found in plasma. In this study, we investigated the relationship between SILs and serum levels of IGF-I and IGFBP-3.

PATIENTS AND METHODS

Study Subjects. Case patients and control subjects were recruited from September 1991 through August 1994. The case patients were selected from a pool of women who were referred to The University of Texas M. D. Anderson Cancer Center Colposcopy Clinic for follow-up of an abnormal Pap smear. The clinic serves minority and economically disadvantaged women referred from the County Health Department clinics of Harris County, Texas. The women were eligible for the study if they had histologically confirmed SILs, no previous history of cervical neoplasia, no treatment for cervical neoplasia or cancer, and no prior hysterectomy. Control subjects were drawn from the population of women attending two Harris County Health Department clinics for family planning and screening services. These clinics were selected because they serve many minority patients. Control subjects were eligible for the study if they had normal cytological smears at the time of recruitment and had no prior history of an abnormal Pap smear, cervical biopsy, or hysterectomy. All study subjects were nonpregnant non-Hispanic white, African-American, or Hispanic women aged 18 years or older who lived in Harris County at the time of recruitment. Control subjects were matched to the case patients on age ($\pm$5 years).

For the cases, each histopathological sample was reviewed by the gynecologic histopathologist on clinical duty at The University of Texas M. D. Anderson Cancer Center, and there was a second blinded review performed by the study pathologist. For the controls, each cytological sample was reviewed by the state laboratory if the patient was seen at the health center, or by The University of Texas M. D. Anderson Cancer Center if the patient was seen at our clinic. One hundred slides were evaluated in a blinded review by the head of the state laboratory and the study cytologist. Of the 100 selected cases, there were 10 cancers, 10 CIS, 10 CIN 3, 10 CIN 2, 10 CIN 1, 10 HPV, 10 atypias, and 30 normals. The weighted κ statistic for the reading of the histological specimens was 0.85. The κ statistic for the readings of the Pap smears between the state laboratory and the M. D. Anderson study pathologist was 0.80.

Data Collection. The Institutional Review Board at The University of Texas M. D. Anderson Cancer Center approved this research study. Each study subject provided written informed consent before participating in the study. Epidemiological data were collected through personal interviews with a trained interviewer. At the completion of each interview, a 20-ml blood sample was collected in red-top (anticoagulant-free) tubes for analysis.

Measurements of IGF-I and IGFBP-3 Levels. Serum was separated from each blood sample by centrifugation at $3000 \times g$ for 10 min at room temperature and stored at $-80°C$. A previous study established that no differences were detected

in IGF-I and IGFBP-3 levels assayed between stored plasma and fresh sample (11). A commercially available ELISA was used to determine the plasma levels of IGF-I and IGFBP-3 (DSL, Webster, TX). The ranges for intra-assay and interassay precision for the coefficient of variation were 4.5–8.6% and 3.3–6.8%, respectively, for the IGF-I assay and 7.3–9.6% and 8.2–11.4%, respectively, for the IGFBP-3 assay. In an earlier study, the levels of IGF-I and IGFBP-3 remained constant through five freeze-thaw cycles of the heparinized plasma samples (14).

Statistical Analysis. The χ^2 test was used to compare the distributions of the studied variables between the case patients and control subjects for categorical data. The two-sample Student's t test was used to calculate the difference between case patients and control subjects for continuous variables with normal distributions. The Wilcoxon rank-sum test was used to determine the difference between the two groups for continuous variables with non-normal distribution. Two-sided P values are reported here. A Spearman correlation coefficient was used to examine the correlation between IGF-I and IGFBP-3. Because the distributions of IGF-I and IGFBP-3 in the groups were skewed, the levels of IGF-I and IGFBP-3 were analyzed categorically on the basis of their quartile distribution in the control group. Smoking was stratified into three categories: never smoker, former smoker, and current smoker. A smoker was defined as one who had smoked at least 100 cigarettes in her lifetime, and a former smoker was defined as one who had a history of smoking but had stopped at least 1 year before diagnosis (or 1 year before enrollment into the study, for control subjects). The ORs and corresponding 95% CIs were calculated to evaluate the association of risk of SILs and various risk factors using univariate and multivariate regression analyses. In the multivariate analyses, the relationship between IGF-I and SIL risk was investigated with adjustment for IGFBP-3, and the relationship between IGFBP-3 and SIL risk was investigated with adjustment for IGF-I because IGFBP-3 has an effect on IGF-I activity. In addition, we adjusted for additional risk factors such as age, ethnicity, and smoking status. The effect of the IGF-I:IGFBP-3 molar ratio [(IGF-I $\times$ 0.13)/(IGFBP-3 $\times$ 0.035)] was also evaluated.

RESULTS

The associations of SILs with IGF-I, IGFBP-3, and select variables are summarized in Table 1. The cases and controls were well matched on age (26.4 and 26.8 years, respectively). There were significantly more non-Hispanic white women (45.69%) among the case patients than the control subjects (24.79%). Case patients were also more likely to be current smokers (35.96%) than control subjects were (20.59%; $P <$ 0.0001). The levels of IGF-I and IGFBP-3 were determined by using an ELISA (Fig. 1). The mean levels of IGF-I were 144.4 ng/ml (95% CI, 137.2–151.6 ng/ml) in the case patients and 98.9 ng/ml (95% CI, 93.1–104.6 ng/ml) in the control subjects ($P <$ 0.0001). The mean IGFBP-3 levels were also significantly higher in case patients than in control subjects [4097.8 ng/ml (95% CI, 3976.7–4218.9 ng/ml) and 3706.5 ng/ml (95% CI, 3583.0–3830.1 ng/ml), respectively; $P <$ 0.0001]. Case patients had a significantly higher molar ratio of IGF-I:IGFBP-3 than control subjects did [0.133 (95% CI, 0.127–0.139) and 0.101

Table 1 Associations of SILs with IGF-I, IGFBP-3, and select variables

Variable	Case patients (N = 267)	Control subjects (N = 238)	P
Mean age, yrs (95% CI)	26.4 (25.6–27.2)	26.8 (26.1–27.6)	0.426
Race, no. (%)			
Non-Hispanic white	122 (45.69)	59 (24.79)	
Hispanic	83 (31.09)	115 (48.32)	
African American	62 (23.22)	64 (26.89)	<0.0001
Smoking status, no. (%)			
Never smoker	145 (54.31)	174 (73.11)	
Former smoker	26 (9.74)	15 (6.30)	
Current smoker	96 (35.96)	49 (20.59)	<0.0001
IGF-I, no. (%)			
1st quartile	15 (5.62)	60 (25.21)	
2nd quartile	42 (15.73)	59 (24.79)	
3rd quartile	57 (21.35)	59 (24.79)	
4th quartile	153 (57.30)	60 (25.21)	<0.0001
IGFBP-3, no. (%)			
1st quartile	29 (10.86)	60 (25.21)	
2nd quartile	49 (18.35)	59 (24.79)	
3rd quartile	81 (30.34)	59 (24.79)	
4th quartile	108 (40.45)	60 (25.21)	<0.0001
Molar ratio[a], no. (%)			
1st quartile	24 (8.99)	60 (25.21)	
2nd quartile	39 (14.61)	59 (24.79)	
3rd quartile	74 (27.72)	60 (25.21)	
4th quartile	130 (48.69)	59 (24.79)	<0.0001

[a] Molar ratio = (IGF-I × 0.13)/(IGFBP-3 × 0.035).

(95% CI, 0.095–0.106), respectively; $P < 0.0001$]. When the data were categorized by quartile for IGF-I level among the controls, 57.3% of the cases were in the highest quartile. Similarly, 40.45% of the cases were in the fourth quartile of IGFBP-3 level. The same pattern was seen for the percentages of individuals in the fourth quartile of IGF-I:IGFBP-3 molar ratio (48.69%).

Because IGFBP-3 regulates IGF-I activity, the risks for SILs were determined by multivariate analyses. Table 2 presents the risk estimates adjusted for age, ethnicity, and smoking status. Independent of IGFBP-3 level, IGF-I levels were associated with increased risk of SILs in a dose-dependent manner. The OR for the fourth quartile of IGF-I levels, relative to the lowest quartile, without adjustment for IGFBP-3 level, was 9.67 (95% CI, 4.89–19.11), and with adjustment for IGFBP-3 level, the OR was 8.54 (95% CI, 4.15–17.60). A similar dose-dependent relationship was also evident between risk of SILs and level of IGFBP-3 without the adjustment for IGF-I, with an OR of 2.89 (95% CI, 1.63–5.14) for the fourth quartile. However, when IGF-I was included in the model, the OR in the fourth quartile was reduced to 1.41 (95% CI, 0.74–2.61). After adjustment for age, ethnicity, and smoking status, the IGF-I:IGFBP-3 molar ratio exhibited a similar dose-dependent relationship to that of the IGF-I analyses.

Both individuals with high-grade SILs and those with low-grade SILs had significantly higher levels of IGF-I and IGFBP-3 than control subjects did (Table 3). When comparing the levels and the molar ratio by grade, those with high-grade SILs exhibited lower IGF-I and IGFBP-3 levels and molar ratio than those with low-grade SILs; however, these differences were not significant.
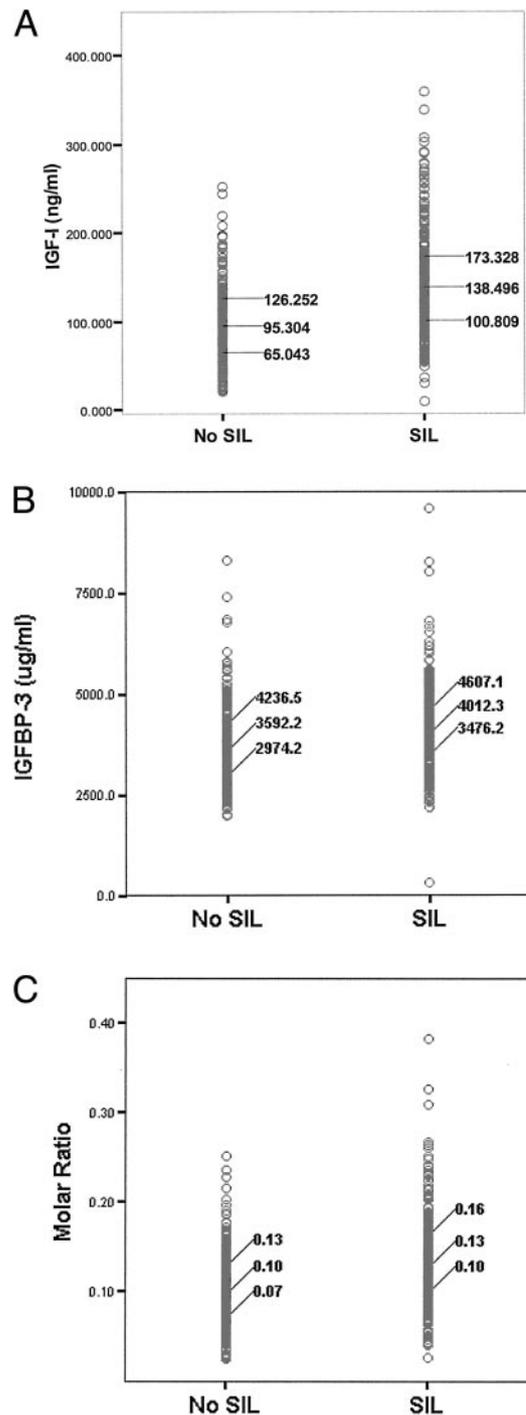

Fig. 1 Scatter plot of the levels of IGF-I (*A*), IGFBP-3 (*B*), and IGF-I: IGFBP-3 molar ratio (*C*) in SIL cases and control subjects. The values of 25%, 50%, and 75% of IGF-I (*A*), IGFBP-3 (*B*), and IGF-I:IGFBP-3 molar ratio (*C*) are marked in the figures (from lowest to highest).

We also evaluated the levels of IGF-I, IGFBP-3, and molar ratio by smoking status (data not shown). No significant differences in IGF-I levels, IGFBP-3 levels, and molar ratios were seen among never smokers, former smokers, and current smokers for either case patients or control subjects.

Table 2 Risk estimates of IGF-I, IGFBP-3, and molar ratio in multivariate analysis

Variable	Adjusted OR[a] (95% CI)	P	Adjusted OR[a] (95% CI)	P
IGF-I	Without IGFBP-3		Including IGFBP-3	
1st quartile	1.00	Ref.[b]	1.00	Ref.
2nd quartile	2.62 (1.29–5.28)	0.007	2.49 (1.22–5.11)	0.013
3rd quartile	3.60 (1.79–7.25)	<0.0001	3.48 (1.70–7.13)	0.001
4th quartile	9.67 (4.89–19.11)	<0.0001	8.54 (4.15–17.60)	<0.0001
Test for trend		<0.0001		<0.0001
IGFBP-3	Without IGF-I		Including IGF-I	
1st quartile	1.00	Ref.	1.00	Ref.
2nd quartile	1.43 (0.78–2.61)	0.25	0.93 (0.48–1.78)	0.82
3rd quartile	2.23 (1.25–4.00)	0.007	1.18 (0.62–2.24)	0.61
4th quartile	2.89 (1.63–5.14)	<0.0001	1.41 (0.74–2.61)	0.30
Test for trend		<0.0001		0.17
Molar ratio[c]				
1st quartile	1.00	Ref.		
2nd quartile	1.61 (0.85–3.07)	0.146		
3rd quartile	2.70 (1.47–4.99)	0.001		
4th quartile	5.29 (2.88–9.70)	<0.0001		
Test for trend		<0.0001		

[a] Adjusted by age, ethnicity, and smoking status.
[b] Reference group.
[c] Molar ratio = (IGF-I $\times$ 0.13)/(IGFBP-3 $\times$ 0.035).

Table 3 Levels of IGF-I, IGFBP-3, and molar ratio by SIL stage

	N	Mean	SD	P	P
IGF-I (ng/ml)					
Control	238	98.87	44.89	Ref.[a]	
High-grade SILs	118	139.61	62.77	<0.0001	Ref.
Low-grade SILs	149	148.18	57.35	<0.0001	0.25
IGFBP-3 (ng/ml)					
Control	238	3706.53	967.60	Ref.	
High-grade SILs	118	4060.39	1010.40	0.0015	Ref.
Low-grade SILs	149	4127.43	1003.66	<0.0001	0.59
Molar ratio[b]					
Control	238	0.101	0.042	Ref.	
High-grade SILs	118	0.130	0.054	<0.0001	Ref.
Low-grade SILs	149	0.136	0.050	<0.0001	0.34

[a] Reference group.
[b] Molar ratio = (IGF-I $\times$ 0.13)/(IGFBP-3 $\times$ 0.035).

DISCUSSION

To our knowledge, this is the first study to investigate the relationships between circulating IGF-I and IGFBP-3 concentrations and risk of a precancerous lesion, especially SILs. We observed a significant difference in the serum levels of IGF-I in case patients and control subjects. After adjustment by age, ethnicity, and smoking status, the IGF-I serum levels remained significantly higher in the case patients than in the control subjects. These findings suggest that IGF-I may play a role in the development of SILs and that it may serve as one biomarker for the detection of predisposition to SILs. We also found a dose-dependent relationship between the serum IGF-I and risk of SILs. The dose-response relationship was still present after adjustment for the serum level of IGFBP-3.

Associations between IGF-I levels and invasive cancer risk have been demonstrated in numerous case-control studies for a variety of epithelial cancers (11–20). The consistency of the results in various studies and types of cancer provides strong evidence that IGF-I is linked to carcinogenesis. IGF-I may play

a number of roles in the development of cancer. *In vitro*, IGF-I can promote growth of cervical cancer cell lines (25). Compared with normal ectocervical cells, IGF-IR expression increased 3- and 5-fold in primary cervical cancer tumor cells and cell lines, respectively (26). In a small study of 11 cervical cancer patients and 27 controls, Ayabe *et al.* (27) found no significant association between the serum levels of IGF-I and cervical cancer risk. However, consistent with our data, they found that the serum levels of IGF-I were higher in young cervical cancer patients than controls (196.3 *versus* 183.6 ng/ml), although the difference was not statistically significant. There is no direct evidence that IGF-I is up-regulated in SIL development, but IGF-I is involved in increased proliferation and angiogenesis and decreased apoptosis, which are hallmarks of high-grade SILs (28). Because IGF-I is a potent growth factor, it may encourage cell proliferation by accelerating DNA synthesis and increasing cyclin D1 production, thus hastening the cell cycle progression from the G_1 to S phase (4, 9). In addition to stimulating cell proliferation, IGF-I can interact with Bax and *Bcl*II to suppress cellular apoptotic pathways and facilitate cell growth (6, 10). Furthermore, IGF-I can promote angiogenesis by inducing the expression of vascular endothelial growth factor, which is involved in regulating the development of new blood vessel formation (29). IGF-I can also interact with epidermal growth factor to promote cell progression, whereas epidermal growth factor receptor is overexpressed in high-grade SILs (30). Therefore, IGF-I might play an important role in SIL development.

It is well established that HPVs are involved in the genesis of cervical cancer (31). The association between HPV infection and IGF levels has not been explored in risk of cervical diseases. Nakamura *et al.* (32) found that the mRNA expression of IGF-I and IGF-IR was not dependent on HPV infection status. However, Berger *et al.* (33) reported that cervical cells transduced with high-risk E6/E7 genes at later cell culture passages exhibited approximately 85-fold increases in IGFBP-3 mRNA levels. Furthermore, *in situ* hybridization of cervical biopsies with an

IGFBP-3 riboprobe revealed high levels of expression in high-grade squamous intraepithelial neoplasia but not in normal cervical epithelium. We speculated that the accumulation of genetic damage in SIL may involve interacting with both viral infection factors (*e.g.,* HPV infection) and humoral factors (*e.g.,* IGFs) that enhance proliferation, resistance to apoptosis, and clonal outgrowth of HPV-infected cells. High levels of IGF-I indicate a potentially high proliferation resulting in clonal outgrowth of HPV-infected cells. Therefore, it is possible that there may be a joint effect of HPV infection and high levels of IGF-I in SIL risk.

In our study, we found that the serum levels of IGF-I and IGFBP-3 were similar in women with either high-grade or low-grade diseases. In fact, women with low-grade SILs tend to have higher levels of both proteins, although the difference in levels between the two grades was not statistically significant. A few studies have suggested that IGF-I may play a role in disease progression. Gurgan *et al.* (34) proposed that IGF-I may be a factor in the development and maintenance of or progression to late-stage endometriosis. They noted that women with late-stage endometriosis had higher serum levels of IGF-I than did women with the early-stage endometriosis (34). A laboratory study revealed that the mRNA expression level of IGF-I increased during prostate cancer progression in a mouse model (35). These studies suggest that IGF-I may also mediate disease progression because the protein modulates cell proliferation and differentiation.

Several studies have reported an association between IGFBP-3 and reduced risk of disease (11–20). In a previous study on lung cancer, we found that higher levels of plasma IGFBP-3 were correlated with reduced risk of the disease after adjustment for IGF-I (13). Studies have also reported that IGFBP-3 serum levels are higher in case patients than in control subjects (11, 36–38). In three case-control studies, IGFBP-3 was higher in the serum of patients with colorectal cancer (37), breast cancer (38), and prostate cancer (12) than in the respective control subjects, but the differences were not significant. These contradictory results may be explained by the dual function of IGFBP-3. IGFBP-3 can inhibit cell growth and induce apoptosis in both IGF-dependent and IGF-independent manners. In the IGF-dependent manner, circulating IGFBP-3 modulates the amount of bioavailable free IGF and inhibits its transfer from the circulation to tissue sites of action by competitively binding to IGFs, thereby preventing their binding to IGF-IR and suppressing cell proliferation (39). IGFBP-3, in this respect, plays a protective role. IGFBP-3 can also sometimes enhance the effects of IGF by presenting and slowly releasing IGF-I for receptor interactions while protecting the receptor from downregulation by high IGF-I exposure (40). In this case, IGFBP-3 serves as a risk factor. The study reported here did not find evidence that IGFBP-3 has a protective role against the development of SILs. After adjustment for IGF-I levels, there was no relationship between IGFBP-3 levels and the risk of SILs.

In summary, this is the first study demonstrating a relationship between serum levels of IGF-I and precancerous SILs. Individuals with either high-grade or low-grade SILs exhibited significantly higher serum levels of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio than did control subjects. Furthermore, there was a dose-dependent relationship between risk for SILs

and levels of IGF-I. Because of the cross-sectional nature of the design, we cannot rule out the influence of disease status in the protein levels and the possibility that serum IGF-I may serve as a cancer marker. However, women with high-grade lesions did not exhibit higher IGF-I levels than women with low-grade lesions. In this study, we did not observe significant association between smoking and protein levels. Also, there is no direct evidence to support that HPV infection modulates protein levels. Our findings suggest an association between elevated IGF-I levels and risk of SILs. It is not known whether elevated levels are in the causal pathway or attributed to preclinical disease. Careful perspective follow-up of the cases before the development of invasive cancer and controls for development of SIL will be needed to address the issue.

ACKNOWLEDGMENTS

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