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ARTÍCULO ORIGINAL

Serum levels of insulin-like growth factor-I and -II and insulin-like growth factor binding protein 3 in women with squamous intraepithelial lesions and cervical cancer

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Introduction. Pap smear has limitations as a screening test for cervical cancer. A marker that allows the identification of women who are at risk of developing cervical cancer would be useful for its prevention. A growing number of studies have demonstrated an association between insulin-like growth factors (IGF) serum levels and increased risk for various cancers.
Objective. To assess whether circulating IGF-I, IGF-II, or IGF binding protein 3 (IGFBP-3) were associated with cervical cancer and low-grade and high-grade squamous intraepithelial lesions (LSIL and HSIL).
Materials and methods. Serum levels of IGF-I, IGF-II and IGFBP-3 were measured by ELISA. Three groups of cases were analyzed: LSIL (n = 37), HSIL (n = 57), and cervical cancer (n = 41). For each case, two controls, matched by age, were included. Control subjects were women with normal, HPV-DNA-negative Pap smear.
Results. Significantly lower values of IGF-I (83.9 ng/ml versus 126.6 ng/ml, $p < 0.001$) and IGF-I:IGFBP-3 molar ratio (0.094 versus 0.137, $p < 0.001$) were observed among cancer cases, as compared to their control group. Women in the highest quartile of IGF-I and IGF-I:IGFBP-3 molar ratio were at an 80% (OR = 0.2, 95% CI [0.06-0.61]) and a 77% (OR = 0.23, 95% CI [0.07-0.73]) lower risk of cervical cancer, respectively, compared with women in the corresponding reference category.
Conclusions. These data suggest that low values of IGF-I and IGF-I:IGFBP-3 molar ratio may be associated with cervical cancer.

Keywords: insulin-like growth factor I, insulin-like growth factor II, insulin-like growth factor binding protein 3, cervical neoplasms, cervical dysplasia .

Niveles séricos de los factores de crecimiento similares a la insulina I y II y su proteína 3 de enlace en mujeres con lesiones escamosas intraepiteliales y cáncer de cuello uterino

Introducción. La citología cervico-uterina como prueba de tamizaje del cáncer cervical tiene limitaciones. Un marcador que permita identificar mujeres en riesgo de desarrollar este cáncer sería de utilidad para prevenir el desarrollo de esta enfermedad. Múltiples estudios demuestran asociación entre los factores de crecimiento similares a la insulina (IGF) y varios tipos de cáncer.
Objetivos. Evaluar si los niveles circulantes de IGF-I, IGF-II, y la proteína 3 de enlace a IGF (IGFBP-3) se asocian con cáncer cervical y lesiones escamosas intraepiteliales de bajo y alto grado (LEIBG y LEIAG).
Materiales y métodos. Los niveles séricos de IGF-I, IGF-II e IGFBP-3 se determinaron por ELISA. Se analizaron tres grupos de casos: LEIBG (n=37), LEIAG (n=57) y cáncer cervical (n=41), apareados por edad con controles, con citología normal, negativa para ADN de VPH.
Resultados. Se observaron valores de IGF-I (83,9 ng/ml versus 126,6 ng/ml, $p < 0,001$) y de la relación molar IGF-I:IGFBP-3 (0,094 versus 0,137, $p < 0,001$) menores en los casos de cáncer,

comparados con sus controles. Las mujeres en el cuartil superior de IGF-I y de la relación molar IGF-I:IGFBP-3 tuvieron un riesgo de cáncer de cérvix 80% (OR = 0.2, IC 95% [0,06-0,61]) y 77% (OR = 0,23, IC 95% [0,07-0,73]) menor, respectivamente, en comparación con las mujeres en la categoría de referencia.
Conclusiones. Estos datos sugieren que valores bajos de IGF-I y de la relación molar IGF-I:IGFBP-3 se asocian con cáncer cervical.

Palabras clave: factor I del crecimiento similar a la insulina, factor II del crecimiento similar a la insulina, proteína 3 de enlace a factor de crecimiento similar a la insulina, neoplasmas del cuello uterino, displasia del cuello uterino.

Cervical cancer is a public health problem in developing countries (1). In Colombia, it is the most common cause of cancer mortality among women (2). Persistent infection with high-risk types of HPV (HR HPV), mainly types 16 and 18, has been identified as the main risk factor for the development of cervical cancer and its precursor lesions, squamous intraepithelial lesions (SILs) (3). SILs precede the development of cervical cancer. SILs have been classified into two groups: low-grade SIL (LSIL) and high-grade SIL (HSIL) (4). Although HPV infections are among the most frequent sexually transmitted diseases, infections are usually self-limited and revert spontaneously, with only a small group of women developing cervical cancer (5). The evolution of infection from LSIL, to HSIL and cancer depends on several factors, many of which are still unknown. Pap smear is the most used screening tool for cervical cancer and SIL. In spite of its wide use, cytology has limitations in sensitivity, specificity and reproducibility (6). HPV detection in cervical scrapings has been proposed as a complementary or alternative test to Pap smear (7), but, due to the high prevalence of HPV infections that do not progress to cancer, HPV testing has a low specificity (5). A marker that allows us to identify those women who are at risk of developing cervical cancer will be a valuable diagnostic tool.

Insulin-like growth factors (IGF-I and IGF-II) are peptides that play a pivotal role in promoting cell proliferation and inhibiting apoptosis in many cell types (8,9). IGF-I and IGF-II actions are mediated through their binding to the IGF-I receptor (IGF-IR) (10). In addition, there are six proteins that bind the IGFs with high affinity (IGFBPs) and are the major determinants of its bioavailability. Among these, IGFBP-3 is the best studied and the most abundant in serum. IGFBP-3 plays a role modulating the interaction between IGFs and IGF-IR and can induce apoptosis and inhibit cell growth independent of IGF-I (11).

Prospective and retrospective studies have demonstrated an association between IGF-I, IGF-II and IGFBP-3 serum levels and increased risk for various cancers (12-15). It has been shown in animal models that circulating IGF-I levels may play a significant role in carcinogenesis (16,17). Some studies have suggested that serum levels of IGF-I, IGF-II or IGFBP-3 may be useful biomarkers for assessing risk of SIL or cervical cancer development (18-21).

In the present study we investigated if circulating levels of IGF-I, IGF-II, and IGFBP-3 are associated with cervical cancer and precancerous lesions (LSIL and HSIL).

Materials and methods

Study Subjects

After receiving IRB approval, women were recruited at five outpatient's gynecological clinics in Bogotá (Liga Colombiana de Lucha contra el Cáncer, Hospital de La Samaritana, Hospital de La Granja, and Instituto Nacional de Cancerología) from April 2002 through April 2003. The inclusion criteria for the study were: women younger than 65 years, not pregnant, no previous history of hysterectomy or cancer, and not under treatment. All subjects signed written informed consents and completed an in-person interview, which elicited

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information on demographic features, reproductive and sexual history, and tobacco use. Clinical and pathological information for both the case and the control groups were obtained by a retrospective chart review. Blood samples and cervical scrapings were obtained; serum was separated from each blood sample by centrifugation and stored at -70°C. Case subjects were patients with confirmed histological diagnostic of SIL or cervical cancer. Control subjects were women with normal, HPV-DNA-negative Pap smear. The sample size was not estimated in this study because there are no reported values of IGF-I, IGF-II and IGFBP-3 serum levels in Colombian women and these levels show important variations and wide distributions among different populations worldwide. Three groups of cases were conformed: LSIL (n = 37), HSIL (n = 57), and cervical cancer (n = 41). For each case, two controls were selected. Controls were age-matched because age is the main determinant of IGF serum levels (22).

IGF-I, IGF-II, and IGFBP-3 levels

Commercially available ELISA kits (DSL, Webster, TX) were used to determine the serum levels of IGF-I (DSL-10-5600), IGF-II (DSL-10-2600), and IGFBP-3 (DSL-10-6600). The mean intra- and inter-assay variation coefficients for the controls were, respectively, 4.1% and 8.6% for IGF-I, 9.8% and 13.8% for IGF-II and 4.2% and 11.9% for IGFBP-3. ELISA was performed according to the manufacturer's instructions. Standards, controls, and samples were tested in duplicate. The IGF-I, IGF-II, and IGFBP-3 serum concentrations were determined from the standard curve by matching the absorbance readings with the corresponding IGF-I, IGF-II, and IGFBP-3 concentrations.

HPV analysis

Cervical cells from the transformation zone of the cervix were collected with a cytobrush; harvested cells were suspended in a saline solution and subsequently centrifuged at 3,000 x g, 10 min. Cellular pellet was suspended in 0.75 ml of Trizol (Gibco) for DNA extraction. Quality of DNA was tested by amplifying the ß-globin gene using the primers PCO3/PCO5, as described by Saiki et al. (23). HPV detection was performed according to the techniques described by Roda-Husman et al. (24). Each sample was tested with a generic primer-mediated PCR with GP5+/GP6+ consensus primers. PCR-amplified products were further tested in a non-isotopic single-strand conformational polymorphism (SSCP) for HPV16 and HPV18, as described by Zehbe et al. (25). PCR-amplified products, excluding positive ones for HPV16 or HPV18 were typed by direct DNA sequencing by using the Termo Sequenase Cy5.5 dye terminator cycle sequencing kit and the SEQ 4x4 automated sequencer (Amersham Pharmacia, Biotech).

Statistical Analysis

Statistical analysis was conducted using SPSS for Windows, version 11.5 (SPSS Inc., 2002). Distributions of age, use of hormonal contraceptives, parity, smoking status and cancer stage were described, as means and standard deviations or percentages. Distributions of IGF-I, IGF-II, and IGFBP3 serum levels, and IGF-I:IGFBP-3 molar ratio ([IGF-I x 0.13] / [IGFBP-3 x 0.035]) were described, as means, standard deviations, medians, and interquartile ranges. Differences in the distributions of these variables between cases and controls were tested by using the McNemar test for categorical variables and the Wilcoxon signed rank test for continuous variables. Circulating levels of IGF-I, IGF-II, IGFBP-3, and IGF-I:IGFBP-3 molar ratio were categorized into quartiles, according to the distribution of values in their respective control groups for analysis by univariate and multivariate conditional logistic models. Distributions of serum levels into quartiles were described by numbers and percentages. Univariate and multivariate logistic regression models were used to assess the association between cervical cancer, LSIL, and HSIL and circulating levels of IGFs. The lowest quartile of each analyzed variable was taken as the reference category, and crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for the other three quartile levels. First, crude OR and 95% CI were calculated in each one of the three groups (LSIL, HSIL, and cancer) for each variable of interest (IGF-I, IGF-II, and IGFBP3 plasma levels and IGF-I:IGFBP-3 molar ratio). Then multivariate models were used to assess the association between each IGF

marker and LSIL, HSIL, and cancer while adjusting for variables associated with cervical cancer risk (use of hormonal contraceptives, smoking status, and parity). IGF-I and IGF-II were adjusted for IGFBP-3, and IGFBP-3 was as well for IGF-I and IGF-II, because IGFBP-3 has an effect on IGF-I and IGF-II activities. Intra-group differences in IGF levels in LSIL and HSIL related to HR HPV presence and intra-group differences in IGF levels in cervical cancer related to stage of disease (I-II versus III-IV) were tested by using the Mann-Whitney test. All p values were two-sided and considered significant at $p < 0.05$.

Results

Tables 1, 2 and 3 summarize the distributions of LSIL, HSIL, and cervical cancer cases and their respective controls by age, tobacco use, reproductive factors, and FIGO stage; and the distributions of IGF-I, IGF-II, and IGFBP-3 levels and IGF-I:IGFBP-3 molar ratio for each group. We observed differences in mean age between groups; in HSIL and cervical cancer, the mean age was higher than in LSIL, which can be related to the progression in time from LSIL to HSIL to cervical cancer. There were no differences in the distribution of tobacco and hormonal contraceptives use. In

Table 1. Characteristics of LSIL cases and controls and IGF serum levels.

Variables	LSIL		*P*
	Cases (n = 37)	Controls (n = 74)	
Mean age (yr) ± s.d.	30.7 ± 8.9	31.2 ± 8.4	
Ever smoked No. (%)	12 (32.43)	21 (28.38)	0.607 [a]
Ever HC use No. (%)	12 (32.43)	28 (37.84)	0.742 [a]
Parity	1.74 ± 1.78	1.95 ± 1.61	0.211 [b]
IGF-I ng/ml			
Mean ± s.d.	197.0 ± 147.0	180.7 ± 100.9	
Median (IQ range)	176.8 (66.0-290.2)	173.9 (115.3-226.3)	0.414 [b]
1st quartile No. (%)	13 (35.14)	18 (24.32)	
2nd quartile No. (%)	5 (13.51)	19 (25.68)	
3rd quartile No. (%)	4 (10.81)	19 (25.68)	0.057 [a]
4th quartile No. (%)	15 (40.54)	18 (24.32)	
IGF-II ng/ml			
Mean ± s.d.	646.84 ± 144.06	674.3 ± 208.3	
Median (IQ range)	619.2 (520.5-743.0)	631.7 (516.2-814.2)	0.520 [b]
1st quartile No. (%)	8 (21.62)	18 (24.32)	
2nd quartile No. (%)	12 (32.43)	19 (25.68)	
3rd quartile No. (%)	12 (32.43)	19 (25.68)	0.517 [a]
4th quartile No. (%)	5 (13.51)	18 (24.32)	
IGFBP-3 ng/ml			
Mean ± s.d.	3513.2 ± 1085.7	3912.9 ± 959.5	
Median (IQ range)	3494.9 (2995.5-4141.4)	3884.0 (3149.3-4557.7)	0.020 [b]
1st quartile No. (%)	13 (35.14)	18 (24.32)	
2nd quartile No. (%)	12 (32.43)	19 (25.68)	
3rd quartile No. (%)	4 (10.81)	19 (25.68)	0.246 [a]
4th quartile No. (%)	8 (21.62)	18 (24.32)	
Molar Ratio			
Mean ± s.d.	0.205 ± 0.135	0.173 ± 0.089	
Median (IQ range)	0.203 (0.076-0.302)	0.161 (0.103-0.212)	0.058 [b]
1st quartile No. (%)	12 (32.43)	18 (24.32)	
2nd quartile No. (%)	3 (8.11)	19 (25.68)	
3rd quartile No. (%)	6 (16.21)	19 (25.68)	0.040 [a]
4th quartile No. (%)	16 (43.24)	18 (24.32)	

yr: year; No., n: number; LSIL: low-grade squamous intraepithelial lesion; HC: Hormonal contraceptives; s.d: standard deviation; IQ: Interquartile; Molar ratio = [(IGF-I x 0.13)/ (IGFBP-3 x 0.035)]; [a] McNemar test; [b] Wilcoxon signed rank test.

Table 2. Characteristics of HSIL cases and controls and IGF serum levels.

Variables	HSIL		*P*
	Cases (n = 57)	Controls (n = 114)	
Mean age (yr) ± s.d.	38.6 ± 10.4	38.9 ± 9.6	
Ever smoked No. (%)	12 (21.10)	28 (24.56)	0.635 [a]
Ever HC use No. (%)	23 (40.35)	39 (34.21)	0.464 [a]
Parity	3.25 ± 2.29	2.39 ± 1.79	0.010 [b]
IGF-I ng/ml			
Mean ± s.d.	154.6 ± 104.3	158.3 ± 93.8	
Median (IQ range)	133.5 (62-235.1)	144.8 (79.9-223.8)	0.700 [b]
1st quartile No. (%)	18 (31.6)	28 (24.6)	
2nd quartile No. (%)	12 (21.1)	29 (25.4)	
3rd quartile No. (%)	12 (21.1)	29 (25.4)	0.717 [a]
4th quartile No. (%)	15 (26.3)	28 (24.6)	
IGF-II ng/ml			
Mean ± s.d.	630.6 ± 191.3	646.5 ± 175.7	
Median (IQ range)	581.58 (498.3-712.2)	626.29 (518.1-729.9)	0.207 [b]
1st quartile No. (%)	17 (29.8)	28 (24.6)	
2nd quartile No. (%)	15 (26.3)	29 (25.4)	
3rd quartile No. (%)	13 (22.8)	29 (25.4)	0.869 [a]
4th quartile No. (%)	12 (21.1)	28 (24.6)	
IGFBP3 ng/ml			
Mean ± s.d.	3793.0 ± 1209.3	3698.4 ± 866	
Median (IQ range)	3727.9 (3092.1-4293.2)	3675.4 (3087-4374.7)	0.805 [b]
1st quartile No. (%)	13 (22.8)	28 (24.6)	
2nd quartile No. (%)	15 (26.3)	29 (25.4)	
3rd quartile No. (%)	16 (28.1)	29 (25.4)	0.976 [a]
4th quartile No. (%)	13 (22.8)	28 (24.6)	
Molar Ratio			
Mean ± s.d.	0.149 ± 0.090	0.162 ± 0.092	
Median (IQ range)	0.144 (0.066-0.226)	0.151 (0.085-0.229)	0.428 [b]
1st quartile No. (%)	17 (29.8)	28 (24.6)	
2nd quartile No. (%)	13 (22.8)	29 (25.4)	
3rd quartile No. (%)	14 (24.6)	29 (25.4)	0.904 [a]
4th quartile No. (%)	13 (22.8)	28 (24.6)	

yr: year; No., n: number; HSIL: high-grade squamous intraepithelial lesion; HC: Hormonal contraceptives; s.d: standard deviation; IQ: Interquartile; Molar ratio = [(IGF-I x 0.13)/ (IGFBP-3 x 0.035)]; [a] McNemar test; [b] Wilcoxon signed rank test.

contrast, parity was significantly higher in HSIL cases and in cervical cancer cases than in their corresponding controls ($p = 0.01$ and $p < 0.001$, respectively).

A statistically significant lower mean circulating level of IGFBP-3 was observed among LSIL cases than among controls (3,513.2 ng/ml *vs* 3,912.9 ng/ml, $p = 0.02$). In contrast, the mean values of IGF-I and IGF-I:IGFBP-3 molar ratio were significantly lower among cancer cases than among controls. Mean levels of IGF-I were 83.9 ng/ml and 126.6 ng/ml ($p < 0.001$), and IGF-I:IGFBP-3 molar ratios were, respectively, 0.094 and 0.137 ($p < 0.001$) for cervical cancer cases and the corresponding controls. We did not find any significant differences in IGF-I, IGF-II, and IGFBP-3 levels in LSIL or HSIL according to HR HPV (Table 4).

Crude and adjusted estimates of the association (OR and 95% CI) between IGF markers and cervical cancer and precancerous lesions (LSIL and HSIL) are presented in Table 5.

In LSIL and HSIL groups we did not find any statistically significant association with serum levels analyzed in the univariate or multivariate logistic regression analysis. In the univariate

Table 3. Characteristics of cervical cancer cases and controls and IGF serum levels.

	Cervical cancer		
	Cases (n= 41)	**Controls (n=78)**	***P***
Mean age (yr) ± s.d.	43.2 ± 8.3	43 ± 8	
Ever smoked, No. (%)	9 (22 %)	23 (29.5%)	0.405 [a]
Ever use of HC, No. (%)	9 (22 %)	27 (34.6 %)	0.188 [a]
Parity	4.46 ± 2.44	2.92 ±1.85	<0.001 [b]
FIGO stage No. (%)			
I	8 (19.5 %)		
II	11 (26.8 %)		
III	18 (43.9 %)		
IV	2 (4.9 %)		
Unknown	2 (4.9 %)		
IGF-I ng/ml			
Mean ± s.d.	83.9 ± 77.3	126.6 ± 76.6	
Median (IQ range)	51.9 (26.6-124.9)	107.7 (68.7-179)	<0.001 [b]
1st quartile No. (%)	26 (63.41)	19 (24.36)	
2nd quartile No. (%)	3 (7.32)	20 (25.64)	<0.001 [a]
3rd quartile No. (%)	7 (17.07)	20 (25.64)	
4th quartile No. (%)	5 (12.20)	19 (24.36)	
IGF-II ng/ml			
Mean ± s.d.	594.3 ± 137.3`	638.3 ± 162	
Median (IQ range)	602 (476.2-703.9)	610 (518.4-707.4)	0.194 [b]
1st quartile No. (%)	11 (26.83)	19 (24.36)	
2nd quartile No. (%)	10 (24.39)	20 (25.64)	0.983 [a]
3rd quartile No. (%)	11 (26.83)	20 (25.64)	
4th quartile No. (%)	9 (21.95)	19 (24.36)	
IGFBP-3 ng/ml			
Mean ± s.d.	3284 ± 840.6	3567.6 ± 831	
Median (IQ range)	3147.8 (2546.3-3955.9)	3519.2 (2944.9-4105.7)	0.066 [b]
1st quartile No. (%)	15 (36.59)	19 (24.36)	
2nd quartile No. (%)	11 (26.83)	20 (25.64)	0.395 [a]
3rd quartile No. (%)	6 (14.63)	20 (25.64)	
4th quartile No. (%)	9 (21.95)	19 (24.36)	
Molar ratio			
Mean ± s.d.	0.094 ± 0.082	0.137 ± 0.085	
Median (IQ range)	0.065 (0.032-0.114)	0.111 (0.075-0.208)	<0.001 [b]
1st quartile No. (%)	22 (53.66)	19 (24.36)	
2nd quartile No. (%)	7 (17.07)	20 (25.64)	
3rd quartile No. (%)	7 (17.07)	20 (25.64)	0.015 [a]
4th quartile No. (%)	5 (12.20)	19 (24.36)	

yr: year; No., n: number; HC: Hormonal contraceptives; s.d: standard deviation; IQ: Interquartile ; FIGO: Federation Internationale de Gynecologie et d'Obstetrique. Molar ratio = (IGF-I x 0.13) / (IGFBP-3 x 0.035); [a] McNemar test; [b] Wilcoxon signed rank test.

logistic regression analysis IGF-I levels and IGF-I:IGFBP-3 molar ratio were found to be inversely associated with cervical cancer. Women in the highest quartile of IGF-I and IGF-I:IGFBP-3 molar ratio were at 80% (OR = 0.2) and 77% (OR = 0.23) lower risk of cervical cancer, respectively, as compared with women in the reference category. After adjusting for variables associated with cervical cancer risk and with IGFBP-3, this inverse association remained statistically significant only for women in the second and third quartiles of IGF-I (OR = 0.12, 95% IC [0.03-0.51] and OR = 0.27, 95% IC [0.09-0.83], respectively). On the other hand, after adjusting for variables associated with cervical cancer risk, the inverse association between IGF-I:IGFBP-3 molar ratio and risk of cervical cancer remained statistically significant for women in the third and fourth

Table 4. Intragroup comparison of IGF-I, IGF-II, IGFBP-3 and IGF-I:IGFBP-3 molar ratio by high-risk HPV presence or absence in LSIL and HSIL.

	LSIL		P*	HSIL **		P*
	HR HPV (-) n = 24	HR HPV (+) n = 13		HR HPV (-) n = 25	HR HPV (+) n = 28	
IGF-I (ng/ml)						
Mean ± s.d.	167.5 ± 111.9	251.5 ± 189.5		156.8 ± 102.1	139.1 ± 106.6	
Median	158.0	278.84	0.306	131.7	110.7	0.454
Interquartile range	58.5 - 271.8	71.5 - 388.5		95.5 - 225.5	51.2 - 223.7	
IGF-II (ng/ml)						
Mean ± s.d.	674.6 ± 152.6	595.6 ± 115.2		665.2 ± 202.8	591.4 ± 157.6	
Median	674.6	619.2	0.212	647.0	536.2	0.128
Interquartile range	572.0 - 799.5	480.5 - 671.4		537.2 - 725.2	480.3 - 693.3	
IGFBP-3 (ng/ml)						
Mean ± s.d.	3482.3 ± 1007.4	3570.2 ± 1259.2		3972.7 ± 1190.9	3622.6 ± 1262.0	
Median	3483.73	3703.83	0.695	3910.5	3440.3	0.193
Interquartile range	3025.0 - 3916.1	2457.0 - 4672.1		3190.5 - 4403.4	2846.9 - 4035.6	
Molar ratio						
Mean ± s.d.	0.181 ± 0.110	0.251 ± 0.167		0.145 ± 0.082	0.137 ± 0.090	
Median	0.182	0.205	0.276	0.134	0.123	0.735
Interquartile range	0.075 - 0.292	0.109 - 0.393		0.084 - 0.226	0.058 - 0.201	

LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion. HR HPV: high-risk human papillomavirus. *: Mann-Whitney test. **: Excluding 4 cases positives for HPV of undetermined type.

quartiles (OR = 0.32, 95% IC [0.1-0.98] and OR = 0.26, 95% IC [0.08-0.89], respectively). For IGF-II and IGFBP-3 serum levels in cervical cancer the differences between cases and controls were not statistically significant (Table 5).

In order to investigate whether the observed inverse association between IGF-I and cervical cancer risk could be related to alterations in catabolism and nutrition associated to advanced stages of cancer, we compared serum levels of IGF-I, IGF-II, and IGFBP-3 and IGF-I:IGFBP-3 molar ratio between stages I-II *vs* III-IV. We did not find any significant differences (Table 6).

Discussion

Many studies have suggested that high circulating IGF-I levels are associated with increased risk of prostate, premenopausal breast, and colon cancer (26); however, an inverse association has also been reported for gastric (27), endometrial (28,29), liver (30), and lung cancer (31). In this study we found an inverse association between cervical cancer risk and IGF-I circulating levels. In concordance with this result, IGF-I:IGFBP-3 molar ratio was also inversely associated with cervical carcinoma. These inverse associations were also observed in HSIL but did not reach statistical significance, maybe due to small sample size. Since IGF-I circulating levels can be affected by the malignant disease itself, the observed lower levels of IGF-I in cancer might be more a consequence of the malignant disease than an associated risk factor.

Over 75% of IGF-I in the circulation is produced in the liver. The main physiological stimuli for hepatic IGF-I synthesis are growth hormone (32) and nutrition (33). It is plausible that the reduced circulating IGF-I levels in cancer patients may be a consequence of alterations in catabolism and nutrition, conditions commonly present in advanced stages of this disease. However, we did not find significant differences in IGF levels according to FIGO stage (I-II versus III-IV).

In this case-control study we cannot establish whether this inverse association is a risk factor or a marker for disease. To solve this question we would need a prospective study, like the reported ones in other cancers like prostate (34), breast (12) and lung (15) cancer. However this kind of study is difficult to carry out for cervical cancer,

Table 5. Odds ratio and 95% confidence intervals for LSIL, HSIL and cervical cancer according to serum levels of IGF-I. IGF-II. IGFBP3 and IGF-I:IGFBP3 molar ratio.

		OR (95% IC) associated with quartile			
Group	**Variable**	**1**	**2**	**3**	**4**
LSIL	**IGF-I**				
	Unadjusted	1.00	0.37 (0.11-1.23)	0.30 (0.08-1.07)	1.15 (0.43-3.08)
	Adjusted[a]	1.00	0.40 (0.11-1.43)	0.40 (0.10-1.60)	3.32 (0.92-12.09)
	IGF-II				
	Unadjusted	1.001	1.42 (0.47-4.25)	1.42 (0.47-4.25)	0.63 (0.17-2.28)
	Adjusted[a]	1.00	1.70 (0.52-5.60)	1.91 (0.58-6.29)	0.83 (0.21-3.20)
	IGFBP3				
	Unadjusted	1.00	0.88 (0.32-2.40)	0.30 (0.08-1.07)	0.62 (0.21-1.84)
	Adjusted[b]	1.00	0.72 (0.25-2.08)	0.23 (0.06-0.90)	0.49 (0.14-1.70)
	Molar ratio				
	Unadjusted	1.00	0.24 (0.06-0.99)	0.48 (0.15-1.53)	1.33 (0.50-3.57)
	Adjusted[c]	1.00	0.20 (0.05-0.89)	0.62 (0.18-2.10)	1.87 (0.63-5.51)
HSIL	**IGF-I**				
	Unadjusted	1.00	0.65 (0.26-1.58)	0.64 (0.26-1.58)	0.83 (0.35-1.97)
	Adjusted[a]	1.00	0.56 (0.22-1.43)	0.67 (0.26-1.71)	0.85 (0.33-2.22)
	IGF-II				
	Unadjusted	1.00	0.85 (0.36-2.02)	0.74 (0.31-1.80)	0.71 (0.29-1.75)
	Adjusted[a]	1.00	0.85 (0.36-2.02)	0.74 (0.28-1.94)	0.63 (0.23-1.72)
	IGFBP3				
	Unadjusted	1.00	1.11 (0.45-2.75)	1.19 (0.48-2.90)	1.00 (0.40-2.53)
	Adjusted[b]	1.00	1.58 (0.60-4.16)	1.45 (0.56-3.80)	1.30 (0.45-3.78)
	Molar ratio				
	Unadjusted	1.00	0.74 (0.31-1.80)	0.80 (0.33-1.91)	0.76 (0.31-1.87)
	Adjusted[c]	1.00	0.66 (0.26-1.67)	0.92 (0.37-2.27)	0.84 (0.34-2.12)
Cancer	**IGF-I**				
	Unadjusted	1.00	0.11 (0.03-0.43)	0.26 (0.09-0.73)	0.20 (0.06-0.61)
	Adjusted[a]	1.00	0.12 (0.03-0.51)	0.27 (0.09-0.83)	0.30 (0.08-1.05)
	IGF-II				
	Unadjusted	1.00	0.86 (0.30-2.50)	0.95 (0.33-2.70)	0.82 (0.28-2.42)
	Adjusted[a]	1.00	1.06 (0.32-3.48)	1.32 (0.38-4.64)	1.38 (0.40-4.80)
	IGFBP3				
	Unadjusted	1.00	0.70 (0.26-1.89)	0.38 (0.12-1.19)	0.60 (0.21-1.70)
	Adjusted[b]	1.00	0.96 (0.30-3.10)	0.28 (0.07-1.08)	1.52 (0.43-5.40)
	Molar ratio				
	Unadjusted	1.00	0.30 (0.11-0.87)	0.30 (0.11-0.87)	0.23 (0.07-0.73)
	Adjusted[c]	1.00	0.38 (0.12-1.15)	0.32 (0.10-0.98)	0.26 (0.08-0.89)

LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion. OR: Odds ratio; 95% CI: 95% confidence intervals; Molar ratio = (IGF-I x 0.13) / (IGFBP-3 x 0.035). [a] Adjusted by smoking. hormonal contraceptives use, parity and IGFBP3. [b] Adjusted by smoking, hormonal contraceptives use, parity and IGF-I e IGF-II. [c] Adjusted by smoking, hormonal contraceptives use and parity.

because Pap smears detect preneoplastic lesions, which ethically forces intervention.

There is one previous report about circulating IGF-I level in cervical cancer. Ayabe et al. (35), in a small study of 11 cervical cancer patients and 27 controls, found not significant association between serum levels of IGF-I and cervical cancer risk. The difference between their results and ours can be mainly related to sample size.

We did not find significant association between precancerous lesions (LSIL and HSIL) and IGF-I serum levels. Conversely, Wu et al. (20), in a study of 267 SIL (HSIL and LSIL) cases and 238 controls, reported an association between elevated serum levels of IGF-I and risk of SIL. This discrepancy cannot be explained by differences in the analytical methods, because the same commercially available assay systems and a

Table 6. Levels of IGF-I, IGF-II, IGFBP-3 and IGF-I:IGFBP-3 molar ratio by cancer stage.

	Cervical cancer stage*		
	Stage I-II (n = 19)	**Stage III-IV (n = 20)**	***P*****
IGF-I ng/ml			
Mean ± s.d.	94.6 ± 82.2	68.6 ± 63.5	
Median	53.6	51.6	0.496
Interquartile range	27.2-145.1	26.1-79.0	
IGF-II ng/ml			
Mean ± s.d.	620.5 ± 136.3	578.0 ± 134.6	
Median	657	571.2	0.258
Interquartile range	525.3-727.4	469.9-678.7	
IGFBP3 ng/ml			
Mean ± s.d.	3369.8 ± 822.6	3251.8 ± 893.0	
Median	3169.9	3032.5	0.588
Interquartile range	2709.7-4131.9	2524.3-3986.2	
Molar ratio			
Mean ± s.d.	0.103 ± 0.088	0.079 ± 0.063	
Median	0.07	0.07	0.380
Interquartile range	0.04-0.14	0.03-0.10	

*: Excluding 2 cases without data for disease stage.
**: Mann-Whitney test.

similar statistical approach were used in both studies, and other factors inherent to the sample, as ethnicity, lifestyle, and diet can affect the result.

For IGF-II and IGFBP-3 serum levels, we did not find any significant differences in SIL or cancer. Mathur et al. (18,19,21) report significantly increased IGF-II and decreased IGFBP-3 serum levels in women with SIL and cervical cancer. In this study we used the same commercial assays, but our statistical approach was quite different and the small sample subdivided into many different groups used by Mathur et al. can explain these discrepancies. In contrast, Wu et al. found higher serum levels of IGFBP-3 in SIL cases than in controls (20). In other cancer types, as breast, prostate, colon and lung, that have been studied in greater extent in relation to IGF serum levels, there are also contradictory results (26). The complexity of the IGF system and its regulation makes it difficult to perform direct comparisons of results, and this hinders the use of serum levels of IGF-I, IGF-II and IGFBP-3 as biomarkers in cervical cancer.

It has been well established that HPVs are involved in the genesis of cervical cancer. Our findings in intra-group comparisons between presence and absence of HPV and serum levels of IGF-I, IGF-II, and IGFBP-3 do not support an association between HPV infection and IGFs.

In summary, our findings suggest an association between lower serum levels of IGF-I and a lower IGF-I:IGFBP-3 molar ratio and cervical cancer. The case-control design of the study did not allow us to determine whether lower IGF-I levels are cause (risk) or consequence (tumor marker) of the malignant disease.

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Conflict of interest

None declared.

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