Full Length Research Article

Vascular Endothelial Growth Factor (VEGF) expression and clinico-pathological characteristics of cervical cancer in the region of Casablanca-Settat

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ABSTRACT

Background: The development of angiogenesis or novel blood vessels is essential to the tumors growth. Inducing and controlling angiogenesis is primarily the function of vascular endothelial growth factor (VEGF).

Methods: The expression of VEGF in tumor tissue from 327 cervical cancer cases treated at the Mohammed VI Center of Cancer Treatment Gynecological Obstetrics service of the Ibn Rochd Hospital (Casablanca) between 2013 and 2017 was investigated using an immunohistochemical technique.

Results: The average age was 50.93 ± 12.52 years. Squamous cell carcinoma accounted for 87.5% of all histological types. More than 76.4% of patients had cervical cancer that was in the middle or advanced stages when they first arrived. The cytoplasmic signal of VEGF was low in 93.3% of patients with high-grade intraepithelial lesions, higher in 87.5% of cases with stage IB2, and slightly lower in 71.4% of instances.

Conclusion: This study identified various issues and requirements of Moroccan women with cervical cancer. Our research will contribute to cervical cancer research in Morocco and aid in the development of novel methods. However, our findings indicate that VEGF is a critical biomarker that serves as a significant clinical predictor of the response of patients with cervix cancer to radiochemotherapy.

INTRODUCTION

Uterine cervical cancer is one of the most common malignant carcinomas that pose a major threat to women's lives. It ranks second in terms of morbidity after breast cancer and accounts for over half of all malignant tumors that affect the female reproductive system [1].

Cervix cancer (CC) is known as a significant issue for Moroccan public health. In terms of incidence and death, it ranks second among Moroccan women, after breast cancer.

With 3,388 new cases and 2,465 deaths recorded each year, the annual incidence rates age-standardized CC and mortality are respectively 17.2 and 12.6 per 100,000 women. CC affects young women and is most often only discovered at an advanced stage.

Due to absence of a national registry, the current statistics could be much higher than published data, which were based and limited in cases registered in some oncology centers [2].

Recent research has confirmed that angiogenesis is directly related to the development, occurrence, metastasis, and invasion of cancers [3,4]. Tumor tissue stops developing when its diameter reaches 1 to 2 mm if blood vessel development is not present. Thus, neoplastic angiogenesis is a necessary condition for the growth of tumors. Vascular growth inhibitors and vascular growth factors work in concert to produce angiogenesis [5].

It appears that angiogenesis occurs early in pre- malignant cervix lesions and guarantees neovascularization and the spread of cancer cells [6,7]. Actually, as the grade of cervical intraepithelial neoplasia grows, so does the microvessel density [3, 8].

A balance between pro- angiogenic and anti- angiogenic elements controls the angiogenesis process in cervical cancer [9]. Angiogenesis is started when this balance is disturbed by an increase in several factors, including hypoxia, which initiates the Hypoxia- Inducible Factor subunit alpha (HIF1α) [9, 10, 11].

Vascular and endothelial cell formation, blood vessel proliferation, and other processes are among the many roles that VEGF performs. Each of these processes promotes the proliferation and spread of tumor cells [12, 13].

VEGF protein expression is an important predictor of cervical cancer prognosis. In fact, VEGF expression was associated with a poor prognosis; it was considered the most significant independent prognostic factor for overall survival and metastasis free survival [14, 15].

VEGF activity in cervical cancer is modulated by HPV-16 E6 oncoprotein [16]. The viral oncoproteins E6 and E7 assure the development of cancer cells in cervical cancer by suppressing the activity of p53 and pRB tumor suppressors [17]. It has been found that treatment of HPV18+ cells lines with Cidofovir and radiation inhibits proangiogenic phenotype of HPV18+ and decrease of E6 and VEGF expression. However, treatment cells with radiation alone significantly increase VEGF mRNA expression [18].

Cervical cancer treatment differs from early to advanced stage of disease. For early stage of cervix cancer, the treatment is based on surgery alone, whereas treatment with brachytherapy and chemoradiotherapy remains very important for patients with advanced cervical cancer [19]. Despite the technological development in terms of cervical cancer treatment, there are still more than one- third of cases who showed a recurrence or metastatic disease because of radioresistance [20]. The presence of hypoxia in tumor microenvironment induces the resistance to radiotherapy due to upregulation of VEGF and HIF1α, which induce tumor growth and survival [20, 21].

The aim of this study is to retrospectively evaluate 327 patients with cervical cancer disease, determine clinicopathological characteristics in a Moroccan population, and analyze correlations between different parameters. Investigate by immunohistochemical analysis the VEGF- A expression during different stages of cervical cancer tissues after treatment with radiochemotherapy.

METHODS

Design and population

From 2013 to 2017, 327 uterine cervical cancer tissues were extracted from patients treated at the Mohammed IV Center for Cancer Therapy, University Hospital of Ibn Rochd of Casablanca. The patients' average age was 50.93 ± 12.52 years. The specimens were embedded in formalin-fixed paraffin for storage at the Pathological Anatomy Service.

The research ethics committee of the organization approved the trial, and each patient signed a written consent form.

Collecting data

The element pertaining to sociodemographic traits (health insurance, residence of patients, Level of education, Socioeconomic level, First Sexual experience, Multi- Sexual Partner and smoker), Clinicopathological parameters (FIGO classification of cervical cancer, lymph node and vascular embolism, tumor size, histological types, Pelvic lymph node, Lymphovascular invasion and HPV infection), type of treatment (surgery, radiotherapy, radiochemotherapy). All of the information was gathered using a standardized form from patient files in the center's archives.

HPV detection

Primers: A 450-bp HPV DNA fragment was amplified using the consensus primers MY09 and MY11 targeting the conserved L1 region of HPV. PCR amplification was performed using a standard thermal cycler. This approach, which uses degenerate primers, allows the detection of more than 40 genital HPV types [22, 24].

PCR conditions: The amplification reaction contained 50 µL of the amplification mixture: 500 nM of each consensus primer, 200 mM of each dNTP, 0.625 U of Taq DNA polymerase, 3 µL of DNA sample in 1X Taq polymerase buffer. The thermocycler program was: 30 cycles of 1 min of denaturation at 95°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C. For five minutes, the extension cycle was held at 72°C [22, 23].

Analysis of PCR results: PCR products were electrophoresed on 2% agarose gels and then stained with 0.8µg/mL of ethidium bromide [22, 23].

Immunohistochemistry

Formalin-fixed, paraffin-embedded, 4µm sections were deparaffinized and rehydrated after being dehydrated for an hour at 70°C and overnight at 37°C. The techniques for retrieving antigens are explained in **Table 1**. Following a 15-minute incubation period in 3% hydrogen peroxidase to inhibit endogenous peroxidase activity, the slides were rinsed with distilled water and then rinsed three times for five minutes each time in PBS. A 1:250 dilution of a mouse monoclonal antibody against VEGF-A (Dako Denmark A/S) was used to immunostain the slides, which were then allowed to sit at room temperature for 30 minutes. The tissues were then exposed for 20 minutes to a secondary antibody that was directed against mouse immunoglobulins. Then, using chromogenic substrate (Chromogen, EnVisionTM Flex substrate buffer) (Dako Denmark A/S) in accordance with the manufacturer's instructions, primary antibody binding was found. It was counterstained with Mayer's hematoxylin.

Immunohistochemical analysis was conducted with invasive cervical adenocarcinoma cases, High-grade intraepithelial lesions and low-grade intraepithelial lesions.

Data analysis

After being checked for precision, all the information collected was coded and inserted into an Excel spreadsheet (Microsoft Corporation). The data were analyzed with the assistance of Chi-square test. Statistical significance was defined as $p < 0.05$.

RESULTS

Epidemiology

This study examined 327 patients diagnosed with cervical cancer over a five-year period (2013–2017). The age range of the study population was 29– 85 years, with a mean age of 50.93 ± 12.52 years. Approximately 58.9% of the patients were over 50 years of age. Only 6.1% of the women were employed, while 91.4% were housewives. Married patients represented 81% of the study population, and 6.7% were widowed. Most of the patients were illiterate, and 87.4% belonged to a low socioeconomic level. In addition, **66.3% of the patients were urban residents**, in accordance with the data presented in **Table 2**.

The data also reveal that 9.4% of the women were HPV-positive, while 90.5% tested negative. The age of first sexual intercourse ranged between 15 and 19 years for 74.3% of the patients. Only a small proportion of the women reported smoking or having multiple sexual partners **(Table 2).**

Commentary of Table 2 :

The data presented highlights various demographic and health-related variables among the surveyed population. Notably, the majority of respondents are housewives (91.4%) and have a low socioeconomic status (87.4%), indicating a significant correlation with the level of education, where a striking 78.8% are illiterate. The chi-square tests reveal significant findings in education and socioeconomic level, with p-values of 0.000, suggesting a strong association with these variables. Conversely, other factors such as marital status, health insurance, and smoking habits did not show significant relationships, as indicated by their higher p-values. This analysis underscores the importance of education and socioeconomic factors in understanding the health landscape of this population. Further research could explore the implications of these findings on health interventions and support systems.

Squamous cell carcinoma (SCC) accounted for 87.5% of the cervical cancer cases in this study, with adenocarcinomas coming in second at 7.9% and other histological categories at 4.5%. 6.4 percent of patients with cervical cancer provided a family history of cervical cancer; 0.4 percent reported a personal history. A family history of cancer was available in only 66.7% of cases, with 38.9% of cases involving a first-grade family connection. Only 24.1% of the patients had early-stage cervical cancer (stage 0: 10.1% and stage I: 13.4%) and 76.4% of the cases were of intermediate to advanced stage (stage II: 68.6%, stage III: 6.5%, and stage IV: 1.3%), according to the FIGO classification. Chemoradiotherapy was used to treat 58.4% of the patients. The tumor diameter was 4 cm or less in 55.3% of cases and larger than 4 cm in 44.7%. The percentage of patients with positive pelvic lymph node metastasis (PLNM) was 21.4% and 15.9% of lymphovascular invasion **(Table 3).**

Data on tumor size were available for only 190 patients (58%). For the remaining 137 patients (42%), tumor size could not be retrieved due to incomplete medical records or the absence of standardized preoperative imaging reports, which is inherent to the retrospective nature of the study.

Immunohistochemical

For Immunohistochemical result, the VEGF-A expression was clearly detected in the cytoplasm of cervical cancer cells. Its staining intensity differed between groups. 93.3% of cases with high grade intraepithelial lesions showed a low cytoplasmic signal of VEGF-A (**Figure 1**). However, the VEGF-A staining was negative in all cases of low grade intraepithelial lesions. For adenocarcinoma invasive cervical cancer, the expression level of VEGF-A was increased at stage FIGO IB (FIGO Classification) for 87.5% of the cases, which represents the strongest expression of VEGF-A. Interestingly, the staining of VEGF-A declined at advanced stages IIB in 71.4% of the cases (**Figure 2**).

Figures

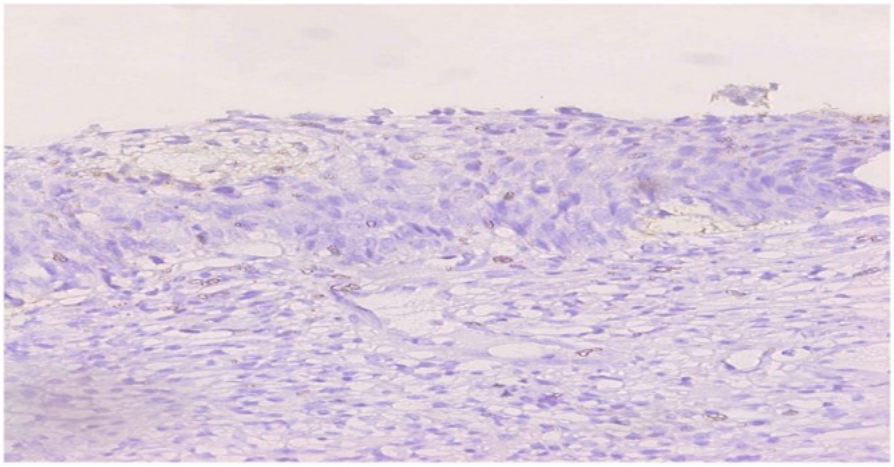

Figure 1: Immunostaining of VEGF-A in pre-invasive cervical lesions (CIN3).

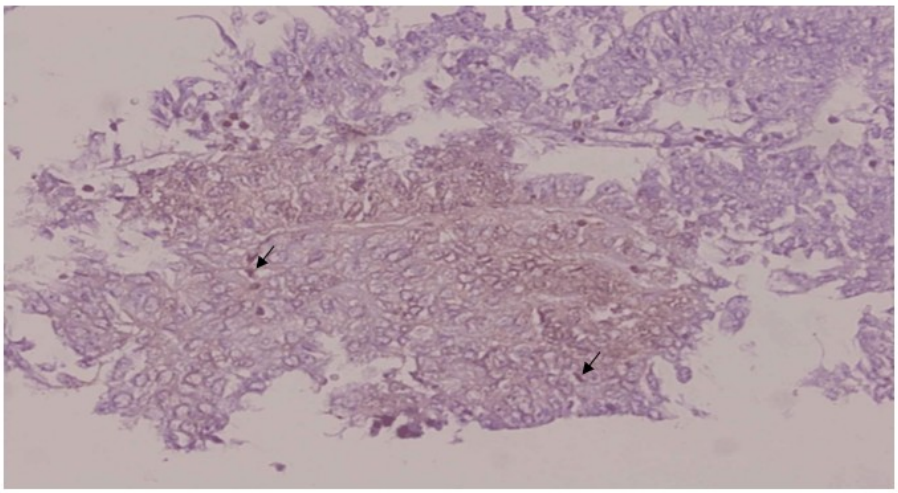

Figure 2: Immunostaining for VEGF-A in cervical adenocarcinoma stage IIB.

Tables

Category	Details
Antigen Retrieval	- Citrate buffer (pH = 6), microwave for 10 min (Target retrieval solution No. S2369). - Proteinase K, room temperature, 7 min (Dako, Glostrup, Denmark).
Primary Antibody	Mouse Anti-Human VEGF (Dako, Denmark A/S).
Positive Control	Colon (colorectal cancer).

Table 1: The three steps of immunostaining are positive control, primary antibody, and antigen retrieval.

Variables	Frequency	Percentage	χ2	ddl	p-value
Profession					
Active	20	6.1%	2.37	2	0.307 (Not significant)
Housewife	299	91.4%			
Unemployed	8	2.4%			
Marital Status					
Single	5	1.5%	4.56	3	0.208 (Not significant)
Married	265	81%			
Divorced	35	10.7%			
Widowed	22	6.7%			
Level of education					
Illiterate	258	78.8%	25.72	3	0.000 (Significant)
Primary	37	6.1%			
Secondary	32	9.7%			
High	0	0%			
Socioeconomic level					
Low	286	87.4%	78.12	2	0.000 (Significant)
Medium	38	11.6%			
High	3	0.9%			
Health insurance	228	69.7%	0.01	1	0.912 (Not significant)
Area of residence (urban):	217	66.3%	0.79	1	0.374 (Not significant)
First Sexual Experience					
< 15 years	35	10.7%	5.52	2	0.063 (Not significant)
15 – 19 years	243	74.3%			
>19 years	49	14.9%			
HPV					
Positive	31	9.4%	0.01	1	0.913 (Not significant)
Negative	296	90.5%			
Smoker	12	3.6%	1.63	1	0.201 (Not significant)
Multi-Sexual Partner	12	3.6%	1.63	1	0.201 (Not significant)

Table 2: Socioeconomic characteristics of cervical cancer patients

Clinicopathologic characteristics	Cases, n (%)	Adjuvant therapy before radical hysterectomy			χ^2	p-value
		Radiotherapy, n (%)	Chemo-Radiotherapy, n (%)	None/Other, n (%)		
Histological types (N=327)						
SCC	286 (87)	22 (8)	170 (59)	94 (33)	5.43	0.066 (NS)
Adenocarcinoma	26 (8)	3 (12)	16 (62)	7 (27)	2.55	0.110 (NS)
Others	15 (5)	0 (0)	7 (47)	8 (53)	5.38	0.068 (NS)
Tumor size (N=190)						
≤4 cm	105 (55)	9 (9)	72 (69)	24 (23)	5.79	0.016 (NS)
>4 cm	85 (45)	4 (5)	54 (64)	27 (32)	5.79	0.016 (NS)
Stage (N=306)						
CIN	31 (10)	0 (0)	4 (13)	27 (87)	4.09	0.043 (S)
I	41 (13)	4 (10)	6 (15)	31 (76)	3.63	0.057 (NS)
II	210 (69)	18 (9)	164 (78)	28 (13)	2.42	0.120 (NS)
III	20 (7)	1 (5)	14 (70)	5 (25)	3.89	0.049 (S)
IV	4 (1)	0 (0)	3 (75)	1 (25)	4.13	0.042 (S)
Pelvic lymph node (N=327)						
Presence	70 (21)	4 (6)	8 (11)	58 (83)	6.92	0.009 (S)
Absence	257 (79)	19 (7)	183 (71)	55 (21)	6.92	0.009 (S)
Lymphovascular invasion (N=327)						
Presence	52 (16)	4 (8)	18 (35)	30 (58)	8.49	0.004 (S)
Absence	275 (84)	20 (7)	175 (64)	80 (29)	8.49	0.004 (S)

Table 3: clinicopathological characteristics for cervical cancer patients who underwent adjuvant radiotherapy or chemo-radiotherapy

DISCUSSION

The purpose of the current study was to assess the predictive markers for patients with cervical cancer from the Casablanca-Settat Region of Morocco. The incidence and mortality of cervical cancer are increased by a number of factors, including the difficulty in accessing medical care once symptoms start to develop [25].

This study is the first to be performed in this context, since the opening of the Mohammed VI Center for Cancer Treatment, Gynecological Obstetrics, Ibn Rochd Hospital Casablanca in 2013. This report is also the continuation of previous research conducted between 2003 and 2007 between the two main oncological centers of Morocco which are The Oncology Department of Ibn Rochd Hospital in Casablanca and the National Institute of Oncology or "INO" Rabat.

Women in our population were mostly from low socioeconomic backgrounds. Of them, 91.4% were housewives and only 6.1% were employed. Socioeconomic factors and the risk of cervical cancer were found to be inversely related in many research [26].

Our pathological findings are consistent with those with a high prevalence of squamous cell carcinoma that have been previously reported [27, 28, 29]. In our study, squamous cell carcinoma (SCC) accounted for 87.5% of the frequent histological forms of cervical cancer, while adenocarcinoma accounted for just 7.9%. However, affluent nations have a little varied incidence of histological type [30, 31].

The tumor is less than 4 cm in 55.3% of the cases. Multiple reports support the perception that controlling a large tumor exceeding 4 cm in its diameter is more difficult than controlling a small tumor. Tumor diameter also has a bearing on the risk of pelvic lymph node metastases which is also influenced by tumor stage at diagnosis with rates of 16%, 26%, 39% and 75% for stages I, II, III and IV, respectively [32, 33, 34].

According to the study's statistical findings, cervical cancer in Morocco is diagnosed later than it should be. Indeed, 76.4% of patients in our study had intermediate or advanced stages of cervical cancer (II, III, and IV). Because treatment is less likely to be successful in advanced stages of cervical cancer, the stage of diagnosis is a crucial prognostic factor [35, 36]. Patients with an advanced stage of cancer had a poorer overall survival rate than those with an early stage [37].

Our analysis revealed a relatively low HPV detection rate (9.4%) using the MY09/11 PCR assay. Among HPV-positive cases, 4.6% were diagnosed with invasive cervical carcinoma and 2.66% with intraepithelial lesions (CIN), all corresponding to squamous cell carcinoma. This low detection rate is most likely attributable to methodological constraints inherent to retrospective molecular investigations. In particular, DNA degradation in formalin-fixed, paraffin-embedded (FFPE) tissue samples may compromise amplification efficiency. Moreover, the analytical sensitivity of the MY09/11 primer set, sequence variability among HPV genotypes, and potential disruption or deletion of the L1 target region following viral integration into the host genome may further reduce detection performance. These technical limitations should therefore be carefully considered when interpreting the present findings. The observed low HPV positivity rate most likely reflects assay-related constraints rather than the absence of a causal association. Importantly, these results do not question the well-established etiological role of HPV in cervical carcinogenesis, but rather highlight the challenges associated with retrospective HPV detection using MY09/11 primers [38].

However, HPV is detected in 99.7% of women with cervical cancer globally [39]. High-risk HPV types have been linked in the past to squamous cell carcinoma and intraepithelial lesions. This is because immune cells change at different stages of cervical cancer, resulting in a pro-inflammatory profile by removing immune and anti-inflammatory mechanisms [40, 41]. Through the upregulation of proangiogenic factors, HPV oncoproteins E6–E7 appear to be crucial in the development of tumors [39]. As the third transforming protein of the Human Papillomavirus (HPV), oncoprotein E5 is becoming more and more well-known. One characteristic of emerging malignancies is their extensive proliferation, and E5 can promote keratinocyte proliferation by upregulating the EGFR signaling pathway [42].

The most famous pro-angiogenic molecule is VEGF, which is increased in cervical neoplasia. It has been shown that high grade intraepithelial lesions and invasive cervical cancer are highly correlated with angiogenesis [43]. On the other hand, different reports display a role for angiogenesis in tumor progression and metastasis. Vascular proliferation is the main characteristic of cancer progression. The new blood vessels nourish the growing tumor, leading to close contact between microvessels and tumor cells. Additionally, a poor prognosis is associated with high VEGF antigenic expression found in cervical cancer lesions [44, 45].

In the second part of the current study, we observed the expression of VEGF among patients with high-grade intraepithelial lesions (CIN3) and the clinical effect of preoperative radiochemotherapy on VEGF expression in the advanced stage of cervical cancer (IB2-IIB1). Immunohistochemical results showed a weak staining of VEGF in CIN3. In patients with stage IB2, the expression of VEGF was higher than in cases with stage IIB. The expression of VEGF after treatment with radiochemotherapy can reflect the progression and growth of cervical

cancer tumors [46]. A previous report showed that expression of VEGF before radiochemotherapy treatment was higher than after treatment [47]. Normal cervix is characterized by hypoxic tissue, which induces the expression of different genes in cervical cancer, especially VEGF and HIF1α, to ensure tumor growth. Moreover, HIFα enhanced radioresistance in cervical cancer cells by upregulating VEGF expression [48].

Previous studies demonstrated that cidofovir with ionizing radiation has an antiangiogenic effect linked to VEGF suppression after TP53 restoration and E6 inhibition [18].

Blood vessel creation depends on the VEGF family, of which VEGFA is a crucial component in tumor angiogenesis [49]. Previous studies revealed that a higher rate of early recurrence is associated with a higher serum level of VEGFA in patients with CC following radiochemotherapy [50]. When used in conjunction with traditional chemotherapy (cisplatin or paclitaxel), the canonical humanized VEGFA monoclonal antibody bevacizumab can increase overall survival in patients with advanced cancer by 3.7 months [51]. Discovering novel molecular pathways that control VEGFA secretion is important because they may be able to stop the progression of CC [52].

In cisplatin-resistant SiHa/DDP cells, linc00958/miR-185-5p/RSF-1 depletion may inhibit VEGFA production and tube formation, according to another study. Linc00958 knockdown significantly reduced the tumor microvessel density biomarkers CD34 and VEGFA in CC mice, indicating that linc00958 knockdown may modulate tumor angiogenesis [53].

It is widely known that the lack of oxygen in the tumor microenvironment induces radioresistance. So, further research is needed to test the restoration of oxygenation before and during radiotherapy treatment by intratumoral injection in cervical cancer patients to evaluate if VEGF will be expressed after oxygenation.

Our study revealed the different problems and needs of cervical cancer women in Morocco. Our findings will be the basis for the development of new strategies for cervical cancer prevention. On the other hand, our results suggest that VEGF is a crucial biomarker that represents an important clinical predictor of radiochemotherapy response in cervical cancer patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

BENHESSOU. M and EL KEROUMI. M: Biopsy specimens and technical assistance

AMINE. A and ELANTRI. S: technical assistance and statistical analysis

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