Time-dependent apoptotic responses to vitamin E in precancerous lesions-on-chip models

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Abstract:
Cervical cancer remains a major global health issue, especially in low- and middle-income countries, with around 660,000 cases and 350,000 deaths in 2022—94% in these regions. Persistent high-risk HPV infection, along with smoking, poor nutrition, inflammation, and immunosuppression, contributes to the progression from LSIL to HSIL and cancer. This study examined how antioxidant treatments—vitamin E and Ialuna®—affect apoptosis in cervical tissue cultured in microfluidic organ-on-a-chip devices, using *Bax*/*Bcl-2* gene expression. Cervical biopsies and a control cell line were cultured in PDMS chips and treated with vitamin E or Ialuna®. Vitamin E triggered an early apoptotic response, especially in LSIL and early HSIL, while Ialuna® induced a slower, sustained effect, notably in HSIL and chronic cervicitis. *Bax*/*Bcl-2* ratios peaked at 144 hours in chronic cervicitis with vitamin E and in HSIL with Ialuna®. This platform offers a promising tool for personalized therapy, supporting a dual antioxidant strategy based on lesion severity and treatment timing.
Keywords: *Bax*/*Bcl-2* ratio, cervical cancer, HSIL, LSIL, microfluidic chip devices

1. Introduction

Cervical cancer remains a significant global health concern, particularly in low- and middle-income countries (LMICs). In 2022, approximately 660,000 women were diagnosed with cervical cancer, and about 350,000 died from the disease [1]. The development of cervical cancer is primarily associated with persistent infection by high-

risk human papillomavirus (HPV) types [2]. While HPV infection is common and often transient, certain factors can contribute to its persistence and progression to cancer. These include smoking, malnutrition, multiple sexual partners, chronic inflammation, and immunosuppression. The interplay of these factors can lead to oxidative stress, promoting cellular changes that may result in malignancy [3]. Cervical cancer typically develops through precancerous stages known as cervical intraepithelial neoplasia, which are graded as LSIL (low-grade), HSIL (high-grade) [4]. LSIL often regress spontaneously; studies indicate that approximately 60–70% regress within one year, and up to 90% within two years. However, persistent lesions can progress to HSIL, which carry a higher risk of developing into invasive cancer if left untreated [5]. Regular screening through Pap smears is crucial for early detection of cervical abnormalities [6].

The regulation of apoptosis is central to cervical carcinoma development, where the balance between pro-apoptotic (Bax) and anti-apoptotic (Bcl-2) proteins determines cell fate. The Bax/Bcl-2 ratio is therefore considered a key marker of apoptotic susceptibility, with a decreased ratio favoring tumor progression and resistance to therapy. Assessing this ratio provides insight into the molecular mechanisms underlying cervical cancer pathogenesis and treatment response [7].

Advancements in biomedical engineering have led to the development of "organ-on-a-chip" technologies, which replicate human organ functions on microfluidic devices. These advanced models promise better insight into tumor mechanisms and improved diagnosis and therapy of cervical cancer through personalized strategies and reduced dependence on animal models [8].

2. Methodlogy

Sixty women undergoing cervical biopsy after abnormal Pap tests were enrolled between August 2023 and March 2024, following approval by the Clinical Center in Kragujevac ethics committee and informed consent. Ethical constraints led to the use of a commercial squamous epithelial cell line as a control group.Patient biopsies included 19 low-grade (LSIL), 6 high-grade (HSIL), and 35 chronic cervicitis samples. Ex-tempore histopathology confirmed benign tissue before allocating part for 3D microfluidic culture.

Mold fabrication used standard UV photolithography on silicon wafers, followed by replica molding in poly(dimethylsiloxane) (PDMS). PDMS was chosen for its optical transparency, gas permeability. Polymer curing used a 10:1 Sylgard 184 (pre-polymer:crosslinker) mix [9].

Samples were treated with Ialuna® vaginalettes (D,L-alpha tocopherol acetate) dissolved at 37 °C and with solution of pure vitamin E. Treatment durations were 24, 72, and 144 hours to monitor temporal gene expression changes. Total RNA was extracted using TRIzol® according to the protocol. cDNA was synthesized from RNA using Thermo Fisher's High-Capacity Reverse Transcription Kit following manufacturer instructions. Using SYBR Green Master Mix, qPCR was performed on a real-time PCR cycler. Expression of apoptosis-related genes Bax and Bcl-2 was quantified, with β-actin

serving as the housekeeping gene [10]. Relative expression was calculated using the validated 2−ΔΔCt method according to Livak & Schmittgen [11].

3. Results and Discussion

This heatmap visualizes the *Bax*/*Bcl-2* ratios over time (24, 72, and 144 hours) for different treatment groups and disease conditions. *Bax* is a pro-apoptotic protein (promotes cell death), *Bcl-2* is an anti-apoptotic protein (prevents cell death). So, a higher *Bax*/*Bcl-2* ratio means more cell death (apoptosis) is happening (Figure 1).

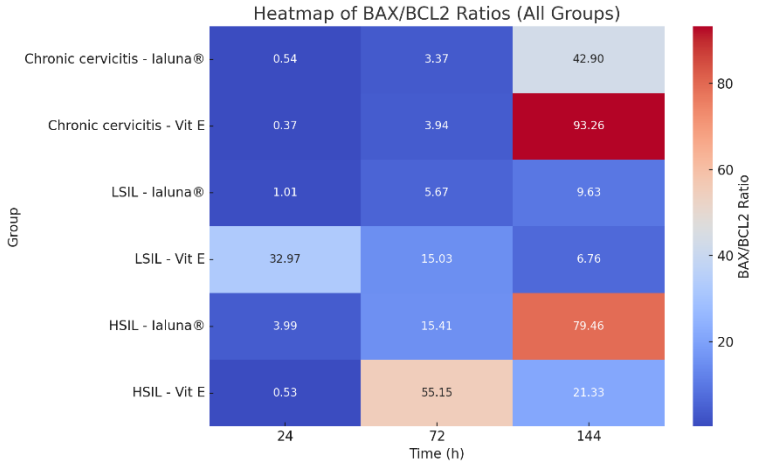

Figura 1. Heatmap of Bax/Bcl-2 ratios

Chronic cervicitis samples treated with vitamin E exhibited the highest Bax/Bcl-2 ratio at 144 hours (93.26), indicating a strong pro-apoptotic response and suggesting that vitamin E effectively promotes cell death in inflamed cervical tissue. Similarly, HSIL samples treated with Ialuna® reached a high ratio of 79.46 at 144 hours, pointing to a gradual yet significant induction of apoptosis over time. In LSIL samples, vitamin E triggered a sharp early apoptotic response at 24 hours with a ratio of 32.97; however, this effect diminished substantially by 144 hours, dropping to 6.76, indicating a transient response. HSIL tissues treated with vitamin E peaked at 72 hours with a ratio of 55.15, followed by a decrease to 21.33 at 144 hours, suggesting a temporary apoptotic effect. In contrast, chronic cervicitis samples exposed to Ialuna® showed a steady increase in apoptosis across all time points, culminating in a peak ratio of 42.90 at 144 hours. These findings highlight distinct temporal dynamics of apoptotic induction depending on both the treatment and lesion type.

4. Conclusions

Vitamin E often induces a stronger and earlier apoptotic response compared to Ialuna®, particularly in low-grade lesions such as LSIL. This suggests that vitamin E may be more effective in initiating rapid cell death in early-stage precancerous tissues, potentially preventing further progression. In contrast, Ialuna® tends to produce a slower but

sustained increase in apoptosis, especially in more advanced lesions like HSIL and in chronic inflammatory conditions. This delayed effect may be advantageous for long-term management of high-grade lesions where a prolonged therapeutic response is needed. The distinct patterns of *Bax*/*Bcl-2* expression indicate that each antioxidant has lesion-specific apoptotic activity. Monitoring these gene expression ratios provides valuable insights into treatment efficacy and the timing of cellular responses. These findings could inform personalized therapeutic strategies, optimizing antioxidant use based on lesion severity and expected response profiles.

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