

Therapeutic Assessment of Vulvar Squamous Intraepithelial Lesions with CO₂ Laser Vaporization in Immunosuppressed Patients

Avaliação terapêutica das lesões intraepiteliais escamosas da vulva com vaporização a laser CO₂ em pacientes com imunossupressão

Julisa Chamorro Lascasas Ribalta¹ Mariana Vendramin Mateussi¹ Neila Maria de Góis Speck¹

¹Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil

Address for correspondence: Julisa Chamorro Lascasas Ribalta, Rua Pirapora 225 Vila Mariana São Paulo, São Paulo, 04008-060, Brazil (e-mail: jclribalta@gmail.com).

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Abstract

Objective A vulvar squamous intraepithelial lesion is deemed to be a preceding lesion to vulvar cancer, especially in women aged under 40 years, holders of an acquired or idiopathic immunosuppression. Several treatments have been used to treat these lesions. One of the aesthetically acceptable therapeutic methods is the CO₂ laser vaporization.

Methods In a transversal study, 46 records of immunosuppressed women bearing a vulvar low grade and/or high grade squamous intraepithelial lesion were selected out of the retrospective analysis, computing age, date of record, date of vulvar lesion treatment with CO₂ laser, the time elapsed between the first and the last visit (in months), the number of visits, the presence or absence of condylomatous lesions in other female lower genital tract sites and whether or not recurrences and persistence of intraepithelial lesions have been noticed during the follow-up.

Results Patients bearing vulvar high-grade squamous intraepithelial lesion and immunosuppressed (serum positive for human immunodeficiency virus [HIV] or with solid organs transplantation) have shown a higher level of persistence of lesions and a higher chance of having other areas of the female lower genital tract involved.

Conclusion While the CO₂ laser vaporization is the most conservative method for the treatment of vulvar high-grade intraepithelial lesions, it is far from being the ideal method, due to the intrinsic infection features considered. The possibility of persistence, recurrences and spontaneous limited regression indicates that a closer surveillance in the long-term treated cases should be considered, in special for immunosuppressed patients.

Keywords

- vulvar intraepithelial neoplasia
- usual vulvar intraepithelial neoplasia
- vulvar high-grade squamous intraepithelial lesion
- CO₂ laser
- laser therapy

Resumo

Objetivo A lesão intraepitelial escamosa da vulva é considerada uma lesão precursora do câncer vulvar, em especial em mulheres com idade inferior a 40 anos, portadoras de imunossupressão adquirida ou idiopática. Vários tratamentos têm sido utilizados para

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Palavras-Chave

- neoplasia intraepitelial vulvar
- neoplasia intraepitelial vulvar usual
- lesão intraepitelial vulvar de alto grau
- CO₂ laser
- terapia a laser

tratar esse tipo de lesão. Um dos métodos terapêuticos esteticamente aceitáveis é a vaporização a laser CO₂.

Métodos Em um estudo transversal, foram selecionados da análise retrospectiva de prontuários arquivados no setor de patologia do trato genital inferior 46 prontuários de mulheres com imunossupressão e portadoras de lesão intraepitelial escamosa de baixo grau e/ou alto grau computando-se: idade, data de registro, data do tratamento da lesão vulvar com laser CO₂, tempo entre a primeira e a última consulta (em meses), número de consultas, presença ou ausência de lesões condilomatosas em outros locais do aparelho reprodutor feminino e a ocorrência ou não de recidivas e persistência de lesões intraepiteliais durante o período de acompanhamento.

Resultados Pacientes com lesão intraepitelial de alto grau vulvar e imunocomprometidas (soropositivas para HIV ou com transplante de órgãos sólidos) mostraram maior índice de persistência das lesões e maior chance de ter outras áreas do órgão genital feminino comprometidas.

Conclusão Embora a vaporização a laser CO₂ seja o método mais conservador para o tratamento das lesões intraepiteliais vulvares de alto grau, está longe de ser o método ideal pelas características intrínsecas da afecção em foco. A possibilidade de persistência, recidivas e regressão espontânea limitada recomenda maior vigilância para os casos tratados a longo prazo, em especial para as pacientes com imunossupressão.

Introduction

The vulvar high-grade squamous intraepithelial lesion (VHSIL), which was termed “vulvar intraepithelial neoplasia usual type” in the 2004 International Society for the Study of Vulvovaginal Disease (ISSVD) terminology, according to its histopathological constitution in 60 to 100% of cases, is related to the presence of the human papillomavirus (HPV), and it is presented in multifocal form.¹ The VHSIL adopts a clinical aspect infection by HPV, with verrucous or plain lesions, mainly in glabrous areas, its color varying from greyish-white to brownish. Its incidence prevails among mature women, in their third or fourth decades of life, being even higher in those holding immunosuppression. The symptoms of VHSIL vary from itching, burning, local discomfort, eventually ulceration and bleeding. However, in most of cases, the patients are symptom-free. These lesions have a discrete potential for regression and can slowly evolve into an invasive cancer, and their frequency is lower than the differentiated vulvar intraepithelial neoplasia.¹⁻⁴

The treatment of these lesions is indicated both to for pain relief and to avoid the progression of the disease into an invasive cancer. Since 1960, the radical vulvectomy was the preferred treatment among the proposed ones. Upon the recognition that this type of lesion presented a biological behavior different from that of an invasive cancer, an option was made for simple vulvectomy. The vulvar anatomy alteration resulting from the surgery and the high recurrence rates, from 30 to 60%, had influence on the search of lesions destructive therapeutic methods, such as electrocoagulation, cryocoagulation, application of chemical agents and immunomodulators. Also, the association of more than one method, such as excision, destruction or both has been used.⁴⁻⁷ The CO₂

laser vaporization is the most conservative form among the destructive methods. The CO₂ laser produces electromagnetic energy in the form of an infrared light issued with a wavelength of 10.6 μm with an average power of 10 to 10,000 W. The tissue destruction results from the raised temperature of cellular contents, transforming the intracellular water into steam, thus leading to cell lysis. With a focused beam, the CO₂ laser can also make the excision of lesion. This fact permits the performance of an anatomopathological exam, if necessary. The cure rate after a vaporization session is of 76.9% of cases, rising up to 78.4% after 2 sessions. Complications are minimal and occur only upon the improper use of the method. Attention has been brought to the high recurrence rates, which vary from 23 to 40% of cases.⁶⁻⁹

The objective of this study was to assess the share of cases that show recurrences in immunosuppressed patients and, in special, the recurrence of VHSIL after a CO₂ vaporization treatment; to assess whether the treatment with CO₂ laser is effective, and, finally, the occurrence of lesions in other regions of the female lower genital tract regions.

Methods

In a transversal study, 46 records of patients attended at the vulva ambulatory, in the sector of lower genital tract pathology of a gynecologic diseases prevention center in the state of São Paulo, Brazil, were retrospectively analyzed within the period from January of 2002 to December of 2014. The project was presented to the Research Ethics Committee of the institution for analysis and was approved (CEP: 0040/2015; opinion: 1045864), and the current proposal is part of a larger project called: Treatment of vulvar intraepithelial neoplasia with CO₂ laser vaporization, developed by the same authors. The

patients signed a Deed of Free and Informed Consent upon the therapeutic method proposal.

The selected patients were classified in accordance with the following criteria: age, date of filing at the gynecological diseases prevention center, date of vulvar lesion treatment with CO₂ laser, number of visits, presence or absence of condylomatous lesions in other sites of the female lower anogenital tract and what sites these were, the presence or absence of lesion persistence or recurrences, and the number of visits occurred. A convention was made to consider as recurrence the appearance of a new lesion after 3 months from the concluded treatment. Persistence would be characterized by the detection of a new lesion since the first clinical control, within 15 days after the treatment. An attempt was made to demonstrate whether the treatment with CO₂ laser vaporization was effective; the type of prevalent lesion, and whether they were more frequent in cases of immunosuppression and lesions in other regions of the female lower anogenital tract.

The collected information was considered in two groups, as follows: a) human immunodeficiency virus (HIV)+: serum positive patients, and b) SOT: patients with solid organ transplantations. Each group was further subdivided into two subgroups: vulvar low-grade squamous intraepithelial lesion (VLSIL) and VHSIL.

The data were tabulated and submitted for statistical analysis by a professional from the statistics service of a university in São Paulo, Brazil.

The statistical analysis of all the information collected for this research was initially performed in descriptive form, through average, median, minimum and maximum values; standard deviation, and absolute and relative frequencies (percentage). The inferential analysis employed with the aim of confirming or rejecting the evidences found in the descriptive analysis were:

- t-Student test for independent samples,¹⁰ or Mann-Whitney test¹¹ for the comparison of the subgroups: VLSIL and VHSIL.
- Fisher Exact test extension¹² for the study of the association between the group of patients bearing virus-caused HIV+ and patients submitted to SOT.

In all the conclusions reached out of the inferential analysis, an α level of significance equal to 5% was utilized. The information was typed in Excel 2010 Windows (Microsoft Corp., Redmond, WA, USA) forms for the proper storage of information. The statistical analysis was performed through the statistic software IBM SPSS Statistics for Windows, version 19.0. (IBM Corp., Armonk, NY, USA)

Results

It was verified that, out of the 46 women exhibiting all the research criteria, 17 (36.95%) patients had VLSIL, against 29 (63.04%) bearing VHSIL.

The VLSIL group comprised 14 women bearing HIV + infection and 3 patients with SOT. Regarding the women presenting VHSIL, 16 were serum positive for HIV and 13 had SOT.

The average age among the patients with VLSIL and HIV was 32.3 years (varying from 16 to 51 years) and among the ones with VHSIL and HIV, it was 34.9 years (varying from 22 to 47 years). Patients bearing VLSIL and SOT, in turn, had an average age of 29 years (16 to 38 years) and those bearing VHSIL and SOT was 36.1 years, varying from 21 to 63 years. The t-Student test assessment for independent samples and the Mann-Whitney test assessment failed to show a statistically significant difference.

The patients bearing VLSIL and HIV+ presented a higher number of affected areas, with 14 women (46.79%) with up to 3 or 4 affected areas. In respect to the VHSIL and HIV+, 16 cases (53.5%) showed up to 3 affected areas ($p = 0.077$). An isolated infection of the vulva occurred in 6 out of 16 patients with VHSIL and HIV+. The same happened to 5 out of 13 bearers of VHSIL and SOT (► **Table 1**).

The application of the Mann-Whitney statistic test evidenced a trend for statistic difference just for the VHSIL and HIV+. By assessing the different time intervals corresponding to the follow-up time of all patients, we found once again a trend for higher significance among those bearing VHSIL, in special those with HIV infection. Most of the patients with VHSIL, both the HIV-serum positive and the transplanted ones, came to 6– 10 visits after the CO₂ laser vaporization, during a variable time from 13 to 25 months (► **Table 2**).

Likewise, an attempt was made to compare whether differences would appear between the number of visits performed by both groups, from the beginning of the treatment until the last recorded notes. It was found that no difference appeared among them, since most of the patients came to return visits after the 19th visit and only one out of three patients bearing organs transplantation and HSIL, with three patients, came until the 48th.

Out of 16 patients bearing HIV and VHSIL, 12 cases (75%) of lesions persistence after the treatment were computed. The same occurred in 14 VLSIL and HIV+ patients, with only

Table 1 Distribution of 46 women bearing vulvar viral lesions under the conditions of immunosuppression, association of affected areas and intra- and intergroups statistical analysis

Association of affected areas					
	Vulva n (%)	2 areas n (%)	3 areas n (%)	4 areas n (%)	Total n (%)
HIV+					30 (100.0)
VLSIL	2 (25.0)	1 (25.0)	10 (58.8)	1 (100.0)	14 (46.7)
VHSIL	6 (75.0)	3 (75.0)	7 (41.2)	0 (–)	16 (53.3)
SOT					16 (100.0)
VLSIL	0 (–)	1 (20.0)	2 (33.3)	0 (–)	3 (18.7)
VHSIL	5 (100.0)	4 (80.0)	4 (66.7)	0 (–)	13 (81.3)

Abbreviations: HIV + , infection by the Human Immunodeficiency Virus; n, number of cases; SOT, Solid organs transplantation; VLSIL, vulvar Low Grade Squamous Intraepithelial Lesion; VHSIL, vulvar High-Grade Squamous Intraepithelial Lesion.

Statistical analysis – HIV+ – VLSIL X VHSIL; $p = 0.077$ Mann-Whitney test; OT – VLSIL X VHSIL; $p = 0.239$ Mann-Whitney test; VLSIL – HIV+ X OT; $p = 0.768$ Mann-Whitney test; VHSIL HIV+ X OT; $p = 0.714$ Mann-Whitney test.

Table 2 Distribution of 46 women bearing vulvar viral lesions, as per the immunosuppression conditions, the follow-up time gap, intra- and intergroup statistical analysis

Follow up time interval in months						
	01 to 29 n (%)	30 to 59 n (%)	60 to 89 n (%)	90 or more n (%)	No inf. n (%)	Total n (%)
HIV+						30 (100.0)
VLSIL	10 (56.3)	1 (25.0)	0 (-)	0 (-)	3 (60.0)	14 (46.7)
VHSIL	7 (43.7)	3 (75.0)	1 (10.0)	3 (100.0)	2 (40.0)	16 (53.3)
SOT						16 (100.0)
VLSIL	2 (25.0)	0 (-)	0 (-)	0 (-)	1 (50.0)	3 (18.7)
VHSIL	6 (75.0)	2 (100.0)	1 (100.0)	3 (100.0)	1 (50.0)	13 (81.3)

Abbreviations: HIV + , infection by the human immunodeficiency virus; n, number of cases; No Inf., no information; SOT, solid organs transplantation; VLSIL, vulvar low grade squamous intraepithelial lesion; VHSIL, vulvar high-grade squamous intraepithelial lesion.

*highest value 136 months - Statistical analysis: HIV (VLSIL X VHSIL) $p = 0.084$ Mann-Whitney test excluding "no information" - OT (VLSIL X VHSIL) $p = 0.352$ Mann-Whitney test excluding "no information" - VLSIL (HIV+ X OT) $p = 0.909$ Mann-Whitney test excluding "no information" - VHSIL (HIV+ X OT) $p = 0.940$ Mann-Whitney test excluding "no information".

5 women (35.7%) showing persistence of lesions. Within the group of patients with SOT, a similar result was noticed. Only three cases of recurrences were computed, with two of the patients being HIV+ and one bearing SOT (►Table 3).

The exact Fisher test extension, excluding the cases with no information, showed a statistically significant difference as to the VHSIL cases persistence for HIV+ women.

Discussion

Variation of age and symptoms, type of intraepithelial lesion, predominant location, psychological factors and, mainly, states of immunosuppression cause patients with vulvar intraepi-

Table 3 Distribution of 46 women bearing vulvar viral lesions under the conditions of immunosuppression in terms of persistence and/or recurrences number and intra- and intergroups statistical analysis

Persistence and/or recurrence in numbers				
	Recurrence n (%)	No of lesions n (%)	No inf. n (%)	Total n (%)
HIV+				30 (100.0)
VLSIL	1 (50.0)	4 (100.0)	4 (57.11)	14 (46.7)
VHSIL	1 (50.0)	0 (-)	3 (42.9)	16 (53.3)
SOT				16 (100.0)
VLSIL	0 (-)	1 (33.5)	1 (25.0)	3 (18.8)
VHSIL	1 (100.0)	2 (66.7)	3 (75.0)	13 (81.3)

Abbreviations: HIV + : infection by the human immunodeficiency virus; n: number of cases; No inf, No information; SOT, solid organs transplantation; T, total number; VLSIL, vulvar low grade squamous intraepithelial lesion; VHSIL: vulvar high-grade squamous intraepithelial lesion.

Statistical analysis: HIV+ (VLSIL X VHSIL) $p = 0.024$ exact Fisher extent excluding "no information" - OT; (VLSIL X VHSIL) $p = 0.576$ exact Fisher extent excluding "no information" - VLSIL (HIV X OT) $p > 0.999$ exact Fisher Extent excluding "no information" - VHSIL (HIV X OT) $p = 0.413$ exact Fisher extent excluding "no information".

thelial lesions to receive different treatments. The possibility of relapses is frequent, and the results depend on the best choice decided in common agreement between patient and doctor.

It has been recommended that low-grade vulvar lesions can only be followed-up or treated conservatively. When there is a need for an active option, destructive, excisional or immunomodulatory treatments may be used.

Because of the elevated potential for malignancy and a higher probability of relapses or persistence, VHSIL deserve greater attention and individualized decisions. Small lesions can be excised easily in the outpatient setting, but multifocal procedures require major care, such as hospitalization for clinical and anesthetic attention. It is recommended that destructive treatments always be performed after histopathological examination that excludes invasion.

Chueri and Brito¹³ report a medical assistance protocol for immunosuppressed patients using topical application of fluorouracil 5%, twice a week, for four weeks, followed by removal of the remaining lesions with CO₂ laser vaporization. Satisfactory responses occur in 75% of the cases. The use of surgery with loops of high frequency waves, when in extensive cases, needs to be performed at a surgical center and can result in unpleasant and uncomfortable scars depending on the extent of the lesions. On the other hand, CO₂ laser vaporization can be performed on an outpatient basis, with topical anesthesia, in more than one session. With this procedure, remissions are reported in 75–80% of cases and can reach 97% when a second procedure is performed.

Leufflen et al,¹⁴ when comparing excisional treatment with laser vaporization, described a remission rate of 91% after one year with excisional treatment compared to 65% with laser therapy.

In recent years, several authors have suggested the use of 5% imiquimod, an immunomodulator with antiviral and antitumor action. It increases the production of interleukin, interferon and tumor necrosis factor. When applied on the lesion 3 times a week, at night, for 16 weeks, it allows complete regression of lesions in 50 to 80% of cases. As with laser

vaporization, there is functional and anatomical preservation of the vulva.

For Rama et al,¹⁵ the clinical response was similar when the treatments with imiquimod cream 5% and CO₂ laser vaporization were compared. No recurrent lesions, but a statistical difference was seen when the severity of recurrent lesions was assessed. Upon the follow-up, the difference was noted in the histological grade of the recurrent lesions ($p = 0.048$). The women submitted to the CO₂ laser treatment presented alterations with high histological grade, while 83.3% of the patients treated with imiquimod 5% presented lesions with lower severity.^{13–15}

The induced immunosuppression, both by an HIV-induced infection or by SOT or other diseases, predispose patients bearing VLSIL and VHSIL to present more polymorphic lesions and persistent conditions, whatever the instituted treatments might be. A higher probability of evolution to invasive neoplastic conditions results. Then, a constant and extended clinical assessment is necessary. This information coincides with those by Speck et al.⁸

The VHSIL patients, both HIV-serum positive and organs transplanted, showed the widest age range compared with the VLSIL patients, but with no statistical significance. Likewise, the infection in other areas in addition to the vulva was more notorious among women bearing VLSIL and HIV+ than in the others. Thus, the importance of the proliferative process of HPV-induced lesions in more extended areas is featured, and on the other hand, the aggressiveness of VHSILs, upon immunosuppression of whatever nature. The same conclusion is shared by such authors as Speck et al,⁵ Parellada et al⁴ and Girardi et al.³

The follow-up after the CO₂ laser application in VLSIL in patients with immunosuppression, both by HIV-induced infections or by SOT, such as kidneys and pancreas, in special, evidenced that patients bearing VHSIL, of both groups, were more concerned with not failing the control than the others, despite all of them receiving the same return guidance.

The persistence of lesions after the CO₂ laser vaporization treatment appeared to be more evident among patients bearing VHSIL and HIV, a fact that was also observed by Penna et al⁷ and Speck et al.⁸ However, an evaluated sample showed recurrence, that is, the reappearance of the lesion after 3 months from the conclusion of the treatment — a conventionally-established term — in only 3 cases, with two of the patients being serum positive and one transplanted.

The short term considered for a recurrence or the lack of return to be established, or the size of sample, or even the lack of a proper catalogue of data might justify the low recurrence index. Those conditions, bound to the frequent persistence of lesions, induce the health professionals to introduce a long-term post-operative adjuvant therapeutic alternative, as suggested by Speck et al, in 2004.⁹

Conclusion

Patients who are immunosuppressed, due to HIV infection or to SOT, and who have an intraepithelial vulvar lesion, do it in a more concerning form, as pre-neoplastic lesions throughout

the third and fourth decades of life; they are more resistant to the laser destructive treatment and exhibit higher persistence index. That fact, therefore, suggests the need of a deeper surveillance in frequency and time gap, aiming to avoid a possible clinical evolution toward an invading neoplasia.

Contributors

Ribalta J. C. L., Mateussi M. V. and Speck N. M. G. contributed with the project and interpretation of data, writing of the article, critical review of the intellectual content and final approval of the version to be published.

Conflicts of Interest

The authors declare that there were no conflicts of interest.

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