Communication

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Abstract: (1) Background: Human papillomavirus (HPV) is diagnosed in most cervical cancers and is the fourth cause of cancer worldwide. Currently, fourteen HPV types are considered carcinogenic (HPV 16/18/31/33/35/39/45/51/52/56/58/59/66/68) with different oncogenicity. Cervical lesions might be due to HR (high risk)-HPV for what is important to identify new methods to genotype cervical lesions. The main goal was to identify, for the first time, HPV-HR in cervical lesions using Anyplex™ Detection kit in paraffin-embedded tissue. (2) Methods: It was performed a retrospective study in 45 women from Academic Hospital of Cova da Beira (CHUCB). DNA extraction was performed and through Anyplex™ II HPV-HR Detection kit HPV-HR were genotyped. (3) Results: HPV-HR were identified in 38 women; HPV-16 (55,26%), HPV-18/39/56/58/59 (5,26%), HPV-31 (21,05%), HPV-35 (7,89%), HPV-51/6 (2,63%) and HPV-52 (10,53%). (4) Conclusions: Our results reflect that Anyplex™ II HPV-HR Detection kit, designed for HR-HPV genotyping of cervical cancer screening, might be properly used to HR-HPV genotyping in histology. The proposed methodology allows an easy and cheaper technique that should be used as a future cervical risk stratification. It is a strong tool to be implemented in clinical practice in order to detect HR-HPV detection in cervical lesions, contributing to a more accurate diagnosis.

Keywords: HR-HPV Detection kit; HR-HPV genotyping; Cervical Cancer

1. Introduction

Human papillomavirus (HPV) is diagnosed in more than 90% of cervical cancers [1], being the fourth cause of cancer in women and the leading cause of cancer mortality among women in 23 countries [2].

The International Agency for Research on Cancer (IARC) considers HPV-16/18/31/33/35/39/45/51/52/56/58/59 as high-risk HPV types (HR-HPV), belonging to Group 1 [3]. HPV-68 and HPV-66 were considered has probably carcinogenic (Group 2A) and possibly carcinogenic (Group 2B), respectively [3].

HPV cytological screening programs in conventional cytology and liquid-based cytology have significantly contributed to cervical cancer incidence reduction [4,5]. However, HPV testing for HR-HPV (HR-HPV-16/18/31/33/35/39/45/51/52/56/58/59/66/68) is more sensitive than cytology for high-grade intraepithelial lesions (HSIL) detection, being the cervical cancer screening test recommended by guidelines of all most scientific societies [6]. To a screen-and-treat approach World-Health Organization (WHO) recommends visual inspection with acetic acid (VIA) and HR-HPV DNA tests, that commonly identify 14 types of HR-HPV (HR-HPV-16/18/31/33/35/39/45/51/52/56/58/59/66/68)

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[5]. Usually, the risk of cancer is assessed by HPV testing and reflex cytology results, if a determined risk higher than 4 or 5%, the women is oriented to immediate colposcopy and biopsy or excision of transformation zone [7].

For histologic high-grade cervical lesions, there are no validated tools to assess the risk of progression for cancer. Lower age (less than 25-30 years-old) have been used as a criteria for no treatment [8].

As HPV type 16 and HPV type 18 have a more aggressive behavior, and are related with more risk for progression to invasive cancer [9], it´s possible that HPV type determination on histologic lesions of HSIL may be a useful tool for risk stratification and treatment optimization. For clinical practice, there is the need to develop easy and cheaper techniques for HPV genotyping of histologic lesions of HSIL.

HR-HPV tests are commonly used to genotype samples collected from Pap tests. Thus, despite the value of screening programs and HR-HPV DNA tests though liquid based cervical cytology it is of main importance to identify the genotypes of HR-HPV when the lesion has already occurred.

The main goal of this work is to stablish, for the first time, a protocol to HR-HPV detection in cervical lesions present on paraffin-embedded tissue samples using an HPV-HR Detection kit designed for cervical cancer screening. The implementation of this protocol in clinical practice might contribute to a more accurate diagnosis and a more effective strategy, which may lead to a better prognosis and outcome. In the future, it can even be included in the algorithms of HPV screen-and-treat approach leading to HPV guidelines update.

2. Results

In the present study 45 paraffin-embedded slides (3µm) of HSIL, were submitted to genomic DNA extraction. Genomic DNA was extracted from 44 samples and it was not possible to extract DNA from 1 sample (sample not considered for the study). Thus, 44 genomic samples were genotyped for HPV, using HR-HPV Detection kit.

Data were analyzed through Seegene Viewer™ (Seegene®) and 38 (86,36%) samples were HPV-positive and 6 (13,63%) samples were HPV-negative (Table 1).

Table 1. HPV status of the 44 samples included in the study.

HPV status	*n* (%)
	44 (100)
HPV-positive	38 (86,36)
HPV-negative	6 (13,63)

The majority of the cases were HPV-16 positive (n=21; 55,26%), 8 cases (21,05%) were positive for HPV-31, 4 cases (10,53%) were positive for HPV-52, 3 (7,89%) cases for HPV-35, 2 (5,26%) cases for each HPV-18/39/56/58/59 and 1 (2,63%) for each HPV-51/66 (Table 2).

Table 2. HR-HPV genotyping.

HPV genotypes	*n* (%)
HPV-16	21 (55,26)
HPV-18	2 (5,26)
HPV-31	8 (21,05)
HPV-33	0
HPV-35	3 (7,89)
HPV-39	2 (5,26)
HPV-45	0
HPV-51	1 (2,63)

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HPV-52	4 (10,53)
HPV-56	2 (5,26)
HPV-58	2 (5,26)
HPV-59	2 (5,26)
HPV-66	1 (2,63)
HPV-68	0

HR-HPV multiple infections were verified in 9 cases (23,68%) and are summarized in Table 3. HR-HPV double infection was verified in 2 cases for HPV-16/18, 1 case for HPV-16/35, 1 case for HPV-16/59, 2 cases for HPV-31/39, 1 case for HPV-35/58 and 1 case for HPV-52/56. HR-HPV triple infection was verified in 1 case, having been identified the HPV-16/31/35 genotypes.

Table 3. Co-infection of HPV genotypes.

Co-infection	***n***
HPV-16 and HPV-18	2
HPV-16 and HPV-35	1
HPV-16 and HPV-59	1
HPV-16, HPV-31 and HPV-35	1
HPV-31 and HPV-39	2
HPV-35 and HPV-58	1
HPV-52 and HPV-56	1

These results indicates that genotyping cervical lesions by Anyplex™ is feasible, easy to perform and maybe of main importance for risk stratification of cervical high-grade lesions, once the majority of the lesions were positive for HR-HPV and some lesions presented multiple infections for HR-HPV.

3. Discussion

Cervical cancer is a significant global health issue, and it is well established that persistent infection with high-risk human papillomavirus strains, such as HPV types 16 and 18, is a major risk factor for the development of cervical cancer. The study addresses the crucial need for early detection and monitoring of high-risk HPV infections, which can lead to early intervention and potentially prevent the progression to cervical cancer.

HR-HPV types are present in the majority of cervical cancers, leading to high mortality rates. The present retrospective study included 45 cases of paraffin-embedded samples of cervical lesions. Genotyping of HR-HPV types was performed for the first time in this type of tissue using the referred HR-HPV detection kit. Although screening programs highly contributes to cervical cancer prevention, this methodology show us to be precise and able to detect HR-HPV types present in cervical lesions.

In the present work, in 44 from 45 cases (97,8%) was possible to extract HPV DNA for analysis, 86,36% of the lesions are positive for at least one type of HR-HPV and 55,26% are positive for HR-HPV-16, being HR-HPV-16 the more prevalent in co-infections. As expected, the frequency of the samples for 66/68 subtypes is low, being in accordance with the literature. Also, 23,8% of the samples are positive for HR-HPV multiple infections, similar results were found by Sousa et al. where 25,7% of the liquid-based cytology samples also have multiple infections [10].

This is the first time that paraffin-embedded slides of cervical lesions are genotyped using this HR-HPV detection kit and it is certainly a step forward for earlier detection of the lesion's precursors of cervical cancer development. The kit used in the present work, was so far used for liquid cytology specimens' biopsies and now shows to be also an effective tool to be used in paraffin-embedded tissues. Traditional methods for HPV detection often involve time-consuming and labor-intensive techniques. Using the Anyplex™ Detection Kit for HPV detection in paraffin-embedded slides can

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provide a more efficient and accurate diagnostic tool. This improved accuracy is vital in ensuring that individuals at risk receive appropriate follow-up and treatment. The use of paraffin-embedded tissue slides is a common practice in pathology labs, as they allow for long-term storage of tissue samples. The study focuses on applying this widely available resource to HPV detection, making it more feasible for retrospective studies and enabling the examination of a larger pool of samples collected over time.

Detecting high-risk HPV in cervical lesions has immediate clinical relevance. It can aid clinicians in identifying patients who may require closer monitoring, more aggressive treatment, or preventive measures such as vaccination. Additionally, it can inform the management of cervical lesions, potentially reducing the need for unnecessary interventions and minimizing healthcare costs. This research contributes to the ongoing efforts in cervical cancer screening and prevention. By developing a more efficient and reliable method for detecting high-risk HPV, this study may have implications for public health programs and policies aimed at reducing the burden of cervical cancer.

Ultimately, the clinical interest of this study lies in its potential to improve patient outcomes, avoiding unnecessary treatments and improving prognosis and quality of life for individuals at risk of cervical cancer.

In summary, this scientific article on high-risk HPV detection using the Anyplex™ Detection Kit in paraffin-embedded slides from cervical lesions addresses a pressing clinical need in cervical cancer prevention and management. It has the potential to enhance diagnostic accuracy, inform clinical decision-making, and contribute to broader public health initiatives focused on reducing the incidence and impact of cervical cancer.

We believe that is investigation opens a new window to future stratification of HSIL risk and therapeutic management. New laboratorial studies are need with higher number of cases should be performed in order to better to understand if the present protocol is pertinent and clinical studies to determine if this technique is feasible and useful, specially to assess progression risk of histologic lesions of HSIL should be added to cervical cancer detection guidelines.

4. Materials and Methods

4.1. Study population

A retrospective study was performed using paraffin-embedded tissue samples from 45 women previously submitted to squamous high-grade cervical lesions excision in Academic Hospital of Cova da Beira (CHUCB). The samples collection occurred at the Child and Women, Gynaecologic Oncology Division of CHUCB, Covilhã, Portugal. The study was approved by the Ethics Committee of Beira Interior University with the code CE-UBI-Pj-2017-027.

4.2. HPV genotyping

Genomic DNA was extracted from the paraffin-embedded tissues using QIAamp DNA FFPE Tissue Kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions.

For HR-HPV genotyping, Anyplex™ II HPV HR Detection kit (Seegene®, Seoul, Korea, acquired to Werfen, Portugal) was used, following manufacturer's instructions [11]. A multiplex real-time PCR (CFX96 PCR from Bio-Rad, USA) was performed allowing the simultaneous genotyping of the 12 HR-HPV types (HPV-16/18/31/33/35/39/45/51/52/56/58/59), HPV-66 (group 2B) and HPV-68 (group 2A) [3,11]. Anyplex™ II HPV HR Detection kit provides an internal control for each sample and a positive and negative control for each plate of the real-time PCR reaction.

The analysis of PCR data was performed using the software Seegene Viewer™ (Seegene®).

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