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Cervical cytology and HPV distribution in Cape Verde: A snapshot of a country taken during its first HPV nation-wide vaccination campaign

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ABSTRACT

Cervical cancer ranks as the third most common female cancer in Cape Verde and is the leading cause of cancer-related deaths among women in the country. While Human Papillomavirus (HPV) vaccination, which started in 2021, is anticipated to significantly reduce disease incidence, cervical screening remains crucial for non-vaccinated women. We retrospectively reviewed gynecologic cytology exams and HPV tests performed in Cape Verde between 2017 and April 2023 and processed at IMP Diagnostics. For this study, we considered 13035 women with cytology examinations performed and, 2013 of these, also with an HPV molecular test. Cytology diagnostics comprised 83 % NILM cases; 12 % ASC-US; 2.7 % LSIL; 1.2 % ASC-H; 0.5 % HSIL and 0.1 % SCC. In 505 (25.1 %) high-risk HPV infection was detected. Prevalence of HPV infection varied with age, peaking at young ages - ≤24 years old (55.5 %) and 25-35-year-old women (31.5 %) - and the lowest after 66 years old (9.7 %). Herein we present a comprehensive study regarding Cape Verde's cervical cytology and HPV distribution, aiming to provide a snapshot of the country's cervical cytology results and HPV distribution in recent years. Moreover, these data may contribute to establish a baseline to assess, in the future, the vaccination impact in the country.

1. Introduction

Cervix uteri cancer is the fourth most prevalent cancer and the fourth cause of cancer-related death in women, worldwide [1]. In many low- and middle-income countries the prevalence and impact of cervical cancer is even higher, and the highest regional incidence and mortality is reported in sub-Saharan African countries [1,2]. Human papillomavirus (HPV) is the major cause of cervical dysplasia and cervical cancer [3]. Importantly, the World Health Assembly has recently proposed a global strategy for cervical cancer elimination, set on three pillars: vaccination, screening and treatment [4]. The triple-strategy (90-70-90) states that all countries must have by 2030: 90 % of girls up to 15 years of age fully vaccinated against HPV; 70 % of women screened with a high-performance test by the age of 35, and again at 45; 90 % of women with diagnosed cervical disease adequality treated [4].

In Cape Verde, a western sub-Saharan country belonging to the community of Portuguese-language African countries (CPLP), cervical cancer is the third most prevalent cancer in women and, importantly, it is the leading cause of cancer-related female mortality [5]. In Cape Verde there are no national recommendations implemented regarding cervical disease screening [6,7]. Nonetheless, it is estimated that around 37 % of women have been screened in the 5 years prior to 2021 (data from Ref. [6]). Regarding HPV vaccination, it was introduced in the country in 2021 and, according to the 2023 WHO Report, full coverage of all the girls under 15 years old was achieved by 2022 [6,8].

Herein, we present cervical cytology results and HPV status in Cape Verde population, to our knowledge constituting the biggest casuistic in the recent literature, contributing to a wider characterization of cervical disease distribution in the country. Importantly, as these cytology exams and HPV tests were performed between 2017 and 2023, partially overlapping with Cape Verde's first HPV nation-wide vaccination campaign, this study may also offer a baseline for a future evaluation of the impact of HPV vaccination in Cape Verde's female population.

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2. Methods

2.1. Study population

We retrospectively reviewed the gynecologic cytology exams and HPV tests from 13035 women from Cape Verde which were sent to IMP Diagnostics Portugal between June 2017 and April 2023. Most women were followed in private gynecologic consults, n = 12493. In addition, 542 exams were performed in local screening context, organized by regional associations against cancer, including examinations conducted among populations residing in more distant or isolated regions ("Associação Caboverdiana de Luta Contra o Cancro"; "Liga Caboverdiana de Luta Contra o Cancro"; "Associação Unidos na Luta Contra o Cancro"). To the best of our knowledge there were 42 private clinical entities performing Gynecology in Cape Verde during the time interval of this study and IMP Diagnostics works with 31 (74 %) of these entities, which are also the ones with the largest caseloads. The information regarding these centers, and their location, is presented in Fig. 1.

The samples considered for this study were collected in Cape Verde and sent to IMP Diagnostics (Portugal) for diagnosis, as depicted in Fig. 2.

2.2. Cytology

Cervical specimens were collected during gynecological examination and placed into a ThinPrep® Pap test vial containing PreservCyt Solution (Hologic®). We used the 2014 Bethesda System for reporting the cervical cytology [9].

2.3. HPV testing

HPV testing was performed in liquid-based cytology samples with Aptima® HPV/Aptima® HPV-GT Assays (Hologic®) (n = 1921 patients) or with PapilloCheck® Test Kit (n = 92 patients), according to the manufacturers' instructions [10–12]. Fig. 3 shows the HPV testing stepwise approach. The Aptima® HPV Assay is a target amplification nucleic acid probe test for the in vitro qualitative detection of E6/E7 viral messenger RNA (mRNA) from 14 high-risk types of HPV (16/18/31/33/35/39/45/51/52/56/58/59/66/68). The Aptima® HPV assay does not discriminate between the 14 high-risk types. The Aptima® HPV-GT uses the same technology for detection of mRNA from HPV genotypes 16, 18, and 45; the assay differentiates the genotype 16 from 18/45 but does not differentiate between 18 and 45. Samples positive with Aptima® HPV were always reflex tested with the Aptima HPV-GT. Test results may be negative for both HPV 16 and HPV 18/45 (meaning the patient has other high-risk HPV subtype), negative for HPV 16 and positive for HPV 18/45, positive for HPV 16 and negative for HPV 18/45 or positive for both HPV 16 and HPV 18/45. Note that if in the Aptima® HPV-GT Assay result is HPV16+ it means the patient has an HPV16 subtype but the patient could have an additional subtype (co-infection) other than 18/45 and the test will not provide this information. PapilloCheck® Test Kit (Greiner Bio-one®) is a genotyping assay, that allows qualitative detection and differentiation of 24 types of HPV (18 high-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82; and 6 low-risk subtypes: 6, 11, 40, 42, 43, 44/55) in DNA-preparations from human cervical samples. The test results specify the subtype(s) of HPV present in each sample. Regarding HPV testing (Aptima® or PapilloCheck®) there were no "insufficient" or "inconclusive" results as, when this happened, the test was always repeated (from a new sample) and no result from the first product was registered. In one patient, besides the PapilloCheck® Test Kit, an HPV Direct Flow Chip Kit test (from Vitro, Master Diagnostica), was also performed (as part of a laboratory trial), detecting an HPV-71 subtype.

2.4. Statistical analysis

Categorical variables are presented as frequencies and percentages. Descriptive statistical analyses were performed using the Microsoft® Excel® 2019 MSO (version 2306 Build 16. 0. 16529. 20164) 64-bit. When a patient had more than one cytology, the exam with the most severe diagnosis was accounted for as "index cytology diagnosis". If all the exams were negative for intraepithelial lesion or malignancy (NILM), the exam accounted for (as "index cytology diagnosis") was either a cytology exam associated with an HPV test or, if no HPV test was performed, the most recent one. Regarding HPV results, in patients with more than one HPV test performed during the study timeframe, the "index HPV result" was chosen, in relative priority, (1) the first positive test; (2) if all tests were HPV negative, we accounted the first test that had a cytology related to it or (3) if none of these rules applied, the most recent test was accounted for. Furthermore, to correlate the cytology

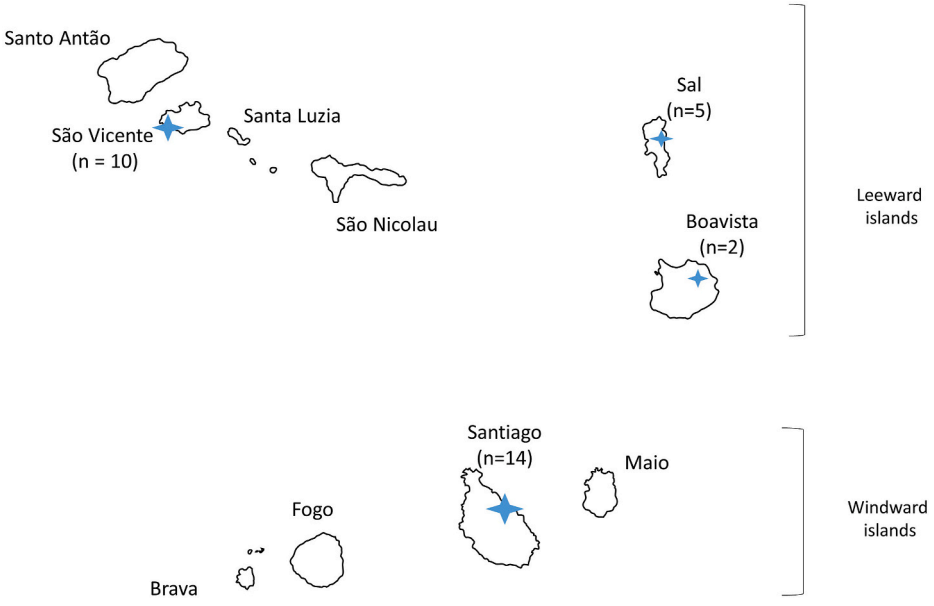

Fig. 1. Private practice clinical entities, by island/region, included in the study. (Stars indicate islands with institutions that work with our laboratory; n, number of institutions).

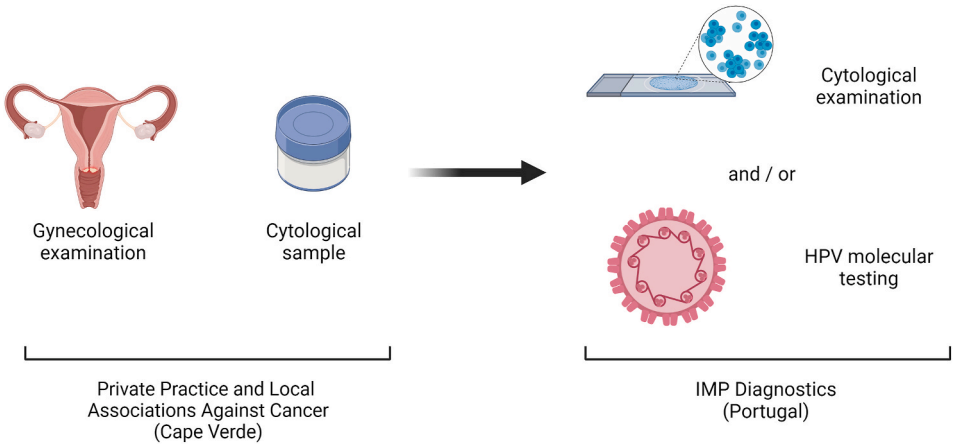

Fig. 2. The cervical specimens collected in Cape Verde are sent to IMP Diagnostics (Portugal), and cytologic examination and/or HPV molecular testing are performed, according to the patient's physician request. If a co-test (both cytology and HPV testing performed at the same time) is ordered, the same sample can be used for both procedures.

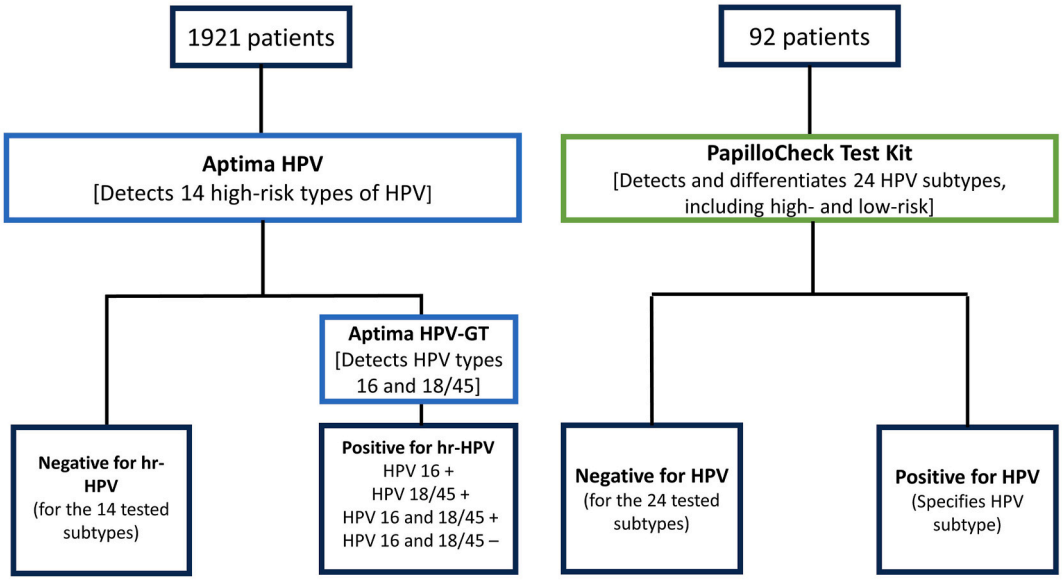

Fig. 3. HPV testing methodologies. The attending physician chooses the molecular test to be performed at IMP Diagnostics. Regarding the Aptima test results, if a positive patient is HPV16/18/45 negative, it means the patient has a different high-risk HPV subtype infection (hr-HPV, high-risk HPV).

results with HPV status, we evaluated patients that had both exams performed in relative time proximity: either co-test (performed on the same examination); reflex cytology (defined, for this purpose, as any cytology following an HPV test within six months of this) or reflex HPV testing (defined, for this purpose, as any HPV test within six months following a cytology exam). This way, if an HPV test was performed with a longer interval in relation to the cytology, these cases were not included in the analysis. For example, if a case was firstly negative for HPV and then had a positive cytology one year (or more) after, it was not included in the analysis, as the patient could have been truly negative and only HPV-infected after. Only cases possible to relate in the stipulated timeframe were compared.

3. Results

The results presented in this study refer to the exams of 13035 women, that were performed between June 2017 and April 2023 in Cape Verde and sent to IMP Diagnostics for diagnosis. These included 15900 cytology exams, as 2299 patients had more than one cytology exam during this period. The age interval of patients was between 13 and 89 years old (median age 38 years old; in 48 cases birth date was not available).

We selected one index cytology test per patient (as described in the Methods section) and the results of cytology diagnoses are detailed in Table 1. Most diagnostics 10829 (83.1 %) were negative for intraepithelial lesion or malignancy (NILM). In 6000 (46 %), NILM diagnoses encompassed specific non-neoplastic findings, such as hyperkeratosis, parakeratosis, microorganisms, etc. ("NILM, other non-neoplastic findings). There were 1590 (12.2 %) ASC-US (atypical squamous cells of undetermined significance) cases and 158 (1.2 %) ASC-H (atypical squamous cells, cannot exclude squamous intraepithelial high-grade lesion) cases. In 355 cases (2.7 %) a low-grade intraepithelial lesion (LSIL) was diagnosed and in 64 (0.5 %) a high-grade lesion (HSIL). Lastly, eight squamous cell carcinomas (SCC) were diagnosed and further 17 cases had a glandular lesion, either atypia or carcinoma, without squamous alterations.

From our cohort, 2013 patients had at least one HPV test performed (1547 were co-tests, i.e., the HPV testing was done at the same time of the cytology). In most patients (n = 1921) Aptima® HPV/Aptima® HPV-GT Assays (Hologic®) were performed, whereas PapilloCheck®

Table 1
Cytology results.

	N (%)
NS	14 (0.11 %)
NILM	4829 (37.05 %)
NILM, non-neoplastic findings	6000 (46.03 %)
ASC-US	1590 (12.20 %)
- ASC-US	[1589]
- ASC-US + AGC, NOS	[1]
LSIL	355 (2.72 %)
ASC-H	158 (1.21 %)
- ASC-H	[156]
- ASC-H + AGC, endocervical	[1]
- ASC-H + AGC, NOS	[1]
HSIL	64 (0.49 %)
- HSIL	[63]
- HSIL + AGC, endometrial	[1]
SCC	8 (0.06 %)
AGC/ADC, endocervical	9 (0.07 %)
AGC/ADC, endometrial or NOS	8 (0.06 %)
Total	13035 (100.00 %)

AGC/ADC, Atypical glandular cells/adenocarcinoma; NOS, Not otherwise specified; NS Non satisfactory. (Square brackets contain the subcategories results, under the wider above category above).

genotyping was executed in 92 patients. Results regarding HPV status are shown in Table 2. High-risk HPV infection was found in 505 women (25.1 %; including 472 women with Aptima® positive results and 33 women with high-risk HPV detected by genotyping). In five additional cases only low-risk HPV infection was detected by genotyping.

As previously mentioned, genotyping was performed in only 92 patients (4.6 %). From the 38 patients with a positive result, 33 had high-risk HPV identified and five patients had only low-risk HPV subtype(s). Twenty patients presented co-infection with two or more HPV types (in two of these patients, only one was a high-risk subtype and the other(s) was a low-risk HPV). There were multiple HPV subtypes detected in this cohort (detailed information is shown on Table 3 and Supplemental Table S1).

Since Aptima® assays were performed in the majority of patients, and this test only identifies HPV 16 and HPV 18/45 (not specifying other HPV subtypes; see the Methods section for details), it was not possible to assess how many of these patients also had co-infection, though we can state that at least four patients had co-infection (with HPV 16 and either HPV18 or 45). In Aptima® testing, 117 (24.8 %) patients had HPV 16, HPV 18 or HPV 45 infection (isolated or co-infection), while 355 (75.2 %) patients had infection caused by other high-risk subtypes (Aptima®+, non-specified subtype). In PapilloCheck® Genotyping, 11 patients had HPV-16 or HPV-18 infection, 33 % of the positive high-risk cases (11/33); while 22 patients had other subtypes identified. As such, 75 % (377/505) of detected infections were caused by different HPV subtypes other than 16/18/45 (355 "Aptima®+, non-specified subtype" results plus 22 patients with different subtypes identified at genotyping). High-risk HPV infection prevalence varied according to age, showing the highest percentage of positive high-risk HPV positive tests at ages ≤24 (55.5 %) and 25–35 years old (31.5 %) of patients who tested HPV positive. At older ages, namely ≥66 years old, the prevalence was lower, 9.7 %. In Fig. 4 the high-risk HPV infection prevalence distribution according to age is shown (one patient was excluded from this analysis due to unavailability of birth date information).

Table 2
HPV testing results.

HPV test type/result	N (%)
- Aptima® HPV -	1449 (71.98 %)
- Aptima®HPV/Aptima® HPV-GT test +	472 (23.45 %)
- Aptima® HPV + (non-specified subtype)	[55 (17.64 %)]
- Aptima® HPV-GT 16+/HPV 18/45-	[67 (3.33 %)]
- Aptima® HPV-GT 16-/HPV 18/45+	[46 (2.29 %)]
- Aptima® HPV-GT 16+/HPV 18/45+	[4 (0.20 %)]
- PapilloCheck® Genotyping -	54 (2.68 %)
- PapilloCheck® Genotyping +[a]	38 (1.89 %)
Total	2013 (100.00 %)

[a] Genotyping: 33 cases (1.64 %) with high-risk subtype of HPV identified and five cases (0.25 %) with low-risk HPV. (Results in grey/square brackets correspond to the detailed results under the wider category above).

Table 3
Genotyping positive results.

Only one high-risk HPV subtype detected per sample	**N**
hr16	2
hr18 or hr45	0
Other high-risk HPV subtypes	11
Co-infection (more than one HPV subtype detected per sample)[a]	**N**
h16 + another HPV subtype(s)	7
h18 + another HPV subtype(s)	2
Co-infection by HPV subtypes other than HPV 16/18/45	11
Only low-risk HPV subtypes detected	5
Total patients	**38**

hr, high-risk subtype; lr, low-risk subtype; N, number of patients.
[a] Patients with more than one HPV subtype per patient, with at least one hr-HPV. Different combinations were found in each patient, for details refer to Supplemental Table 1.

The burden of cervical HPV infection, for each cytology category, is depicted in Table 4, for the 1973 patients whose both exams were performed in proximity (either as co-test or reflex testing; see the Methods section for details). In 40 patients the HPV test was done isolatedly, with no cytology test timely associated to it (neither as co-test or as reflex testing), thus these were excluded from this analysis. Most patients (89.7 %) with NILM cytology were negative for high-risk HPV, meaning that 10.3 % of these patients were high-risk HPV positive. Conversely, the majority of patients with detected lesions in cytology (LSIL/HSIL) had high-risk HPV infection detected. In Table 4 the cytology results are also distributed according to the hr-HPV type encountered (HPV 16/18/45 vs. other hr-HPV subtypes). In most patients in our cohort with ≥ASC-US cytology, the associated HPV was a high-risk subtype other than 16/18/45. None from the SCC or glandular lesion cases were tested for HPV in our cohort.

4. Discussion

Cape Verde has a population of 220065 women aged 15 years or more, who are at risk of developing cervical cancer [6] and, as the cohort of our study comprehends 13035 women, it represents 6 % of this population. To analyze our study's results, we begin by examining the prevalence of cervical lesions in our cohort, comparing it to other similar studies. Next, we explore the prevalence of high-risk HPV infections and contrast it with global and regional data. Additionally, we highlight the most common HPV genotypes identified in our cohort. Finally, we acknowledge this study's limitations and comment on its relevance.

Overall, 83.1 % of the patients in our cohort had a NILM diagnosis and 16.9 % had an altered cytology result (≥ASC-US). A large study, conducted in the Limpopo province, South Africa, reported that, in 84466 screened women, 19.6 % had an abnormal cervical cytology result [13], a percentage that is slightly higher than the one we report in our study. On another recent study from Njagi SK et al., with data from a smaller cohort of 480 women from Kenya, a 37 % prevalence of cervical lesions was found in cytology [14], a percentage that is higher than the one we report. To the extent of our knowledge there are no published data regarding the specific distribution of cytology diagnoses in Cape Verde for comparison. In our cohort, the percentage of LSIL and HSIL was 2.7 % and 0.5 %, respectively. According to "The Bethesda system for reporting cervical cytology", benchmark data obtained by the College of American Pathologists (CAP) showed that the median rate of LSIL was between 2.5 and 2.9 % and of HSIL was 0.5 % [9]. In addition, the

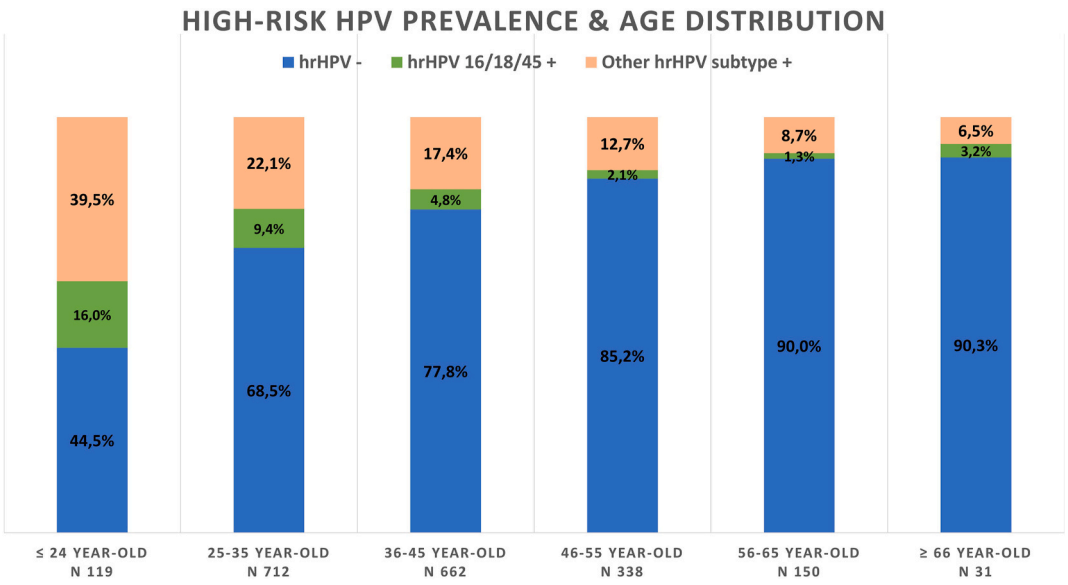

Fig. 4. High-risk HPV prevalence according to age distribution (results are presented as % of total tests performed in each age interval; N, number of patients tested).

Table 4
Cytology results and high-risk HPV status.

Cytology	hr-HPV-(N = 1486)	HPV 16/18/45 +(N = 126)	Other hr-HPV subtype + (N = 361)	Total
	N (%)	N (%)	N (%)	N
NILM	1086 (89.7 %)	24 (2.0 %)	101 (8.3 %)	1211
ASC-US	383 (59.6 %)	63 (9.8 %)	197 (30.6 %)	643
LSIL	16 (21.9 %)	17 (23.3 %)	40 (54.8 %)	73
ASC-H	1 (2.9 %)	18 (52.9 %)	15 (44.1 %)	34
HSIL	0 (0.0 %)	4 (33.3 %)	8 (66.7 %)	12
Total				1973

hr, high-risk subtype.

Risk Estimates supporting the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) consensus guidelines reported that 1.7 % of cytology exams were LSIL and 0.3 % HSIL [15,16]. As such, even though there are limitations that hinder a direct comparison, as our data was not collected via a nationwide screening initiative, but derive mostly from occasional gynecological consults, the percentages we found in Cape Verde are in line with these reports. Regarding HPV prevalence, 25.1 % of our cohort had high-risk HPV infection. This finding is lower than reported in previous studies, where an average HPV prevalence of 29.6%–32.3 % in African countries has been reported [17,18]. According to ICO/IARC HPV Information Center there is no available data regarding the HPV burden in the general population of Cape Verde [5]. In Portugal, which shares an historical link and a proximity relationship with Cape Verde, a 12.5 % prevalence of high-risk HPV was recently reported, data from the northern regional cervical screening program [19]. In the Portuguese study, involving 462 401 women, the five most common high-risk HPV genotypes were HPV-68 (16.09 %), HPV-31 (15.30 %), HPV-51 (12.96 %), HPV-16 (11.06 %), and HPV-39 (11.01 %) [19]. In Africa, the four most common genotypes reported are HPV-16, HPV-18, HPV-52 and HPV-35 [20]. In our cohort, 75 % (377/505) of high-risk HPV positive cases were caused by subtypes other than 16/18/45, indicating that, overall, different subtypes have noteworthy impact in Cape Verde population. Due to the fact that most HPV tests were performed with Aptima® HPV tests, our study does not allow the specific identification of which subtype was the most common by itself and cannot preclude to be one of the 16/18/45 subtypes. Specifically, HPV-16 was identified in a total of 80 cases, accounting for 15.8 % of infections. Importantly, the Aptima® HPV test detects messenger RNA (mRNA) oncogenic transcripts (HPV E6/E7 mRNA), which may be a better marker of significant disease while maintaining a similar sensitivity as DNA-based testing [21].

A systematic review and meta-analysis, by Ogembo RK et al., determined the overall HPV prevalence rates in African women with normal cervical cytology (29.3 %), ASC-US (46.5 %), LSIL (74.2 %) and HSIL (84.8 %) [20]. In our study, the results follow the same trend - women with a diagnosis of ASC-US had 40.4 % HPV prevalence; LSIL had 78.1 % HPV burden; and HSIL 100 %, even though the percentage of HPV positive cases in women with normal cytology was lower (10.3 %). All the HSIL cases that had an associated HPV test were positive for high-risk HPV infection, but these corresponded to only 12 patients. None of the cases with SCC (n = 8) or with glandular lesions (n = 17) had an HPV test performed in our laboratory. Although this study presents some limitations related to HPV testing (only 2013 women, out of 13035, had molecular testing, and assessment of HPV subtype was limited, due to variation between testing methodologies), it is, to our knowledge, the biggest comprehensive casuist to date reporting on cytology diagnostic results and HPV burden among women from Cape Verde.

5. Conclusion

Our study characterizes the cervical health landscape in Cape Verde, based on cytology and HPV testing performed between June 2017 and April 2023, and contributes with valuable insights to the global understanding of HPV prevalence and cytological diagnoses in the country. Further research, with larger and more representative cohorts, coupled with refined HPV molecular testing methodologies would deepen our understanding of the specific subtypes of HPV present in Cape Verde, as this may have an impact on vaccination choice. Our findings, encompassing the timeframe both preceding and immediately following the initiation of Cape Verde's vaccination programme in 2021, might represent a valuable baseline for a future evaluation of the impact of vaccination in the country.

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CRediT authorship contribution statement

Rita Vieira: Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Diana Montezuma:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Carla Barbosa:** Writing – review & editing. **Isabel Macedo Pinto:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The following authors are employees in IMP Diagnostics: Rita Vieira, Diana Montezuma.

Isabel Macedo Pinto is an owner at IMP Diagnostics.

No other disclosures.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tvr.2024.200280.

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