











**Figure 3:** Intraoperative image. After performing tracheotomy, double functional bilateral neck dissection, excision of the lesion with macroscopic margins above the centimeter and subsequent reconstruction with radial forearm flap was performed. Histological results reveals a pT3N0M0 human papillomavirus 16+ squamous cell carcinoma, with close resection margins, 6 mm thickness, with no vascular or perineural infiltration. After surgery, the patient received adjuvant radiotherapy. She is currently without signs of loco-regional recurrence

Regarding to etnogeographical differences, some authors suggest that Japanese studies tend to have the highest rate of HPV,<sup>[70,71]</sup> while Africans tend to have the lowest rate.<sup>[59]</sup> Kreimer *et al.*<sup>[14]</sup> established that HPV+ prevalence was higher among studies from North America compared with those from Europe and Asia. In 2016, Mehanna *et al.*<sup>[72]</sup> conducted a prospective study of 801 patients with head and neck cancers. They established the geographic variability (differences between continents) as an independent risk factor for HPV+ prevalence of OPSCC. It is most prevalent in Western Europe, when compared to Eastern Europe (37%, 155 of 422 vs. 6%, 8 of 144;  $P < 0.0001$ ) and Asia (37% vs. 2%, 4 of 217;  $P < 0.0001$ ).

Regarding the genotype, the most prevalent is HPV16 (68.2-90%)<sup>[14,33,66]</sup> [Figures 1-3] followed by HPV18 (34.1%).<sup>[14,73]</sup> But this varies depending on the series analysed and the techniques used, and that proportion may be reversed, being higher HPV18.<sup>[28,59]</sup> Although the association between HPV and OSCC is described,<sup>[32,62,68,74-77]</sup> it is important to note that high-risk genotypes HPV16 have been detected in normal oropharyngeal mucosa,<sup>[78,79,62]</sup> questioning this causal relationship. In 2001, Mork *et al.*<sup>[80]</sup> defined HPV infection as a risk factor for OSCC, whose exposure may precede the occurrence of OPSCC in 10 years and older.

In the oropharynx there is no hard evidence linking HPV with alcohol or tobacco use, and the absence of synergism is the most accepted hypothesis,<sup>[81]</sup> suggesting two ways for the development of OPSCC, one derived from smoking with or without alcohol and another derived from the HPV induced genomic instability.<sup>[31]</sup>

### Most frequent location

HPV has a predilection for the oropharynx and the Waldeyer ring.<sup>[14,24,59]</sup> It is estimated that the most frequent location

for detecting papillomavirus DNA is the palatine tonsil and the base of the tongue, with a strong causal association,<sup>[14,82]</sup> independently of the influence of smoking or alcohol. Oropharyngeal HPV+ tumours appears in up to six times more often than in other tumours of the head and neck.<sup>[6]</sup> Snijders *et al.*<sup>[83]</sup> were the first to suggest the amygdala is linked with the HPV, in 1992.

### Detection, diagnosis and typing techniques

Molecular assays are the gold standard for HPV identification,<sup>[84]</sup> mainly polymerase chain reaction (PCR),<sup>[85]</sup> specifically the reverse transcription PCR (RT-PCR) to measure viral mRNA E6 and E7 in fresh tissue.<sup>[86]</sup> It has a high sensitivity.<sup>[80,85-88]</sup> It is even able to detect latent infections. Other tests that have been used for detection of HPV are “Southern Blot” (less sensitivity than PCR)<sup>[89]</sup> and *in situ* hybridization (ISH) (less sensitive and less expensive than PCR). Some authors have proposed the combination of PCR with ISH, combining the advantages of the two tests: the high sensitivity of PCR and the ability of ISH to identify and localize genomic sequences linked to HPV in this kind of tumours.<sup>[90,91]</sup>

P16 is a protein used by some authors as a biomarker for HPV infection, which can be expressed when viral DNA is integrated into the host cell. It reflects the functional effects derived from the inactivation of pRb, induced by E7. It is detected by immunohistochemistry staining and it can be used as a predictor of HPV infection in OPSCC, even being proposed by some authors the detection of p16INK4A as an initial test, followed by the detection of HPV in which are positive for this.<sup>[92-94]</sup>

Regarding to the sample being sent for testing, the most commonly accepted it is taking biopsies or tumour specimen analysis [Figure 1]. This allows not only molecular analysis











