



Oral Human Papillomavirus (HPV) and sexual behaviors in a young cohort of oral cancer survivors

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8 **Oral Human Papillomavirus (HPV) and sexual behaviors in a young cohort of oral cancer**
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10 **survivors**
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5 **Keywords:** Oral squamous cell carcinoma (OSCC); Risk factor; Human papillomavirus
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8 (HPV); Oral sex; Young patients
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2 The most common risk factors for oral cancer are tobacco use, alcohol abuse and the
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4 chewing of betel and areca nuts (Mello *et al*, 2019). Although oral squamous cell carcinoma
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6 (OSCC) mostly occurs in adults after the fifth-sixth decade of life, young individuals are
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8 not exempted, accounting around 6% of all cases (Paderno *et al*, 2018). The incidence of
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10 oral cancer is increasing among people younger than 45-years old, who often do not show
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12 the traditional risk factors, or show a relatively short exposure to them (Fan *et al*, 2014;
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14 Xu *et al*, 2019; Caldeira *et al*, 2019).

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16 Human papillomaviruses (HPV) are small double-stranded DNA viruses that consist of more
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18 than 100 subtypes belonging to the Papillomaviridae family. HPV-16 and -18 have been
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20 recently associated with oropharyngeal carcinomas, especially in young males, with a
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22 higher number of sexual partners and with a negligible history of tobacco smoking (Salazar
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24 *et al*, 2014; Fakhry *et al*, 2018). HPV has been considered a potential risk factor for OSCC,
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26 too. However, despite many OSCC localized at the lingual base and palatine tonsils are
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28 positive for HPV, carcinomas affecting other sites of the oral cavity show, instead, a very
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30 low frequency of the viral presence (less than 10%) (Combes and Franceschi, 2014; Vidal
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32 Loustau *et al*, 2019), with an unclear clinical significance.

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34 The importance of better understanding the presence and the role of HPV DNA and sexual
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36 behaviors in OSSC of young patients can help in elucidating oral carcinogenesis and some
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38 clinical aspects. Since evidence is largely lacking, the aim of our observational cross-
39
40 sectional study was to investigate the frequency of HPV oral infection and the sexual
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42 habits of an Italian cohort of oral cancer survivors, who received the diagnosis at a young
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44 age (before 45 years).

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2 We retrieved demographic and clinical data of patients referred, between 1994 and 2016,
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4 to the oral medicine clinic of the dental school of the Università degli Studi di Milano,
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6
7 Ospedale San Paolo (Italy). All the oral cancer survivors, with a maximum age of 45 years
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9 at the time of the oral cancer diagnosis, were included.
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13 Patients were contacted by phone for scheduling a follow-up visit, during which specific
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15 questionnaires on sexual behavior (NHANES - National Health and Nutrition Examination
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17 Survey - <https://www.cdc.gov/nchs/nhanes/index.htm>) and on traditional risk factors
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19 were anonymously filled by the patient. An oral medicine specialist carried out the
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21 complete intra-oral examination, and collected the oral mucosal smear for the further
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23 cytological analysis to identify the presence of oral HPV DNA. For each patient, the
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25 histological paraffin-embedded samples, used for the histopathological diagnosis of OSCC,
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27 were also retrieved and used to perform the molecular tissue analyses for HPV detection.
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29 Briefly, total DNA was extracted from cytological smear and from tissue specimens using
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31 a commercial kit (QIAamp DNA Blood Mini KIT®, Qiagen, Hilden, Germania), then
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33 multiplex-Polymerase Chain Reaction (PCR) was carried out and HPV DNA search was
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35 conducted by amplifying both the L1 and the E6/E7 regions.
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39 Thirty subjects potentially eligible for the study were identified: 16 males and 14 females
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41 (age: 37 years \pm 8.1; range: 19 - 45 years). Fourteen were excluded (9 males and 5 females;
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43 age: 39.2 years \pm 7; range: 20 - 45 years): two patients died, 4 did not accept to participate,
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45 8 could not be contacted. Eventually, 16 patients were included: 9 females (56%) and 7
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47 males (44%). The mean age was 35.1 \pm 8.85 (range: 19 - 45 years). Four patients reported
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49 systemic diseases, i.e. arterial hypertension, liver transplant for cirrhosis, hypothyroidism
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2 and gastritis. One patient referred past mandibular osteoradionecrosis after the head-
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4 neck radiotherapy, which was completely resolved at the time of the study. Table S1
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6 summarizes findings on traditional oral cancer risk factors, i.e. tobacco smoking and
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8 alcohol habits, before and after oral cancer diagnosis. At the time of diagnosis, just 4
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10 patients were heavy smokers (more than 10 cigarettes daily), and two of them were also
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12 heavy drinkers (more than 3 international units consumed daily). After the oral cancer
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14 diagnosis, 5 patients stopped smoking, while no changes in drinking habits could be found.
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16 Two male patients reported a mixed diet with no assumption of fruits and vegetables
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18 daily, while all the others (except two for whom the data was not available) declared a
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20 mixed, equilibrated dietary pattern, which included daily intake of fresh fruits and
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22 vegetables.
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32 Altogether, these findings support the hypothesis that the traditional risk factors can be
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34 responsible for just a small proportion of cases of oral cancer among young people, and
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36 that factors other than tobacco and alcohol are implicated (Llewellyn *et al*, 2003, 2004;
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38 Fan *et al*, 2014; Al-Amad *et al*, 2014; Toporcov *et al*, 2015; Guidry *et al*, 2018; Dalla Torre
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40 *et al*, 2018; Vidal Loustau *et al*, 2019; Purwanto *et al*, 2020; Lee *et al*, 2020).
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46 Fifteen questionnaires on sexual habits were analyzed; one was excluded because the
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48 patient misunderstood the form and filled it incorrectly. Table 1 shows that most of the
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50 patients did not report previous genital infection (including HPV infection). All the fifteen
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52 patients reported at least one sexual experience before cancer diagnosis. The first sexual
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54 intercourse occurred at the mean age of 17.5 ± 3 (range: 13 - 23 years), while the mean
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56 number of sexual partners was 11.7 ± 13.5 (range: 1 - 48). Most of them experienced oral
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2 (n=14) and anal (n=10) sex (Tab. 2). The increased lifelong exposure to oral sex, in terms
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5 of number of oral sex partners (more than six) and earlier age at first intercourse, has
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7
8 been associated with HPV-related head and neck carcinomas (Martín-Hernán *et al*, 2013;
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10 Rettig *et al*, 2015). Before and after OSCC diagnosis, most of patients reported a similar
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13 “seldom use” of protections during oral sex practices (Tab. 3), although their protective
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16 role during oro-genital sex have been advocated (Gupta *et al*, 2019).

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19 The detection of HPV DNA in the histopathological specimens was feasible for 13 out of 16
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21 patients. Indeed, for the three of them who had received the OSCC diagnosis in other
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23 hospitals, the tissue specimens were not available. The presence of HPV DNA was not
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26 detected in the histopathological specimens or in the cytological oral smears of any of the
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29 13 patients (Tab.4). Our findings are consistent with previous studies, which did not
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31
32 identified HPV DNA in tissue samples of patients affected by oral cancer (Kantola *et al*,
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35 2000; Dahlstrom *et al*, 2003; Dahlgren *et al*, 2004; Siebers *et al*, 2008; Brägelmann *et al*,
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38 2013).

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40 Within the limitations of this study (small sample size with about 50% of participation rate,
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43 retrospective design) that largely hinder the external validity of the study, these findings
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46 support the absence of HPV DNA in a young Italian cohort of oral cancer survivors and a
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49 controversial role of sexual behaviors. Our results confirm the need to further elucidate
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52 oral carcinogenesis and risk factors in young patients affected by oral cancer, in order to
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55 organize further larger studies for better elucidating the OSCC pathogenesis in young, and
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58 to set precise preventive measures.
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5 **Author Contributions:** E.V. designed the work, contributed to data analysis and
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8 interpretation, and drafted the manuscript; L.M and R.F. designed the work, analyzed and
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10 interpreted data, revised critically the paper for important intellectual content; N.L.,
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13 F.D.A., A.S. analyzed data and interpreted data, drafted the manuscript; V.N. and B.C.
14
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16 acquired, analyzed and interpreted data; G.L. conceived and designed the work, analyzed
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19 and interpreted data, revised critically the paper for important intellectual content.
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24 All Authors gave the final approval of the version to be published and agree to be
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27 accountable for all aspects of the work in ensuring that questions related to the accuracy
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30 or integrity of any part of the work are appropriately investigated and resolved.
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35 **Ethical committee approval and informed consent:** The study was performed in
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38 compliance with the recognized international standards, including the principles of the
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41 Declaration of Helsinki. Approval of the ethical committee from Authors' Institution was
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43
44 obtained. Data and sample were collected under patient's informed written consent,
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47 guaranteeing anonymity.
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Table 1. Sexual transmitted infections reported by patients (n=15)

		Have you ever had a diagnosis of genital herpes?	Have you ever had a diagnosis of genital warts?	Have you ever had a diagnosis of HPV?	Have you ever had a diagnosis of gonorrhoea?	Have you ever had a diagnosis of chlamydia?
Overall (n=15)	No	14 (93.3%)	15 (100%)	13 (86.7%)	14 (93.3%)	14 (93.3%)
	Yes	1 (6.7%)	0 (0%)	2 (13.3%)	1 (6.7%)	0 (0%)
	I don't know	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6.7%)
<i>Females (n=9)</i>	No	9 (100%)	9 (100%)	8 (88.9%)	8 (88.9%)	8 (88.9%)
	Yes	0 (0%)	0 (0%)	1 (11.1%)	1 (11.1%)	0 (0%)
	I don't know	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (11.1%)
<i>Males (n=6)</i>	No	5 (83.3%)	6 (100%)	5 (83.3%)	6 (100%)	6 (100%)
	Yes	1 (16.7%)	0 (0%)	1 (16.7%)	0 (0%)	0 (0%)
	I don't know	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 2. Sexual behavior of oral cancer survivors: answers related to overall sexual experience and practices before receiving diagnosis of OSCC (n=15)

Patient answers	Have you ever had any kind of sexual experience?	Have you ever had vaginal sex?	Have you ever had oral sex?	Have you ever had anal sex?
Yes	15 (100%)	15 (100%)	14 (93.3%)	10 (66.7%)
No	0 (0%)	0 (0%)	0 (0%)	5 (33.3%)
I don't want answer	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)

Patient answers	Throughout your life, how many sexual partners have you had before the diagnosis of OSCC?			
	Mean number of partners	Mean number of partners for vaginal sex	Mean number of partners for oral sex	Mean number of partners for anal sex
Mean	11.7	7.6	4.8	1.4
- Mean in males	20.2	10.4	9	1.75
- Mean in females	7	6.1	2.9	1.2
I don't know	1	1	2	2

How old were you when you had first sexual experience including oral, vaginal, anal sex? (years old)	
Mean	17.5 years old
- Mean in males	17.3 years old

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- Mean in females 17.5 years old

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Table 3. Sexual behavior and use of protection: answers related to before and after receiving diagnosis of oral cancer (n=15)

Patient answers		Use of protection during vaginal sex		Use of protection during oral sex		Use of protection during anal sex	
		Before diagnosis of OSCC	After diagnosis of OSCC	Before diagnosis of OSCC	After diagnosis of OSCC	Before diagnosis of OSCC	After diagnosis of OSCC
Overall (n=15)	Usually	6 (40%)	4 (26.7%)	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)
	Seldom	8 (53.3%)	6 (40%)	13 (86.6%)	11 (73.3%)	7 (46.7%)	5 (33.4%)
	Always	1 (6.7%)	2 (13.3%)	1 (6.7%)	2 (13.3%)	1 (6.7%)	4 (26.7%)
	I don't know	0 (0%)	1 (6.7%)	0 (0%)	1 (6.7%)	3 (19.9%)	2 (13.3%)
	I don't want to answer	0 (0%)	2 (13.3%)	1 (6.7%)	1 (6.7%)	1 (6.7%)	2 (13.3%)
	No sex	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (13.3%)	2 (13.3%)
Females (n=9)	Usually	5 (55.6%)	2 (22.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Seldom	4 (44.4%)	4 (44.5%)	8 (88.9%)	7 (77.8%)	5 (55.6%)	3 (33.4%)
	Always	0 (0%)	1 (11.1%)	0 (0%)	1 (11.1%)	0 (0%)	2 (22.2%)
	I don't know	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	I don't want to answer	0 (0%)	2 (22.2%)	1 (11.1%)	1 (11.1%)	2 (22.2%)	2 (22.2%)
	No sex	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (22.2%)	2 (22.2%)
Males (n=6)	Usually	1 (16.7%)	2 (33.3%)	0 (0%)	0 (0%)	1 (16.7%)	0 (0%)
	Seldom	4 (66.6%)	2 (33.3%)	5 (83.3%)	4 (66.6%)	2 (33.3%)	2 (33.3%)
	Always	1 (16.7%)	1 (16.7%)	1 (16.7%)	1 (16.7%)	1 (16.7%)	2 (33.3%)
	I don't know	0 (0%)	1 (16.7%)	0 (0%)	1 (16.7%)	2 (33.3%)	2 (33.4%)

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I don't want to answer	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No sex	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

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Table 4. Detection of HPV DNA in the histopathological specimens (used for OSCC histopathological diagnosis) and cytological smears (n=13).

HPV status	Histopathological samples	Cytological smears
HPV +	0 (0%)	0 (0%)
HPV -	13 (100%)	13 (100%)

Supplementary files

Table S1. Traditional oral cancer risk factors related to lifestyles: smoking and alcohol habits (N/A= answer not available, since no reply was provided by the patient; * Heavy smoking cut-off: >10 cigarettes/die; Ψ Stopped smoking after OSCC diagnosis; x Heavy alcohol drinker cut-off: >3 units/die)

Patient number	Smoking habit before oral cancer diagnosis *	Smoking habit after oral cancer diagnosis *	Alcohol habit before oral cancer diagnosis x	Alcohol habit after oral cancer diagnosis x
1	Former light smoker	Former light smoker	Occasionally drinker	Occasionally drinker
2	Light smoker	Former light smoker Ψ	Occasionally drinker	Occasionally drinker
3	Heavy smoker	Former heavy smoker Ψ	Former heavy drinker	Former heavy drinker
4	N/A	N/A	N/A	N/A
5	Light smoker	Former light smoker Ψ	Occasionally drinker	Occasionally drinker
6	No smoker	No smoker	Never drinker	Never drinker
7	Heavy smoker	Occasionally smoker Ψ	Occasionally drinker	Occasionally drinker
8	No smoker	No smoker	Occasionally drinker	Occasionally drinker
9	No smoker	No smoker	N/A	N/A
10	Heavy smoker	Heavy smoker	Heavy drinker	Heavy drinker
11	Heavy smoker	Heavy smoker	Heavy drinker	Heavy drinker
12	No smoker	No smoker	Light-moderate drinker	Light-moderate drinker
13	No smoker	No smoker	Occasionally drinker	Occasionally drinker
14	N/A	N/A	N/A	N/A
15	Light smoker	Former light smoker Ψ	Occasionally drinker	Occasionally drinker

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16	No smoker	No smoker	Light-moderate drinker	Light-moderate drinker
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