Role of Oral Microbiome on Oral Cancer

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Oral cancer (OC) is the most common neoplasm in the head and neck region. OCs are defined as cancers of the tongue, the floor of the oral cavity, the lining of the cheeks, the roof of the oral cavity, the gums, and the lips but do not include laryngeal and pharyngeal cancers. The majority of OCs comprise oral squamous cell carcinomas (OSCCs). The oral microbiome hosts more than 750 common oral species. A healthy microbiome usually consists of Streptococci, Staphylococci, Neisseria species, and about 50 other aerobes. The consensus is that a normal mouth microbiome consists mainly of aerobes, while the percentage of anaerobes increases with OC (and other pathological oral conditions). The connection that exists between microbes and carcinoma is complex and influenced by a number of factors, such as the vulnerability and genetic makeup of the host, as well as environmental conditions, including the host's dietary regime, oral hygiene, and tobacco and alcohol use.

Keywords: microbiome ; alcohol ; acetaldehyde ; auto-brewery syndrome ; oral cancer ; risk factors

1. Oral Cavity Histology

The structure of the mucous membrane of the oral cavity comprises two strata: (a) the keratinised or non-keratinised stratified squamous epithelium and (b) the lamina propria of the mucosa. The stratified keratinised squamous epithelium covers the lamina propria in areas exposed to mechanical stimuli related to chewing (the gums, hard palate, and the back of the tongue) and consists of four layers (basal, spinous, granular, and keratinising). The non-keratinised stratified squamous epithelium covers the inner surfaces of the lips, cheeks, and sublingual area and consists of three layers (basal, spinous, and superficial). The lamina propria of the mucosa is made of fibrous connective tissue and contains small salivary glands (labial, buccal, palatal, and lingual). The proper mucosa contains clusters of lymphoid tissue (tonsils and loose lymphocytes), which protect against harmful factors from air and food [1].

2. Oral Cancer Risk Factors

Drinking alcohol, smoking tobacco, and infection with the human papillomavirus (HPV) (there are about 14 high-risk types of HPV, including HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; two of these, HPV 16 and HPV 18, are responsible for most HPV-related cancers, and HPV 16 in particular is responsible for oropharyngeal carcinoma) are most commonly recognised as the most critical risk factors for oral cancer (OC) [2][3][4][5][6][7][8][9][10][11][12]. All the above-mentioned factors are controllable: it is a matter of lifestyle choice in the case of the first two and being vaccinated in the case of the latter. It should be noted that the meta-analysis by Arif et al. implied a negative synergy between HPV infection and smoking tobacco as well as HPV infection and high alcohol consumption, increasing the risk of primary OC development in a tiered and cumulative effect analysis [13]. Other risk indicators include Epstein–Barr virus (EBV, for nasopharyngeal carcinoma) [14][15], *Candida albicans* [16][17], a diet low in vitamin A and carotenoids, inadequate dental hygiene, including the regularity of tooth brushing, and chronic mouth trauma [18].

Another possible risk factor is the use of mouthwash [19]. Research studies examining this last correlation have conflicting outcomes, with some confirming and others disputing it. Carr and Aslam-Pervez noted that there is currently insufficient proof to reach any conclusive answers about the link between the use of an alcohol-containing mouthwash and the risk of OC [20].

However, among the many possible risks of HNC, alcohol and tobacco are the factors with the strongest associations [21] [22]. Mello et al. state in a systematic review and meta-analysis that alcohol consumption and smoking tobacco are the primary risk factors for developing OC, with substantial synergistic effects [23]. This fact is not new and has been known for many years.

The earliest evidence of alcoholic fermentation comes from 7000 BC [24]. As a matter of fact, over 60 various diseases and conditions have been causally associated with alcohol consumption [25]. In 2016, the World Health Organisation

(WHO) evaluated that alcohol consumption was responsible for 3 million deaths worldwide [26]. It is worth noting that alcohol use exerts pleiotropic effects on human health which are highly dependent on the dose. Alcohol abuse can seriously affect mental [27][28][29] or physical health [30][31][32].

Even though alcohol is a notorious carcinogen, it remains underestimated by the population at large [33]. French pathologist Lamu published the first report on this relationship in Paris in 1910. It concerned a significant correlation between heavy drinking (absinthe—an anise-flavoured spirit derived from several plants, comprising 45–74% alcohol, also known as “The Green Fairy”), smoking, and oesophageal cancer [34]. Numerous longitudinal and case–control studies provide ample evidence linking such cancers to alcohol use [35].

Overall, regardless of the variety of beverages, ethanol intake accounts for an estimated 5% of total cancers, predominantly of the liver [36], upper respiratory tract, gastrointestinal tract, pancreas, breast, and lung [4][37]. Jayasekara et al. reported that their findings support dose-dependent associations linking chronic alcohol use with cancers such as breast, upper respiratory tract, digestive tract, and colorectal carcinoma [38]. Until now, many epidemiological studies, including several meta-analyses [39][40][41][42][43], strongly support the connection between alcohol use and the possibility of OC, yet it should be mentioned that certain studies have not found a significant link between alcohol and the incidence of oral tumours [44]; however, such studies are rare.

Ceasing the use of alcohol reduces the risk of HNC every year. After 20 years of abstinence, the risk becomes comparable to the risk of non-drinkers. The results showed that in upper aerodigestive tract (UADT) cancer survivors, drinking was linked to an over twofold increased risk of second UADT cancers versus not drinking [45].

Though alcohol use is a significant risk factor for head and neck cancer, only a few drinkers eventually develop HNC, which indicates that other conditions may influence the development of HNC [46]. Even though an association appears to exist between long-term alcohol consumption and the progression of OC, people have not yet entirely understood the exact role of alcohol in the pathogenesis of the disease. Not all OC sufferers use alcohol, and not all alcohol users develop OC [47].

3. Mechanism of Alcohol Toxicity

The ethanol molecule itself has not been proven to be genotoxic, mutagenic, or carcinogenic [48]. However, ethanol is metabolised into acetaldehyde (ACH) via alcohol dehydrogenase (ADH, EC 1.1.1.1); in turn, this is metabolised into the non-harmful molecule acetate via acetaldehyde dehydrogenase (ALDH, EC 1.2.1.10) which, after conversion into acetyl coenzyme A (Acetyl-CoA), enters a tricarboxylic acid cycle (the Krebs cycle or the TCA cycle). ACH causes DNA mutations and is accepted to have a major impact on carcinogenesis [48][49][50][51]. ACH fulfils four of the ten essential properties generally displayed by recognised human carcinogens—ACH is electrophilic and harmful to genetic material, interferes with DNA repair, and causes oxidative stress [52]. ACH’s in vitro and in vivo genotoxicity is generally known [48], and it has also been shown in other species [53].

Hence, the IARC has classified ACH from alcohol consumption as belonging to Group 1 carcinogens (“carcinogenic to humans”) [45][54][55]. The conclusions of the IARC refer to the ACH metabolically generated from ethanol and the “free” ACH found in alcoholic drinks [56]. ACH is also the most common carcinogen in cigarette smoke, which solubilises in saliva during smoking [57].

Although alcohol metabolism predominantly occurs in the liver, the oral tissue layer and kidneys can also metabolise ethanol [58]. Cytoplasmic and mitochondrial ALDH break down ACH so efficiently that neither peripheral nor liver venous blood contain measurable ACH amounts after a dose of alcohol in persons with regular ALDH activity, and the amount of ACH is only slightly elevated (<10 mM) in those with ALDH deficiency [59][60][61]. Inversely, ACH concentrations were found in saliva after the consumption of alcohol at concentrations greater than what is needed to induce a mutagenic effect.

Typically, measurable amounts of ACH are not found in human saliva [62]. However, ethanol consumption (or tobacco smoking) causes an accumulation of ACH in the saliva which is dependent on the concentration, in seconds, that persists from 10 to 15 min following every sip of an alcoholic drink. Both ADH and ALDH dehydrogenases are expressed in the oral mucosa; however, ALDH is less active than ADH. Therefore, transformation into the relatively non-harmful acetate molecule is minimal in the oral cavity. This may lead to the build-up of mutagenic concentrations of ACH in saliva (compared to blood, its levels are 10 to 100 times higher) [63][64]. The locally generated ACH is mainly produced by microbes representing the normal gastrointestinal flora [65][66].

It must be added that during the treatment of patients with regular ALDH expression with 4-methylpyrazole (4-MP), an inhibitor of human ADH, no substantial alterations in ACH levels were observed in the saliva or blood. This proves that in people with normal ALDH and ADH activities, the generation of ACH in the saliva is mainly of microbial provenance [65][67]. Approximately 10- to 20-fold larger concentrations of 4-MP are required to achieve an inhibition of microbial ACH production of 40 to 50% in vitro than for an identical inhibition of ethanol elimination in humans [67].

Yokoyama et al. intensively investigated a widespread ALDH gene polymorphism with a dormant mutated enzyme [68]. Inactive ALDH polymorphisms and reduced ACH clearance are strongly associated with the risk of cancer (oropharyngolaryngeal, esophageal, stomach, colon, and lung but not liver or other cancers). A single-point mutation of the ALDH2 gene lowers the activity of the main enzyme that metabolises ACH (ALDH2). Consequently, people with an ALDH2 deficiency after alcohol consumption are exposed to 2- to 3-fold concentrations of ACH in their saliva and 5- to 6-fold concentrations in their gastric juice compared to people who have an active ALDH2 enzyme [61][64][69][70]. In parallel with an increased local exposure to ADH, the risks of oral, pharyngeal, oesophageal, and stomach cancers among alcohol users with an ALDH2 deficiency are many times higher than in individuals with a functioning ALDH2 enzyme. The current epidemiological and biochemical evidence for people with an ALDH2 deficiency presents a rare and measurable model of the risk ACH presents to humans because it is not corrupted by interfering factors that hinder the majority of epidemiological research on cancer attributable to alcohol (ethanol particles are not carcinogens; ACH linked with alcohol drinking is carcinogenic (Group 1); salivary ACH levels are zero in the absence of ethanol or tobacco; the oral tissue layer is devoid of ALDH enzymes; the low or lack of ability of the oral microflora to eliminate ACH; the lack of disparity between ALDH2-deficient and ALDH2-positive persons when comparing the ability of the mouth microbiota to generate ACH from ethanol as well as the ability of the mouth microbiota to eliminate ACH) [70]). After drinking alcohol, local ACH exposure occurs immediately due to ACH's immediate and mainly microbial formation [65][71]. Prolonged exposure represents ACH created from ethanol, which re-diffuses from the blood into the saliva within 30 min of the last sip of alcohol. Since the microbes of the mouth have a limited ability to eliminate ACH, as mentioned above, and as they cannot eliminate ACH microbially formed from ethanol, there are no indications of differences between ALDH2-deficient and ALDH2-positive persons. Thus, drinking causes the accumulation of mutagenic concentrations of ACH in the saliva of both ALDH2-positive and ALDH2-deficient persons insofar as alcohol remains in the human body [72].

4. The Role of the Microbiome

The connection that exists between microbes and carcinoma is complex and influenced by a number of factors, such as the vulnerability and genetic makeup of the host, as well as environmental conditions, including the host's dietary regime, oral hygiene, and tobacco and alcohol use [73][74]. Dysbiosis is usually given as a reason for oral carcinogenesis, as it promotes inflammation on one hand and ACH production on the other.

Changes in the microbiome's composition can modify the microenvironment of the oral cavity, resulting in inflammation and malignancies [75]. The tumour microenvironment is strongly affected by cells connected to inflammation, which is an essential factor in carcinogenesis [42]. Many studies show that poor oral health fuels a persistent macrobiotic imbalance and is linked to dysplasia and the formation of cancer in the upper gastrointestinal passageway [76]. In contrast, the relationship of the oral microbiota with HNC was examined in a 4-year study by Hayes et al., and the study did not show a substantial risk related to particular types of bacteria or the makeup of the oral microbiome in subjects with this type of cancer [77].

Salaspuro reported that due to their ADH activity, a number of microbes that make up the normal oral microbiota can oxidise ethanol to ACH [78]. Peak salivary ACH levels following alcohol consumption may differ significantly among individual subjects, ranging from 18 to 260 µM [79]. The differences are caused by changes in the microbial composition of the oral cavity as well as the salivary ethanol concentration after alcohol consumption. In the oral cavity, the interindividual diversity of the microbial flora occurs primarily at the species or strain levels [74][80]. Homann et al. reported that salivary ACH formation from ethanol under in vitro conditions could be twice as high in those with poor oral health than in individuals with sound oral hygiene [63]. Rinsing the mouth with an antimicrobial rinse before consuming alcohol has been shown to reduce the number of microorganisms in the saliva and ACH rates by approximately 50%, proving the prominent role of the mouth's bacterial microbiota in this process [81].

Counts of bacteria and yeast may seem important, but to evaluate the individual risk of the presence of ACH in the oral cavity it is crucial to determine the makeup of the mouth microbiome. Therefore, when examining the microbial structure of the oral cavity, in addition to checking the number of bacteria, it is vital to study the microbial species to determine whether they are known producers of ACH.

It should be mentioned that not only does poor or good oral hygiene influence the composition of the microbiome but alcohol consumption is also known to modify it [65][74][82][83]. Regarding the effect of alcohol use on the population of oral cavity microbiota, a major study of Americans found that the microbial composition is altered, especially in heavy drinkers [84]. Notably, the number of Lactobacillus bacteria, which appear to be linked to anti-inflammatory and antioxidant properties, has dropped considerably. Smoking is also known to change the microbiome makeup and may increase the incidence of fungal infections caused by yeasts such as *Candida albicans* (*C. albicans*). Homann et al. reported that the microbiome of cigarette smokers has a greater vital capacity to generate acetaldehyde from alcohol both in vitro and in vivo [63].

Of the bacterial species that usually inhabit the oral cavity, the species Neisseria mucosa (Gram-negative aerobic bacteria, generally associated with good oral health) has an exceptionally elevated ADH activity and generates substantial levels of ACH when cultured in the presence of ethanol in vitro [22]. Muto and co-workers reported that Neisseria's ability to produce ACH was more than 100 times more remarkable than other species tested [85]. Topical ACH generation by Neisseria mucosa in the mouth has been linked to the development of cancer. That said, a recent article noted an inverse correspondence between high levels of Neisseria and the ability of the mouth microflora to create ACH [86].

Streptococci (Gram-positive aerobic bacteria) present in the healthy oral microbiota (for example, *S. salivarius*, *S. gordonii*, *S. intermedius*, and *S. mitis*) also exhibit a considerable enzymatic activity of ADH. Among 16 Streptococcus strains studied by Kurkivuori et al., *S. salivarius*, *S. intermedius*, and *S. mitis* showed significant ADH activity. Moritani et al. evaluated the production of ACH in vitro by 41 bacterial species from 16 genera chosen for their dominance and presence in the saliva of 166 healthy individuals. Among the examined species, all Neisseria, Rothia mucilaginosa, Streptococcus mitis, and Prevotella histicola were capable of generating ACH from alcohol in amounts over 50 µM [74][87].

The role of the yeasts inhabiting the oral cavity in ACH creation was first investigated in 1999. Of the yeasts capable of metabolising ethanol to ACH, positive evidence only exists for Candida species. Notably, both *C. albicans* and non-C. albicans (for example, Candida glabrata and Candida tropicalis) generate mutagenic quantities of ACH from alcohol (>100 µM) which can further contribute to epithelial dysplasia and oral cancer [22][74][88][89]. Yeast colonisation increased for the group with elevated salivary ACH production (78%) in comparison to the group with low (47%) salivary ACH production [90].

Long-term Candida infections are associated with mouth and oesophageal carcinogenesis in susceptible subjects [91]. Alnuaimi and colleagues examined the capacity of Candida isolates from people living with oral cancer and healthy individuals to generate ACH. The findings showed that Candida isolates generating larger quantities of ACH were more common in people with oral cancer than in healthy participants, additionally supporting the importance of Candida in ethanol-induced oral carcinogenesis, as well as the significance of identifying strains of microbiota for the assessment of oral ACH exposure [92][93].

References

1. Lowe, J.; Anderson, P.; Anderson, S. Stevens & Lowe's Human Histology, 5th ed.; Elsevier—Health Sciences Division: München, Germany, 2019.
2. Llewellyn, C.D.; Johnson, N.W.; Warnakulasuriya, K.A. Risk factors for squamous cell carcinoma of the oral cavity in yo ung people—A comprehensive literature review. Oral Oncol. 2001, 37, 401–418.
3. Available online: https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-and-cancer (acces sed on 30 April 2023).
4. Anantharaman, D.; Marron, M.; Lagiou, P.; Samoli, E.; Ahrens, W.; Pohlabeln, H.; Slamova, A.; Schejbalova, M.; Merlett i, F.; Richiardi, L.; et al. Population attributable risk of tobacco and alcohol for upper aerodigestive tract cancer. Oral On col. 2011, 47, 725–731.
5. Kumar, M.; Nanavati, R.; Modi, T.G.; Dobariya, C. Oral cancer: Etiology and risk factors: A review. J. Cancer Res. Ther. 2016, 12, 458–463.
6. Barul, C.; Fayosse, A.; Carton, M.; Pilorget, C.; Woronoff, A.S.; Stucker, I.; Luce, D.; Icare Study Group. Occupational e xposure to chlorinated solvents and risk of head and neck cancer in men: A population-based case-control study in Fra nce. Environ. Health 2017, 16, 77.
7. Pezzuto, F.; Buonaguro, L.; Caponigro, F.; Ionna, F.; Starita, N.; Annunziata, C.; Buonaguro, F.M.; Tornesello, M.L. Upd ate on Head and Neck Cancer: Current Knowledge on Epidemiology, Risk Factors, Molecular Features and Novel Ther

apies. Oncology 2015, 89, 125–136.

8. Strycharz, M.; Polz-Dacewicz, M. Rola alkoholu w etiopatogenezie raka jamy ustnej—Przegląd piśmiennictwa. Stom. Współ. 2006, 13, 20–23.
9. Maso, L.D.; Torelli, N.; Biancotto, E.; Di Maso, M.; Gini, A.; Franchin, G.; Levi, F.; La Vecchia, C.; Serraino, D.; Polesel, J. Combined effect of tobacco smoking and alcohol drinking in the risk of head and neck cancers: A re-analysis of case –control studies using bi-dimensional spline models. Eur. J. Epidemiol. 2016, 31, 385–393.
10. Sapkota, A.; Gajalakshmi, V.; Jetly, D.H.; Roychowdhury, S.; Dikshit, R.P.; Brennan, P.; Hashibe, M.; Boffetta, P. Smoke less tobacco and increased risk of hypopharyngeal and laryngeal cancers: A multicentric case-control study from India. Int. J. Cancer 2007, 121, 1793–1798.
11. Gillison, M.L.; Chaturvedi, A.K.; Anderson, W.F.; Fakhry, C. Epidemiology of Human Papillomavirus–Positive Head and Neck Squamous Cell Carcinoma. J. Clin. Oncol. 2015, 33, 3235–3242.
12. Sand, L.; Jalouli, J. Viruses and oral cancer. Is there a link? Microbes Infect. 2014, 16, 371–378.
13. Rawan, T.; Arif, R.T.; Mogaddam, M.A.; Merdad, L.A.; Farsi, N.J. Does human papillomavirus modify the risk of orophar yngeal cancer related to smoking and alcohol drinking? A systematic review and meta-analysis. Laryngoscope Investig. Otolaryngol. 2022, 7, 1391–1401.
14. Shimakage, M.; Horii, K.; Tempaku, A.; Kakudo, K.; Shirasaka, T.; Sasagawa, T. Association of Epstein-Barr virus with o ral cancers. Hum. Pathol. 2002, 33, 608–614.
15. Drop, B.; Strycharz-Dudziak, M.; Kliszczewska, E.; Polz-Dacewicz, M. Coinfection with Epstein–Barr Virus (EBV), Hum an Papilloma Virus (HPV) and Polyoma BK Virus (BKPyV) in Laryngeal, Oropharyngeal and Oral Cavity Cancer. Int. J. Mol. Sci. 2017, 18, 2752.
16. Gall, F.; Colella, G.; Di Onofrio, V.; Rossiello, R.; Angelillo, I.F.; Liguori, G. Candida spp. in oral cancer and oral precanc erous lesions. New Microbiol. 2013, 36, 283–288.
17. Develoux, M. Cancer and Mycoses and Literature Review. Bull. Soc. Pathol. Exot. 2017, 110, 80–84.
18. Albuquerque, R.; López-López, J.; Marí-Roig, A.; Jané-Salas, E.; Roselló-Llabrés, X.; Santos, J.R. Oral tongue squamo us cell carcinoma (OTSCC): Alcohol and tobacco consumption versus non-consumption. A study in a Portuguese popul ation. Braz. Dent. J. 2011, 22, 517–521.
19. Chang, J.S.; Lo, H.I.; Wong, T.Y.; Huang, C.C.; Lee, W.T.; Tsai, S.T.; Chen, K.C.; Yen, C.J.; Wu, Y.H.; Hsueh, W.T.; et al. Investigating the association between oral hygiene and head and neck cancer. Oral Oncol. 2013, 49, 1010–1017.
20. Carr, E.; Aslam-Pervez, B. Does the use of alcohol mouthwash increase the risk of developing oral cancer? Evid. Base d Dent. 2022, 23, 28–29.
21. Bouquot NDA. Oral and Maxillofacial Pathology, 2nd ed.; Saunders Elsevier: Philadelphia, PA, USA, 2002; p. 5055.
22. Ferraguti, G.; Terracina, S.; Petrella, C.; Greco, A.; Minni, A.; Lucarelli, M.; Agostinelli, E.; Ralli, M.; de Vincentiis, M.; R aponi, G. Alcohol and Head and Neck Cancer: Updates on Role of Oxidative Stress, Genetic, Epigenetics, Oral Microbi ota, Antioxidants, and Alkylating Agents. Antioxidants 2022, 11, 145.
23. Mello, F.W.; Melo, G.; Pasetto, J.J.; Silva, C.A.B.; Warnakulasuriya, S.; Rivero, E.R.C. The synergistic effect of tobacco and alcohol consumption on oral squamous cell carcinoma: A systematic review and meta-analysis. Clin. Oral Investig. 2019, 23, 2849–2859.
24. McGovern, P.E.; Zhang, J.; Tang, J.; Zhang, Z.; Hall, G.R.; Moreau, R.A.; Nuñez, A.; Butrym, E.D.; Richards, M.P.; Wan g, C.S.; et al. Fermented beverages of pre-and pro-to-historic China. Proc. Natl. Acad. Sci. USA 2004, 101, 17593–175 98.
25. GBD 2020 Alcohol Collaborators. Population-level risks of alcohol consumption by amount, geography, age, sex, and y ear: A systematic analysis for the Global Burden of Disease Study 2020. Lancet 2022, 400, 185–235.
26. Global Status Report on Alcohol and Health. Available online: https://www.who.int/publications/i/item/9789241565639 (accessed on 30 April 2023).
27. Ledda, R.; Battagliese, G.; Attilia, F.; Rotondo, C.; Pisciotta, F.; Gencarelli, S.; Greco, A.; Fiore, M.; Ceccanti, M.; Attilia, M.L. Drop-out, relapse and abstinence in a cohort of alcoholic people under detoxification. Physiol. Behav. 2019, 198, 6 7–75.
28. Ceccanti, M.; Hamilton, D.; Coriale, G.; Carito, V.; Aloe, L.; Chaldakov, G.; Romeo, M.; Iannitelli, A.; Fiore, M. Spatial le arning in men undergoing alcohol detoxification. Physiol. Behav. 2015, 149, 324–330.
29. Coriale, G.; Gencarelli, S.; Battagliese, G.; Delfino, D.; Fiorentino, D.; Petrella, C.; Greco, A.; Ralli, M.; Attilia, M.L.; Mes sina, M.P.; et al. Physiological Responses to Induced Stress in Individuals Affected by Alcohol Use Disorder with Dual Diagnosis and Alexithymia. Clin. Ter. 2020, 171, e120–e129.

30. Ceci, F.M.; Ceccanti, M.; Petrella, C.; Vitali, M.; Messina, M.P.; Chaldakov, G.N.; Greco, A.; Ralli, M.; Lucarelli, M.; Angeloni, A.; et al. Alcohol Drinking, Apolipoprotein Polymorphisms and the Risk of Cardiovascular Diseases. Curr. Neurovascular Res. 2021, 18, 150–161.
31. Ceccanti, M.; Inghilleri, M.; Attilia, M.L.; Raccah, R.; Fiore, M.; Zangen, A. Deep TMS on alcoholics: Effects on cortisolemia and dopamine pathway modulation. A pilot study. Can. J. Physiol. Pharmacol. 2015, 93, 283–290.
32. Messina, M.P.; D'Angelo, A.; Battagliese, G.; Coriale, G.; Tarani, L.; Pichini, S.; Rasio, D.; Parlapiano, G.; Fiore, M.; Petrella, C.; et al. Fetal alcohol spectrum disorders awareness in health professionals: Implications for psychiatry. Riv. Psychiatr. 2020, 55, 79–89.
33. Kiviniemi, M.T.; Orom, H.; Hay, J.L.; Waters, E.A. Limitations in American adults' awareness of and beliefs about alcohol as a risk factor for cancer. Prev. Med. Rep. 2021, 23, 101433.
34. Lamu, L. Etude de statistique clinique de 131 cas de cancer de l'oesophage et due cardia. Arch. Mal. Appar. Dig. Mal. Nutr. 1910, 4, 451–456.
35. Wiseman, M. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: A global perspective. Proc. Nutr. Soc. 2008, 67, 253–256.
36. Vonghia, L.; Michielsen, P.; Dom, G.; Francque, S. Diagnostic challenges in alcohol use disorder and alcoholic liver disease. World J. Gastroenterol. 2014, 20, 8024–8032.
37. Baan, R.; Straif, K.; Grosse, Y.; Secretan, B.; El Ghissassi, F.; Bouvard, V.; Altieri, A.; Cogliano, V. Carcinogenicity of alcoholic beverages. Lancet Oncol. 2007, 8, 292–293.
38. Jayasekara, H.; MacInnis, R.J.; Room, R.; English, D.R. Long-Term Alcohol Consumption and Breast, Upper Aerodigestive Tract and Colorectal Cancer Risk: A Systematic Review and Meta-Analysis. Alcohol Alcohol. 2016, 51, 315–330.
39. Bagnardi, V.; Rota, M.; Botteri, E.; Tramacere, I.; Islami, F.; Fedirko, V.; Scotti, L.; Jenab, M.; Turati, F.; Pasquali, E.; et al. Alcohol consumption and site-specific cancer risk: A comprehensive dose-response meta-analysis. Br. J. Cancer 2015, 112, 580–593.
40. Turati, F.; Garavello, W.; Tramacere, I.; Bagnardi, V.; Rota, M.; Scotti, L.; Islami, F.; Corrao, G.; Boffetta, P.; La Vecchia, C.; et al. A meta-analysis of alcohol drinking and oral and pharyngeal cancers. Part 2: Results by subsites. Oral Oncol. 2010, 46, 720–726.
41. Goldstein, B.Y.; Chang, S.C.; Hashibe, M.; La Vecchia, C.; Zhang, Z.F. Alcohol consumption and cancers of the oral cavity and pharynx from 1988 to 2009: An update. Eur. J. Cancer Prev. 2010, 19, 431–465.
42. Islami, F.; Tramacere, I.; Rota, M.; Bagnardi, V.; Fedirko, V.; Scotti, L.; Garavello, W.; Jenab, M.; Corrao, G.; Straif, K.; et al. Alcohol drinking and laryngeal cancer: OVERALL and dose–risk relation–A systematic review and meta-analysis. Oral Oncol. 2010, 46, 802–810.
43. Turati, F.; Garavello, W.; Tramacere, I.; Pelucchi, C.; Galeone, C.; Bagnardi, V.; Corrao, G.; Islami, F.; Fedirko, V.; Boffetta, P.; et al. A meta-analysis of alcohol drinking and oral and pharyngeal cancers: Results from subgroup analyses. Alcohol Alcohol. 2013, 48, 107–118.
44. Lin, W.J.; Jiang, R.S.; Wu, S.H.; Chen, F.J.; Liu, S.A. Smoking, alcohol, and betel quid and oral cancer: A prospective cohort study. J. Oncol. 2011, 2011, 525976.
45. Druesne-Pecollo, N.; Keita, Y.; Touvier, M.; Chan, D.S.; Norat, T.; Hercberg, S.; Latino-Martel, P. Alcohol Drinking and Second Primary Cancer Risk in Patients with Upper Aerodigestive Tract Cancers: A Systematic Review and Meta-analysis of Observational Studies. Cancer Epidemiol. Biomark. Prev. 2014, 23, 324–331.
46. Seitz, H.K.; Stickel, F. Molecular mechanisms of alcohol-mediated carcinogenesis. Nat. Rev. Cancer 2007, 7, 599–612.
47. Reidy, J.; McHugh, E.; Stassen, L.F.A. A review of the relationship between alcohol and oral cancer. Surgeon 2011, 9, 278–283.
48. Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment. Mutagenicity of Alcohol (Ethanol) and Its Metabolite Acetaldehyde; Public Health England: London, UK, 2016.
49. Seitz, H.K.; Becker, P. Alcohol metabolism and cancer risk. Alcohol Res. Health 2007, 30, 38–47.
50. Smith, M. Genetics of human alcohol and aldehyde dehydrogenases. Adv. Hum. Genet. 1986, 15, 249–290.
51. Salaspuro, M. Key role of local acetaldehyde in upper GI tract carcinogenesis. Best Pract. Res. Clin. Gastroenterol. 2017, 31, 491–499.
52. Smith, M.T.; Guyton, K.Z.; Gibbons, C.F.; Fritz, J.M.; Portier, C.J.; Rusyn, I.; DeMarini, D.M.; Caldwell, J.C.; Kavlock, R.J.; Lambert, P.F.; et al. Key characteristics of carcinogens as a basis for organising data on mechanisms of carcinogenesis. Environ. Health Perspect. 2016, 124, 713–721.

53. Guo, Y.; Wang, X.; Zhang, X.; Sun, Z.; Chen, X. Ethanol promotes chemically induced oral cancer in mice through activ ation of the 5-lipoxygenase pathway of arachidonic acid metabolism. Cancer Prev. Res. 2011, 4, 1863–1872.

54. WHO. International Agency for Research on Cancer. In IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; Alcohol Drinking; IARC: Lyon, France, 1988.

55. International Agency for Research on Cancer (IARC). Personal Habits and Indoor Combustions. In A Review of Human Carcinogens; IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; IARC: Lyon, France, 2012; pp. 3 73–499.

56. Secretan, B.; Straif, K.; Baan, R.; Grosse, Y.; El Ghissassi, F.; Bouvard, V.; Benbrahim-Tallaa, L.; Guha, N.; Freeman, C.; WHO International Agency for Research on Cancer Monograph Working Group; et al. A review of human carcinoge ns—Part E: Tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol. 2009, 10, 1033–1034.

57. Haussman, H.J. Use of hazard indices for a theoretical evaluation of cigarette smoke composition. Chem. Res. Toxicol. 2012, 25, 794–810.

58. Wang, Y.; He, W. Endogenous mitochondrial aldehyde dehydrogenase-2 as an antioxidant in liver. In Liver; Chapter 21; Elsevier Inc.: Amsterdam, The Netherlands, 2018.

59. Nuutinen, H.U.; Salaspuro, M.P.; Valle, M.; Lindros, K.O. Blood acetaldehyde concentration gradient between hepatic a nd antecubital venous blood in ethanol-intoxicated alcoholics and controls. Eur. J. Clin. Investig. 1984, 14, 306–311.

60. Crabb, D.W.; Matsumoto, M.; Chang, D.; You, M. Overview of the role of alcohol dehydrogenase and aldehyde dehydro genase and their variants in the genesis of alcohol-related pathology. Proc. Nutr. Soc. 2004, 63, 49–63.

61. Yokoyama, A.; Tsutsumi, E.; Imazeki, H.; Suwa, Y.; Nakamura, C.; Mizukami, T.; Yokoyama, T. Salivary acetaldehyde co ncentration according to alcoholic beverage consumed and aldehyde dehydrogenase-2 genotype. Alcohol. Clin. Exp. R es. 2008, 32, 1607.e14.

62. Salaspuro, V.; Salaspuro, M. Synergistic effect of alcohol drinking and smoking on in vivo acetaldehyde concentration i n saliva. Int. J. Cancer 2004, 111, 480–483.

63. Homann, N.; Tillonen, J.; Meurman, J.H.; Rintamäki, H.; Lindqvist, C.; Rautio, M.; Jousimies-Somer, H.; Salaspuro, M. I ncreased salivary acetaldehyde levels in heavy drinkers and smokers: A microbiological approach to oral cavity cancer. Carcinogenesis 2000, 21, 663–668.

64. Nieminen, M.T.; Salaspuro, M. Local acetaldehyde—An essential role in alcohol-related upper gastrointestinal tract car cinogenesis. Cancers 2018, 10, 11.

65. O'Grady, I.; Anderson, A.; O'Sullivan, J. The interplay of the oral microbiome and alcohol consumption in oral squamou s cell carcinomas. Oral Oncol. 2020, 110, 105011.

66. Jokelainen, K.; Matysiak-Budnik, T.; Mäkisalo, H.; Höckerstedt, K.; Salaspuro, M. High intracolonic acetaldehyde values produced by a bacteriocolonic pathway for ethanol oxidation in piglets. Gut 1996, 39, 100–104.

67. Vakevainen, S.; Tillonen, J.; Salaspuro, M. 4-methylpyrazole decreases salivary acetaldehyde levels in aldh2-deficient subjects but not in subjects with normal ALDH2. Alcohol. Clin. Exp. Res. 2001, 25, 829–834.

68. Yokoyama, A.; Muramatsu, T.; Ohmori, T.; Yokoyama, T.; Okuyama, K.; Takahashi, H.; Hasegawa, Y.; Higuchi, S.; Maru yama, K.; Shirakura, K.; et al. Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. Carcino genesis 1998, 19, 1383–1387.

69. Yokoyama, A.; Kamada, Y.; Imazeki, H.; Hayashi, E.; Murata, S.; Kinoshita, K.; Yokoyama, T.; Kitagawa, Y. Effects of A DH1B and ALDH2 genetic polymorphisms on alcohol elimination rates and salivary acetaldehyde levels in intoxicated J apanese alcoholic men. Alcohol. Clin. Exp. Res. 2016, 40, 1241–1250.

70. Lachenmeier, D.W.; Salaspuro, M. ALDH2-deficiency as genetic epidemiologic and biochemical model for the carcinog enicity of acetaldehyde. Regul. Toxicol. Pharmacol. 2017, 86, 128–136.

71. Helminen, A.; Väkeväinen, S.; Salaspuro, M. ALDH2 genotype has no effect on salivary acetaldehyde without the prese nce of ethanol in the systemic circulation. PLoS ONE 2013, 8, e74418.

72. Pavlova, S.I.; Jin, L.; Gasparovich, S.R.; Tao, L. Multiple alcohol dehydrogenases but no functional acetaldehyde dehyd rogenase causing excessive acetaldehyde production from ethanol by oral streptococci. Microbiology 2013, 159, 1437–1446.

73. Mager, D.L. Bacteria and cancer: Cause, coincidence or cure? A review. J. Transl. Med. 2006, 4, 14.

74. Stornetta, A.; Guidolin, V.; Balbo, S. Alcohol-Derived Acetaldehyde Exposure in the Oral Cavity. Cancers 2018, 10, 20.

75. Healy, C.M.; Moran, G.P. The microbiome and oral cancer: More questions than answers. Oral Oncol. 2019, 89, 30–33.

76. Abnet, C.C.; Kamangar, F.; Islami, F.; Nasrollahzadeh, D.; Brennan, P.; Aghcheli, K.; Merat, S.; Pourshams, A.; Marjani, H.A.; Ebadati, A.; et al. Tooth loss and lack of regular oral hygiene are associated with higher risk of esophageal squamous cell carcinoma. Cancer Epidemiol. Biomark. Prev. 2008, 17, 3062–3068.
77. Hayes, R.B.; Ahn, J.; Fan, X.; Peters, B.A.; Ma, Y.; Yang, L.; Agalliu, I.; Burk, R.D.; Ganly, I.; Purdue, M.P.; et al. Association of Oral Microbiome with Risk for Incident Head and Neck Squamous Cell Cancer. JAMA Oncol. 2018, 4, 358–365.
78. Salaspuro, M.P. Acetaldehyde, microbes, and cancer of the digestive tract. Crit. Rev. Clin. Lab. Sci. 2003, 40, 183–208.
79. Homann, N.; Jousimies-Somer, H.; Jokelainen, K.; Heine, R.; Salaspuro, M. High acetaldehyde levels in saliva after ethanol consumption: Methodological aspects and pathogenetic implications. Carcinogenesis 1997, 18, 1739–1743.
80. Bik, E.M.; Long, C.D.; Armitage, G.C.; Loomer, P.; Emerson, J.; Mongodin, E.F.; Nelson, K.E.; Gill, S.R.; Fraser-Liggett, C.M.; Relman, D.A. Bacterial diversity in the oral cavity of 10 healthy individuals. ISME J. 2010, 4, 962–974.
81. Pikkarainen, P.H.; Baraona, E.; Jauhonen, P.; Seitz, H.K.; Lieber, C.S. Contribution of oropharynx microflora and of lung microsomes to acetaldehyde in expired air after alcohol ingestion. J. Lab. Clin. Med. 1981, 97, 631–636.
82. Yokoyama, A.; Tsutsumi, E.; Imazeki, H.; Suwa, Y.; Nakamura, C.; Yokoyama, T. Contribution of the alcohol dehydrogenase-1b genotype and oral microorganisms to high salivary acetaldehyde concentrations in Japanese alcoholic men. Int. J. Cancer 2007, 121, 1047–1054.
83. Thomas, A.M.; Gleber-Netto, F.O.; Fernandes, G.R.; Amorim, M.; Barbosa, L.F.; Francisco, A.L.N.; de Andrade, A.G.; Setubal, J.C.; Kowalski, L.P.; Nunes, D.N.; et al. Alcohol and tobacco consumption affects bacterial richness in oral cavity mucosa biofilms. BMC Microbiol. 2014, 14, 250.
84. Fan, X.; Peters, B.A.; Jacobs, E.J.; Gapstur, S.M.; Purdue, M.P.; Freedman, N.D.; Alekseyenko, A.V.; Wu, J.; Yang, L.; Pei, Z.; et al. Drinking alcohol is associated with variation in the human oral microbiome in a large study of American adults. Microbiome 2018, 6, 59.
85. Muto, M.; Hitomi, Y.; Ohtsu, A.; Shimada, H.; Kashiwase, Y.; Sasaki, H.; Yoshida, S.; Esumi, H. Acetaldehyde production by non-pathogenic neisseria in human oral micro-flora: Implications for carcinogenesis in upper aerodigestive tract. Int. J. Cancer 2000, 88, 342–350.
86. Yokoyama, S.; Takeuchi, K.; Shibata, Y.; Kageyama, S.; Matsumi, R.; Takeshita, T.; Yamashita, Y. Characterization of oral microbiota and acetaldehyde production. J. Oral Microbiol. 2018, 10, 1492316.
87. Moritani, K.; Takeshita, T.; Shibata, Y.; Ninomiya, T.; Kiyohara, Y.; Yamashita, Y. Acetaldehyde production by major oral microbes. Oral Dis. 2015, 21, 748–754.
88. Nieminen, M.T.; Uittamo, J.; Salaspuro, M.; Rautemaa, R. Acetaldehyde production from ethanol and glucose by non-Candida albicans yeasts in vitro. Oral Oncol. 2009, 45, e245–e248.
89. Uittamo, J.; Siikala, E.; Kaihovaara, P.; Salaspuro, M.; Rautemaa, R. Chronic candidosis and oral cancer in APECED-patients: Production of carcinogenic acetaldehyde from glucose and ethanol by Candida albicans. Int. J. Cancer 2009, 124, 754–756.
90. Tillonen, J.; Homann, N.; Rautio, M.; Jousimies-Somer, H.; Salaspuro, M. Role of Yeasts in the Salivary Acetaldehyde Production from Ethanol Among Risk Groups for Ethanol-Associated Oral Cavity Cancer. Alcohol. Clin. Exp. Res. 1999, 23, 1409–1411.
91. Delsing, C.E.; Bleeker-Rovers, C.P.; van de Veerdonk, F.L.; Tol, J.; van der Meer, J.W.; Kullberg, B.J.; Netea, M.G. Association of esophageal candidiasis and squamous cell carcinoma. Med. Mycol. Case Rep. 2012, 1, 5–8.
92. Alnuaimi, A.D.; Ramdzan, A.N.; Wiesenfeld, D.; O'Brien-Simpson, N.M.; Kolev, S.D.; Reynolds, E.C.; McCullough, M.J. Candida virulence and ethanol-derived acetaldehyde production in oral cancer and non-cancer subjects. Oral Dis. 2016, 22, 805–814.
93. Alnuaimi, A.D.; Wiesenfeld, D.; O'Brien-Simpson, N.M.; Reynolds, E.C.; McCullough, M.J. Oral Candida colonisation in oral cancer patients and its relationship with traditional risk factors of oral cancer: A matched case-control study. Oral Oncol. 2015, 51, 139–145.