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candida incr cancer risk by producing acetaldehyde (ACH)?

Letters to the Editor

Chronic candidosis and oral cancer in APECED-patients: Production of carcinogenic acetaldehyde from glucose and ethanol by *Candida albicans*

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Dear Sir,

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare autosomal recessive disease caused by mutations of the AIRE (autoimmune regulator) gene.[1–3] It is associated with a limited T lymphocyte defect and an autoimmune response to various tissues, particularly endocrine glands. It mainly causes a set of three abnormal features; chronic mucocutaneous candidosis, hypoparathyroidism and adrenal insufficiency.[4] Most patients have chronic oral candidosis since early childhood. Of all the APECED patients in Finland that are beyond the age of 25, 10% have developed oral or oesophageal carcinoma at the site of chronic mucositis.[5] It is the only malignancy diagnosed in these patients. The age at cancer diagnosis was markedly low (29–44 years), significantly lower than for oral or oesophageal squamous cell carcinoma in general. The pathogenetic mechanism behind APECED associated oral cancer has so far remained unclear.[6–9]

The most important risk factors for upper digestive tract cancers are tobacco smoking, alcohol intake, and poor oral hygiene.[10–12] They all associate with increased acetaldehyde (ACH) levels in saliva, and there is strong evidence supporting the role of ACH as a common dominator.[13–18] The mechanism leading to increased ACH levels in saliva after alcohol consumption is local microbial ACH production, *i.e.* the oxidation of salivary ethanol (EtOH) to ACH by microbial alcohol dehydrogenase (ADH)-enzyme.[15–17] Previous studies have shown that *Candida albicans* can produce significant amounts of carcinogenic ACH in clinically relevant EtOH concentrations.[19]

It has been demonstrated that polyamines are able to facilitate the formation of mutagenic DNA-adducts in biologically relevant ACH concentrations (50–100 μM).[14] The local *in vivo* carcinogenic effect of ACH is derived from epidemiological and biochemical studies on aldehyde dehydrogenase-2 (ALDH2)-deficient Asians. The mitochondrial ALDH2-enzyme is responsible for most of the ACH oxidation to acetate. Among ALDH2-deficient subjects this enzyme is partly inactive, which results in the accumulation of ACH in saliva after alcohol drinking.[20] In Asian heavy drinkers ALDH2-deficiency is associated with a 10-fold risk of oral cancer when compared to those with the normal ALDH2-enzyme.[21] However, the relationship between glucose and ACH production by *Candida albicans* has not been investigated previously. *C. albicans* may play a role in metabolizing glucose into carcinogenic ACH in the mouth and could participate in the pathogenesis of oral cancer in non-alcohol drinkers. The aim of this study was to compare the production of ACH from EtOH and glucose by oral candida isolates from APECED patients with control isolates.

inability to eliminate ACH incr cancer risk 10x

A total of 67 clinical oral isolates and one reference strain (ATCC 90029) of *Candida albicans* were selected for this study. Of these, 44 were isolated from 21 APECED patients during the years 1994–2007 (1–4 isolates/patients) mainly as reported earlier.[22] Twelve isolates from patients with oral carcinoma were designated as cancer-isolates and 11 isolates obtained from patients with oral candidosis but without any mucosal neoplasia were designated as control-isolates. The *C. albicans* isolates were identified from patient samples using conventional culture and identification methods at the Clinical Microbiology Laboratory of the Helsinki University Central Hospital. The identification of *C. albicans* was based on colony morphology on CHROMagar® Candida medium (CHROMagar, Paris, France), and the negative Bichro-Dubli® latex co-agglutination test result (Fumouze Diagnostics, Levallois Perret, France). *C. albicans* strains were subcultured on Sabouraud Dextrose agar (SP; Saboraud Dextrose Agar (Lab M), Bacto Agar (Difco) supplemented with penicillin (100 000 ui/ml) and streptomycin) for 48 hr at 35°C. Colonies were suspended in phosphate buffered saline (PBS) and adjusted to an optical density (OD) of 0.4 at 492 nm (Multiscan RC spectrophotometer, Labsystems, Helsinki, Finland) corresponding to 1×10^7 colony forming units per millilitre (CFU/ml) as controlled by dilution plating.

A total of 400 μl of the yeast suspension was transferred into a gas chromatograph vial. Thereafter, 50 μl of PBS-buffer containing EtOH or glucose was added and the vial was immediately closed tightly. The final EtOH concentration was adjusted to 11 mM and the final glucose concentration to 100 mM. Samples were incubated for 30 min at 37°C, and the reaction was stopped by injecting 50 μl of perchloric acid

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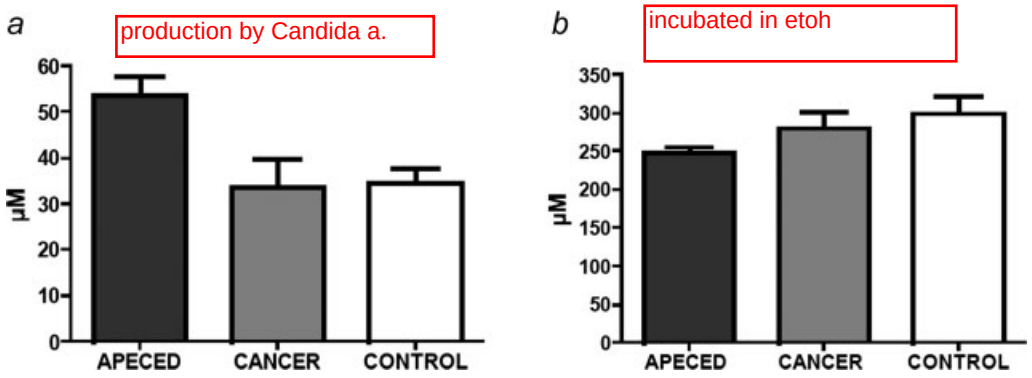

FIGURE 1 – The acetaldehyde production of *Candida albicans* isolated from APECED ($n = 44$), cancer ($n = 12$) and control patients ($n = 11$) in glucose (*a*) and ethanol (*b*) incubation. Mean (+SEM) μM of each group presented. When incubated in 100 mM glucose (*a*) the APECED isolates produced significantly higher amounts of acetaldehyde than the cancer isolates ($p < 0.0001$) and the control isolates ($p < 0.0001$). When incubated in 11 mM ethanol (*b*) the cancer and control isolates produced significantly higher amounts of ACH from ethanol than APECED isolates ($p = 0.0072$ and $p = 0.0003$, accordingly). The differences between the cancer and the control isolates were not significant.

(PCA, 6M) through the rubber septum of the test vial. Every isolate was assayed in triplicate and the mean was used for the analyses. To measure the baseline and artefactual ACH, 50 μl of PCA was immediately added to control vials and the suspension was equally incubated for 30 min at 37°C. The formed ACH was measured by gas chromatography as reported earlier.[23]

Results are presented as mean ± standard error of mean (SEM). Data was analyzed by using Graph Pad Prism version 5.00 (GraphPad Inc. San Diego, California, USA). The two-tailed Mann Whitney *U*-test was used for the comparisons between the patient groups. The two-tailed paired *t*-test was used for the comparisons within patient groups. *p* values of less than 0.05 were considered statistically significant.

When incubated in glucose the mean ACH production by the 44 *C. albicans* isolates from APECED patients was 53.5 μM (±2.3 μM). The means for the cancer and control isolates were 33.7 μM (±3.5 μM) and 34.6 μM (±1.9 μM), respectively (Fig. 1*a*). The APECED isolates produced significantly higher amounts of ACH than the cancer isolates ($p < 0.0001$) and the control isolates ($p < 0.0001$). The differences between the cancer and the control isolates were not significant. The mean ACH production by the reference strain of *C. albicans* ATCC 90029 was 38.02 μM ± 2.06 μM in glucose incubation.

When incubated in EtOH the mean ACH production of the APECED isolates was 247.9 μM (±4.2 μM). The means for the cancer and control isolates were 280.2 μM (±11.8 μM) and 299.1 μM (±12.7 μM) (Fig. 1*b*). The cancer and control isolates produced significantly higher amounts of ACH from EtOH than APECED isolates ($p = 0.0072$ and $p = 0.0003$, accordingly). The differences between the cancer and the control isolates were not significant. The mean ACH production by the reference strain of *C. albicans* ATCC 90029 was 157.43 μM (±1.57 μM) in EtOH incubation. All *C. albicans* isolates produced significantly more ACH in EtOH than glucose incubation ($p = <0.0001$).

From 14 APECED patients multiple isolates (2–4 per patient) from years apart were tested. There was no significant difference in the ACH production from EtOH or glucose between the earlier and more recent isolates from 6 patients. There were some differences between the earlier and more recent isolates from 8 patients, mostly when incubated in glucose, but there was no obvious temporal trend nor were the differences significant.

In this study we were able to show that *C. albicans* isolated from APECED patients produced potentially mutagenic amounts of ACH when incubated in 100 mM glucose. The amount of ACH produced was significantly higher when compared to *C. albicans* strains isolated from groups of patients with oral cancer or from healthy controls. 100 mM glucose is equivalent to 18 g/L, which can commonly be found in food and drinks.[24] Consumption of sweeter products may lead to even higher ACH levels and prolonged exposure. 100 mM glucose has also been shown to increase biofilm formation and adhesion of *C. albicans* in the oral cavity that may enhance the local mucosal exposure to ACH.[24,25]

glucose 100mM ~ 18g/L = 1.8%

The mean age at diagnosis of oral cancer in Finland is over 60 years and oral cancer is very uncommon among healthy young adults.[26] In APECED patients, however, the mean age at diagnosis is only 37 years.[5] The early onset of oral cancer in APECED patients could be partially due to their immune defect and other intrinsic factors. However, both extrinsic and intrinsic factors may operate, and with many of the factors, only long exposure might be carcinogenic. Most APECED patients suffer from refractory oral candidosis since early childhood and the oral carcinoma typically develops at the site of chronic mucositis. This supports the role of *C. albicans* in the pathogenesis of the carcinoma.[5]

According to our results all *C. albicans* isolates analysed were capable of producing carcinogenic levels of ACH from EtOH. The isolates from cancer and control subjects produced significantly higher amounts of ACH than those from APECED patients but the clinical relevance of this difference is questionable since all isolates produced very high amounts. The 11 mM EtOH concentration used can be found in saliva for hours after social alcohol consumption or naturally in many products produced by fermentation.[17]

The main finding of this study is that *C. albicans* may play a role in metabolizing glucose to carcinogenic ACH in the mouth and could participate in the pathogenesis of oral cancer in non-alcohol drinkers. The observation supports the concept of a novel microbially mediated mechanism in the pathogene-

sis of oral cancer and could partly explain why chronic oral candidosis is carcinogenic in APECED patients and why they have a high risk for oral cancer at an early age.

Yours sincerely,

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