

Is Alcohol an independent risk factor for Oro-Pharyngeal and Pulmonary Carcinogenesis - An Acetaldehyde concentrations based Double Blinded Randomized Control Trial

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ABSTRACT

Background: There is increasing evidence that a major part of the tumour-promoting action of alcohol is mediated via its first, toxic and carcinogenic metabolite acetaldehyde.

Materials & Methods: The double blinded randomized control trial was designed for 82 male volunteers aged 20-29 years. Exclusion criteria were individual under antibiotic therapy, smokers, mutant Aldehyde Dehydrogenase deficient subject or any other systemic disease. Subjects were randomized in experimental (alcohol + soft drink) and control group (soft drink) from each pair of equal body weighted volunteers. The amount of alcohol consumed was calculated to be equivalent to 0.7 g alcohol/kilogram of body weight. Samples of breath for Acetaldehyde concentration (AC) were captured with the aid of a highly reproducible fuel cell gas-sampling device (PST-M1; Lions Laboratories, Cardiff, Wales). In Statistical analysis, mean AC was compared among both groups at different interval using paired t-test and Analysis of variance.

Results: Mean acetaldehyde level was recorded higher ($> 50.00 \mu\text{mol/L}$) among interventional group which can be produced from ethanol during metabolism or by oro-pharyngeal microbes. After 15 minutes of drink, the AC was $71.36 \pm 17.46 \mu\text{mol/L}$ in ethanol group compared to $22.75 \pm 9.12 \mu\text{mol/L}$ in soft-drink group. There was significant increase in AC after 1 hour ($P < 0.001$) which was $77.58 \pm 19.43 \mu\text{mol/L}$ in ethanol group compared to $21.16 \pm 8.73 \mu\text{mol/L}$ in soft-drink group.

Conclusion: Although acetaldehyde is metabolite of alcohol, its organ specific production with risk for oro-pharyngeal and pulmonary carcinogenesis makes alcohol an independent risk factor of carcinogenesis.

Key Words: Oro-Pharyngeal Carcinogenesis, Pulmonary Carcinogenesis, Acetaldehyde, Alcohol.

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INTRODUCTION

For millennia, the consumption of alcoholic beverages has contributed to the pleasure of eating and drinking in many cultures of the world. While the optimal or non-injurious levels of alcohol intake are difficult to estimate, they have been thought to be quite low, approx-

imately 10-19 g/day for men and less than 10 g/day for women¹. When taken in excess, alcohol has devastating effects on human health by leading to breakdown of bodily functions and damaging virtually every organ of the body.

The mechanism for the increased cancer risk associated with alcohol consumption is not clear, but has been believed due to the carcinogenic action of the first metabolite of ethanol i.e. Acetaldehyde²⁻³. It has been concluded that there is sufficient evidence for the carcinogenicity of alcoholic beverages in humans⁴, but there is no experimental evidence to indicate that alcohol itself is a carcinogen⁵. So far this carcinogenic phenomenon has been thought to arise from systemic effects of elevated blood acetaldehyde⁶⁻⁸.

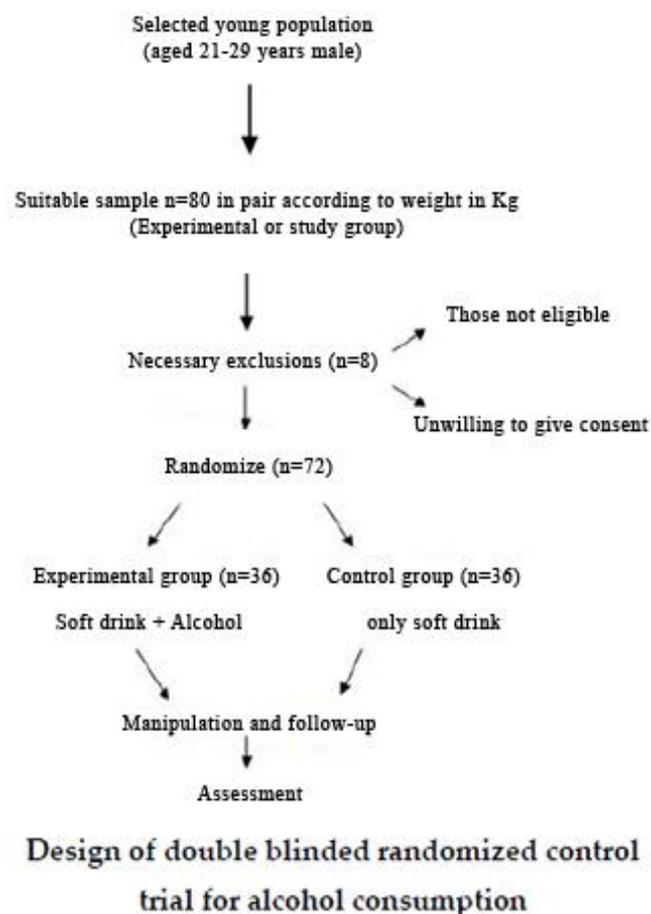
Acetaldehyde has been shown to be carcinogenic both to test animals and humans⁹. The acetaldehyde dehydrogenase enzyme (ALDH2) of the liver removes the acetaldehyde thus generated; no alcohol accumulates in the liver, where the metabolism of alcohol mainly takes place. Researchers are unable to establish alcohol as independent risk factor in oral-pharyngeal cancer; due to scarcity of data on organ specific production of acetaldehyde (a known carcinogen).

Alcohol effect on oro-pharyngeal region can be locally and systemically. As majority of evidence had suggested that systemically alcohol have potential carcinogenic effect on organs and tissues with rich blood supply⁸, its local effect was not proved. There is rich blood supply in oro-pharyngeal region and local effect of alcohol can also be expected for carcinogenesis due to the fact that the regular use of mouthwashes with an alcohol content had shown increased risk of oro-pharyngeal cancer compared to non-alcoholic mouthwash¹⁰. Acetaldehyde is produced from ethanol in the epithelia by mucosal alcohol dehydrogenases, but much higher levels derive from microbial oxidation of ethanol by the oral microflora¹¹. The proposed tumorigenic effect of ethanol may be linked to local production of acetaldehyde from ethanol through exhaled breath and the production by oral microflora. Hence, the objectivity of present study was to assess the risk of carcinogenesis via acetaldehyde production (by alcohol and microbes both) in oral, pharyngeal and pulmonary region through its exhaled breath concentration. The morbidity and mortality in carcinogenesis associated with acetaldehyde level in these vital regions could be helpful in their management and as a prerequisite for their prevention.

MATERIALS AND METHODS

In this study among human volunteers, informed consent was obtained from subjects after approval by the authorized ethical committee. The age of the male volunteers ranged from 21 to 29 years (mean 24.3 years). The double blinded randomized control design for the participants is explained by a diagrammatic presentation. The exclusion criterion comprised of the subjects who have undertaken antibiotic therapy, smoker, chronic alcoholic (>40 g/day), poor oral hygiene, individuals deficient in Aldehyde Dehydrogenase (ALDH2*2 allele) or any other systemic disease.

The amount of alcohol consumed for intervention group was calculated to be equivalent to 0.7 g alcohol/kilogram of body weight. The study was performed on 3 different occasions, between 5 p.m. and 9 p.m., with the consumption of alcohol beginning at 6 p.m. The breath samples were collected as follows: The first sample was collected after 15 min consumption of alcohol (B0). The second



sample (B1) was collected when the AC was estimated to reach a maximum level (i.e., 45 minutes after alcohol consumption), and the last sample (B2) was collected 1 hour after the second sample (B1).

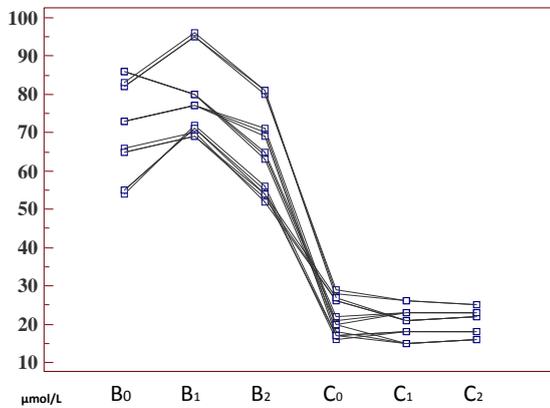


Fig. 1: Acetaldehyde concentration among alcohol group (B0, 1,2) and control.

The alcohol was consumed for 15 minutes. The participants were not allowed to eat or drink anything 4 hours prior, during and in the periods between saliva collections. The alcohol was consumed in soft-drink (pH = 6.5) while in control group alcohol was not added. Samples of breath were captured with the aid of a highly reproducible fuel cell gas-sampling device (PST-M1; Lions Laboratories, Cardiff, Wales)¹². Each subject were instructed to take a moderately deep inhalation of room air and then exhale as much breath as possible. A reusable plastic sample cup was used as a single-use mouthpiece tube for fast, non-invasive AC testing. The combined oral, pharyngeal and breath acetaldehyde levels in were recorded just seconds later on a large, easy to read display. The reading is then stored in memory, with date and time, for later download to a computer system for analysis purpose. An optional data-logging system (e.g. operator, subject, sex and age) was also used. In the fuel cell an electro-chemical reaction between acetaldehyde and oxygen produces an electric current proportional to the AC in air-blow¹². The process of analysis of a breath sample for AC by the fuel cell is as follows. 1. The exhaled breath passes through pharyngeal and oral region, is introduced to the fuel cell. 2. The acetaldehyde in the sample is chemically

oxidized at the anode. 3. At the same time, oxygen (from the atmosphere) is chemically reduced at the cathode. 4. A current flow, proportional to the concentration of acetaldehyde, is produced between the

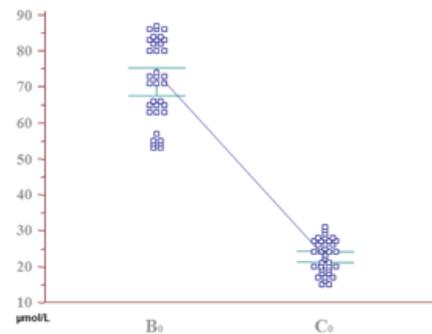


Fig. 2: Acetaldehyde concentration among individual participant after 15 minutes of drinking from alcohol group(B0) and control(C0) in µmol/L.

two electrodes. Fuel Cell Sensor has high specificity to acetldehyde, unaffected by other possible oral and breath contaminants.

For statistical analysis, mean AC was compared among both groups at different interval using paired t-test and Analysis of variance using SPSS software (15 ver., Chicago inc., USA).

RESULTS

Mean acetaldehyde level was recorded higher (> 50.00 µmol/L) among alcohol group which can be produced from ethanol during metabolism and by oro-pharyngeal microbes. The results are described in table 1, figure 1 and figure 2. After 15 minutes of drink, the acetaldehyde concentration(B0) was 71.36 ± 17.46 µmol/L in ethanol group compared to 22.75 ± 9.12 µmol/L in soft-drink group. There was significant increase in ethanol concentration after 1 hour (B1) (paired t test, $P < 0.001$, results not displayed in table) which was 77.58 ± 19.43 µmol/L in ethanol group compared to 21.16 ± 8.73 µmol/L in soft-drink group. This was the peak value recorded followed by lower value after 45 minutes of B1. In B2 the concentration reduced to 64.27 ± 18.86 µmol/L in ethanol group compared to 21.33 ± 9.87 µmol/L in soft-drink group.

Table 1: Mean acetaldehyde concentration (\pm S.D of mean) among alcohol and control group at different time interval

Acetaldehyde concentration (AC) ($\mu\text{mol/L}$)	Ethanol group (A)	Control group (C)	Paired T-test	ANOVA (F-test)
B ₀ (15 minutes)	71.36 \pm 17.46	22.75 \pm 9.12	P< 0.001	F=3.66, P<0.001
B ₁ (1 hour)	77.58 \pm 19.43	21.16 \pm 8.73	P< 0.001	F=4.72, p<0.001
B ₂ (45 minutes after S1)	64.27 \pm 18.86	21.33 \pm 9.87	P< 0.001	F=3.98, P<0.001

T-test for comparison of mean, F-test for comparison of standard deviate, P<0.01 = highly significant (100 $\mu\text{mol/L}$ Acetaldehyde = 4.4 mg/L)

DISCUSSION

Although many researches has been done for alcohol consumption, for organ specific morbidity very few were able to conclude its carcinogenicity with specific reasons. Persson et al (1991)¹³ concluded that alcohol increases mucosal permeability and promotion of bacterial overgrowth. Moreover, Alcohol dehydrogenase (ADH) catalyses the reversible oxidation of many alcohols to corresponding aldehydes¹⁴. In case of ethanol, the reaction is as follows:



The results of the present study were also similar to Doll et al. (1999)⁵, who had shown that cancers of the mouth, esophagus, and larynx are associated with alcohol consumption but he also suggested that the risk increases in a dose-dependent manner.

The potential confounding factors of acetaldehyde production were individual deficient ALDH2, smokers, chronic drinkers and poor oral hygiene were excluded in present study. It has been found in Asian drinkers, who have a familial low-activity modification of the aldehyde dehydrogenase-2 (ALDH2) enzyme, have an increased risk of developing a cancer of the mouth, the pharynx and the digestive tract after consuming alcohol¹⁵. Even more common is the ADH3*1 gene/allele (ADH1C at present), which predisposes the heavy drinkers, who have this gene to the upper digestive tract cancers because of increased local acetaldehyde contents¹⁶. In human, acetaldehyde is formed from alcohol as a consequence of the hepatic metabolism and locally in the digestive tract via microbial alcohol dehydrogenase of mucous membrane cells¹⁷. ALDH2 deficient were excluded

from the present study as cancer risk is markedly increased among heavy drinking Asian individuals with a genetically deficient ability to remove acetaldehyde¹⁸.

Smokers and poor oral hygiene were excluded from the study. Smoking is well-known independent risk factors for oral cancer. There is epidemiological evidence indicating that alcohol and tobacco act together in a multiplicative rather than additive manner and, accordingly seem to have synergistic tumor-promoting effects¹⁹. This implies that smokers, even after moderate alcohol intake, produce much higher levels of carcinogenic acetaldehyde in the oral cavity than non-smokers. Moreover oral hygiene is also important as, yeasts and bacteria found in higher loads in poor oral hygiene among individuals and more frequently in the high acetaldehyde-producing saliva group than the low group. Since *Candida albicans* was the dominating yeast species isolated from the saliva and *C. albicans* produced on average higher acetaldehyde levels than other yeast species detected, this can be regarded as the most common and important yeasts species with respect to acetaldehyde production capacity from ethanol.

Acetaldehyde can be used as bio-marker for analyzing risk associated with alcohol consumption as it reaches all target tissues of the upper oro-digestive tract, including the larynx, pharynx, oral cavity, and esophagus, via normal distribution and evaporation. Due to its high reactivity, toxicity and carcinogenicity, acetaldehyde can be expected to cause organ damage wherever it exists at high concentrations. This hypothesis is strongly supported by our very recent finding demonstrating oro-pharyngeal cancer among Asians with mutant ALDH2*2 allele had 2-3 times

higher oral acetaldehyde levels after a moderate dose of ethanol than those Asians with a normal ALDH 2*1 genotype throughout the whole follow-up period of 240 minutes¹⁵.

According to the recent concept given by Eriksson CJ (2007)²⁰, the acetaldehyde in the breath most likely reflects pulmonary blood acetaldehyde, microbial as well as tissue acetaldehyde production in the Oro-pharyngeal tract, hence it can be considered as acetaldehyde level of oral, pharyngeal and breath as a whole. Breath analysis is a technique rapidly gaining ground as a non-invasive tool to diagnose and monitor various aspects of lung diseases. Measurement of exhaled breath is safe, rapid, simple to perform, and effort independent. Oro-pharyngeal AL were recorded in present study as exhaled breath passes through these region during flow, and hence the score for each blow can be considered as collective AC of all these regions²¹. This EBC(Exhaled Breath Consolidate) is a biological matrix currently encountering both much enthusiasm due to its noninvasiveness, and also a lot of skepticism due to the problems related to difficulties concerning the standardization of its collection procedures and the absence of a gold standard²¹. On account of instrument accuracy the Fuel Cell based AC measuring is approved and used in various countries as an evidential instrument used for medicine, for medico-legal purpose by police, in industry and in many other applications too. Under microaerophilic or aerobic conditions and in the presence of excess ethanol, some microorganisms can oxidize ethanol to acetaldehyde. In the past this bacteriocolonial pathway for ethanol oxidation was primarily thought to be mediated via reversed microbial ADH reaction²². The specification for evidential breath testing equipment requires that foreign gases (such as methanol, isopropanol, acetone, ethyl acetate and toluene) that could be in a breath sample together with alcohol should cause no cross-sensitivity in the evidential breath test, or cause no excessive variation, failing which the test would be automatically discontinued.

CONCLUSION

In our study the alcohol dose was kept similar for the subjects according to weight and potential confounding factors were controlled. Still then,

acetaldehyde production was different among individuals suggesting many other factors also might also be involved other than alcohol in acetaldehyde production affecting carcinogenesis. Hence, future researches are still required in this direction.

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