

## Differential Expression of ADH and ALDH2 can be a Diagnostic Marker in Gastroesophageal Cancer

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### Abstract

Acetaldehyde interacts covalently with DNA to form major stable acetaldehyde–DNA adduct N2- ethyl-2'- de-oxyguanosine, which may be critical initiating event in the multistage process of chemical carcinogenesis primarily observed in gastroesophageal cancers. In the present study, RT-PCR experiment results showed increased expression of ADH and decreased expression of ALDH2 in GE cancerous conditions when compared with the normal GE tissue indicating the variation in the catabolism of acetaldehyde to acetate in the cancerous tissue. These results were further corroborated by enzyme assay where increased ADH enzyme activity was observed ( $0.445 \pm 0.02 \mu\text{M/ml/minute}$ ) in cancerous tissue compared to normal tissue ( $0.335 \pm 0.01 \mu\text{M/ml/minute}$ ) whereas; ALDH2 enzyme activity ( $0.15 \pm 0.01 \mu\text{M/ml/minute}$ ) decreased appreciably in cancerous tissue and compared to normal tissue ( $0.356 \pm 0.04 \mu\text{M/ml/minute}$ ). Further, SDS-PAGE results too indicated differential expression of 40KD and 55KD proteins in normal and in GE cancerous tissue. Therefore, detection of ADH and ALDH2

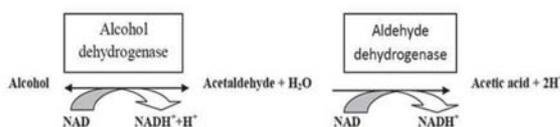
expression levels in alcoholics will assist in the early diagnosis of gastro esophageal cancers.

**Key Words:** Acetaldehyde, DNA adducts, Gastroesophageal cancer, N2-ethyl-2'-deoxyguanosine

### Introduction

Alcohol consumed by individuals is catabolised in the body with the help of Alcohol dehydrogenase (ADH) and Aldehyde dehydrogenases (ALDH). ADH located in the cytosol, promotes the oxidation of ethanol into acetaldehyde, where they release H<sup>+</sup> ions along with reduction of NAD to NADH. Multisubunit superfamily enzyme ALDH catalyzes the oxidation of acetaldehyde to acetate during ethanol metabolism Fig 1. In humans, there are multiple forms of ALDH that consist of nine major families, ALDH1 to ALDH9 (1-3). The best studied isoenzymes are the cytosolic and mitochondrial forms, designated as ALDH1 and ALDH2, respectively (4). ALDH1 and ALDH2 are both tetrameric with individual subunits comprising 499–500 amino acids and they share 68% sequence identity with each other (4).

Among them, mitochondrial ALDH2 plays a major role in human acetaldehyde metabolism because of its very low  $K_m$  ( $< 5 \mu\text{M}$ ) comparing with the other isoforms (5) Even though, aldehyde formation is majorly through by ADH, cytochrome P4502E1 (CYP2E1) and catalase also contributes its formation (6).



**Fig 1.** Mechanism of alcohol consumption in human beings

All human are not alcoholics but alcohol may derive from fermented foods and metabolites of microbial flora. More than 90% of ingested alcohol is eliminated via metabolic degradation mainly in the liver. The International Agency for Research on Cancer (IARC/WHO) has recently concluded that acetaldehyde derived from the alcoholic beverage itself or formed endogenously is carcinogenic to humans (7-10), which is a highly reactive intermediate that may cause cellular and DNA damage. Several possible mechanistic pathways through which drinking alcohol may cause cancer are: alcohol's contact-related local effects on the upper gastrointestinal tract, the induction of microsomal enzymes involved in carcinogen metabolism, the generation of oxygen radicals and lipid peroxidation products, nutritional deficiency, especially vitamin and mineral deficiencies and suppressed immune function (11). The inhalation of acetaldehyde produced tumors of the respiratory tract, specifically adenocarcinomas and squamous cell carcinomas of the nasal mucosa in rats (12) and laryngeal carcinomas in hamsters (13), in which this metabolite also served as a promoter in carcinogenesis attributable to benzo(a)pyrene (14). Acetaldehyde interacts covalently with DNA to

form a major stable acetaldehyde–DNA adduct, N2-ethyl-2'- deoxyguanosine, which is a critical event in the multistage process of chemical carcinogenesis (15, 16). N2-ethyl-2'- deoxyguanosine can be used efficiently by eukaryotic DNA polymerase (17). Alcoholic patients' levels of acetaldehyde adduct in lymphocyte and granulocyte DNA was much higher than the corresponding levels in healthy control individuals (18, 19). In view of importance of alcohol metabolism; in the present study ADH and ALDH2 expressions were evaluated in human gastro esophageal cancer for the probable use as prognostic marker.

#### Materials and Methods

**Biopsy collection:** For this study, the cancerous and normal human gastro esophageal tissues of 6 patients were obtained from the Department of gastroenterology, Sri Venkateswara Institute of Medical Sciences (SVIMS).

#### Reverse Transcription -Polymerase Chain Reaction (RT-PCR):

The total RNA was extracted from both normal and cancerous tissues and from them the mRNA was isolated by oligo dT cellulose column chromatography. Thus, 1 $\mu\text{g}$  of total mRNA from both normal and diseased tissues were isolated, the first strand of cDNA synthesis was carried by using 1 unit of AMV-reverse transcriptase (as per manufacturer's protocol -Promega) for 1hr, the above reaction mixture was used as template for RT-PCR which was performed in a Thermal cycler (Eppendorf master cycler gradient) by using specific primers and conditions mentioned in Table 1. The results were recorded in Vilber Lourmat gel documentation system (20-22).

**Tissue Homogenization:** 20 mg of diseased and normal tissues were homogenized by adding 1ml homogenizing buffer (0.1M Tris-HCl PH = 7.5; 15 mM EDTA; 0.25N sucrose) each.

**Table 1.** The details of Forward and Reverse Primers and PCR reaction conditions

	<b>Forward primer</b> 5' '1 3'	<b>Reverse primer</b> 5' '1 3'	<b>PCR Product size</b>
ADH	GTGCTTGTGCCAAGGTTTC	CAGGCCACGTGCTTGGCAG	0.762Kb
ALDH2	CAGCAGCAATGCCCCCA	CCAGCTGGGCTCACCTT	0.917Kb
<b>PCR conditions</b>		<b>Temperature</b>	<b>Time</b>
Initial denaturation		94°C	5 min
Denaturation		94°C	1 min
Annealing		52°C	45 sec
Amplification X 40 cycles		72°C	1 min
Extension temperature		72°C	30sec

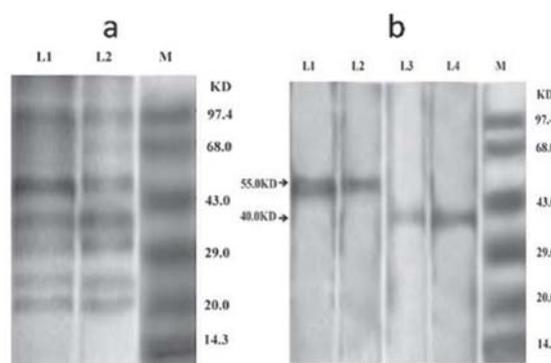
Homogenized solutions were collected, centrifuged for 2min at 1,500 g at 4°C cell debris were removed, and supernatant was again centrifuged at 33,000 g for 10 min at 4°C. The supernatant was again centrifuged at 1,00,000 g for 90 min at 4°C and the supernatant thus, obtained was used for enzyme assay.

**Enzyme Assay for ADH and ALDH2:** Enzymes ADH and ALDH2 were assayed by comparing their respective normal and diseased tissues to estimate their enzyme activities, Km towards substrates. 3ml assay mixture contained 100mM Tris HCl pH 8.0, 1mM NAD, 50  $\mu$ l of ADH / 50 $\mu$ l of ALDH2 enzyme (source tissue extract), 0.5M ethanol (for ADH assay) / 1mM acetaldehyde (for ALDH2 assay) were taken and incubated for 10 min and O.D. values read at 340nm. Enzyme activities, specific activities, Vmax and Km were determined through Hanes-Woolf plot. On X-axis (So) substrate concentration and on Y-axis [(So)/ Vo] were plotted.

**Proteins Profile Analysis:** The extracts of both normal and diseased tissue were electrophoresed in 10% SDS-PAGE and analysed by silver staining (23, 24).

**Result**

In the present study human Gastro esophageal cancerous tissue was obtained from Department of Gastroenterology, Sri Venkateswara Institute of Medical Sciences; Tirupati, which was evaluated and ascertained clinically. From the cancerous and normal tissues total RNA was extracted and from that, total mRNA was fractionated using Oligo-dT cellulose column chromatography. Thus obtained mRNA



**Fig. 2.** Detection of ADH and ALDH2 mRNA expression in gastroesophageal cancer tissue compared with normal tissue. a. Lanes L1 and L2 RT-PCR products of ADH from normal tissue and lanes L3 and L4 ADH RT-PCR products from cancerous tissue. b. Lanes L1 and L2 RT-PCR product of ALDH2 from normal tissue and lanes L3 and L4 ALDH2 RT-PCR product from cancerous tissue.

from the column was about 0.1% of the total RNA and this total mRNA was used as template in RT-PCR experiment. The results indicated an increased expression of ADH gene in cancerous condition compared to normal (Fig 2a). Similarly, another important enzyme of alcohol metabolism ALDH2 was also evaluated using RT-PCR which showed low expression level compared to normal (Fig 2b).

These results were further evaluated using enzyme kinetics (Table 2). The enzyme activity of ADH was found to be EA =  $0.335 \pm 0.01 \mu\text{M/ml/min}$ ;  $K_m = 0.193 \pm 0.01 \mu\text{M}$  and ALDH2 was found to be  $0.356 \pm 0.04 \mu\text{M/ml/min}$ ;  $K_m = 0.0299 \pm 0.003 \mu\text{M}$  in the normal tissue. However, in the Gastro esophageal cancerous tissue the enzyme kinetics for ADH and ALDH2 were EA =  $0.445 \pm 0.02 \mu\text{M/ml/min}$  and  $0.150 \pm 0.01 \mu\text{M/ml/min}$ ;  $K_m = 0.082 \pm 0.01 \mu\text{M}$  and  $0.2916 \pm 0.003 \mu\text{M}$  respectively. The SDS PAGE analysis of normal and cancerous tissue showed high expression at 40KD. This study was performed in order to ensure increased protein content due to higher expression of ADH since, the molecular weight of human ADH is about 40KD. These

results clearly explain probable accumulation of acetaldehyde in the cancerous tissue.

### Discussion

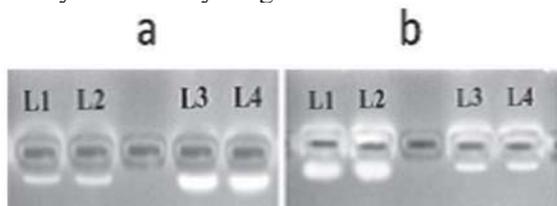
Acetaldehyde, the major ethanol metabolite that is extreme toxic and reactive than ethanol, has been postulated to be responsible for alcohol-induced tissue and cell injury. The problem arises when there is any malfunction of ALDH2 i.e., prevention of oxidizing the acetaldehyde (toxic) to acetate (non toxic) thus, the accumulation of acetaldehyde, a Carcinogen in the body leads to cancer. Many researchers have shown that accumulation of acetaldehyde in the body induces chromosomal aberrations, micronuclei and sister chromatid exchanges in cultured mammalian cells (25). It can also interact covalently with DNA to form DNA adducts, which may be involved in cancerous conditions. The experiments in mice explaining the formation of N2-ethyl-2'- deoxyguanosine, one major stable acetaldehyde-DNA adduct (26), was detected in the liver of ethanol-treated mice (27) and it has been observed that N2- ethyl-2'- de-oxyguanosine is used more efficiently by eukaryotic DNA polymerase to incorporate in

**Table 2.** The kinetics of ADH and ALDH2

	ADH			ALDH2	
	Normal tissue	Cancerous tissue		Normal tissue	Cancerous tissue
<b>Enzyme activity</b>	$0.335 \pm 0.01 \mu\text{M/ml/min}$	$0.445 \pm 0.02 \mu\text{M/ml/min}$	<b>Enzyme activity</b>	$0.356 \pm 0.04 \mu\text{M/ml/min}$	$0.150 \pm 0.01 \mu\text{M/ml/min}$
<b>Specific activity, <math>V_o</math></b>	$134 \pm 4 \mu\text{M/mg/min}$	$30.90 \pm 2 \mu\text{M/mg/min}$	<b>Specific activity, <math>V_o</math></b>	$24.72 \pm 1 \mu\text{M/mg/min}$	$60 \pm 3 \mu\text{M/mg/min}$
<b><math>V_{max}</math></b>	$4.84 \pm 0.04 \mu\text{M/mg/min}$	$1.36 \pm 0.06 \mu\text{M/mg/min}$	<b><math>V_{max}</math></b>	$0.598 \pm 0.05 \mu\text{M/mg/min}$	$8.333 \pm 0.09 \mu\text{M/mg/min}$
<b><math>K_m</math></b>	$0.193 \pm 0.01 \mu\text{M}$	$0.082 \pm 0.01 \mu\text{M}$	<b><math>K_m</math></b>	$0.0299 \pm 0.003 \mu\text{M}$	$0.2916 \pm 0.03 \mu\text{M}$

SD  $\pm$  for four determinations

DNA (28). In Alcoholic patients, levels of acetaldehyde adduct in lymphocyte and granulocyte DNA was much higher than the corresponding levels in healthy control individuals (29). *In vitro* experiments have shown that lymphocytes from habitual drinkers with the inactive form of ALDH2, which cannot detoxify acetaldehyde efficiently, have higher frequencies of sister chromatid exchanges than lymphocytes from individuals with normal, active ALDH2 (30). In the present study corroborated results shown increased ADH activity with very low  $K_m$  and decreased ALDH2 activity with very high  $K_m$  in the cancerous tissue compared to normal tissue (Table 2). Very low  $K_m$  also indicates very high  $K_{cat}$  (31, 32) leading to high amount of ADH expression and thus, increased formation of acetaldehyde (Fig. 1). These results were further substantiated in RT-PCR experiment where higher and lower expression levels of ADH and ALDH2 respectively were observed in the cancerous tissue (Fig. 3). Hence, the ADH and ALDH2 activity and expression in the system could be handy in the early diagnosis of GE cancer.



**Fig. 3.** SDS PAGE analysis of total protein extracted from cancerous tissue and normal tissue. Lane L1 cancerous tissue total protein and L2 normal tissue total protein.

### Conclusion

The present study clearly suggests that the increased consumption of alcohol has profound effect resulting in Gastro esophageal cancer thus; it is imperative to diagnose GE cancers at an early stage. In this respect the differential levels of ADH and ALDH2 can be diagnostic markers.

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