

# ERYTHROCYTE ALDEHYDE DEHYDROGENASE = A POTENTIAL MARKER FOR ALCOHOL DEPENDENCE.

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## ABSTRACT

The present study explored the utility of erythrocyte aldehyde dehydrogenase (ALDH) as a peripheral marker in alcoholism. The ALDH levels in alcoholics, their first degree nonalcoholic relative and controls were compared. ALDH was found to be significantly lower in alcoholics ( $3.38 \pm 1.70$  mU  $p < 0.001$ ) and their first degree relatives ( $4.04 \pm 1.55$  mU  $p < 0.05$ ) compared to controls ( $5.06 \pm 1.55$  mU). Low ALDH levels among alcoholics persisted despite abstinence. The levels did not correlate with indices of hepatic dysfunction or with severity of alcoholism. These findings indicate that low erythrocyte ALDH may be considered as a biochemical trait marker associated with alcoholics, and the alcohol abuse would further decrease enzyme activity. To evaluate this proposition, longitudinal studies involving high risk progeny of alcohol dependents is being planned.

Key Words : Aldehyde dehydrogenase, Familial Alcoholism, Alcohol Dependence.

## INTRODUCTION

The familial nature of alcoholism and the associated markers of the disease have been the subject of study by several groups (Ballenger et al 1979, Monteiro et al. 1988, Begleiter and Porjesz 1988). However, most of the laboratory based diagnostic parameters are unsatisfactory because of nonspecificity. Hence, there is a need for an ideal biochemical marker of alcoholism. While the ideal biochemical marker of alcoholism would serve as a "state" marker confirming diagnosis and to assess the state of the disease before initiating therapeutic measures, the "trait" marker would be of immense use in identifying the population at risk and to take preventive measures. The search is still on, for a reliable marker which could help physicians in their ability to diagnose alcoholism, monitor abstinence, identify high risk individuals and plan intervention strategies.

In recent times, one such marker that has aroused much interest is aldehyde dehydrogenase (ALDH), the enzyme which metabolises acetaldehyde, the intermediary metabolite of ethanol. Hepatic ALDH is present in both cytosolic and mitochondrial compartments, and the is present in both cytosolic and mitochondrial compartments, and the erythrocyte ALDH

represents the cytosolic counterpart in liver.

Selective reduction of cytosolic ALDH (hepatic and erythrocyte) have been demonstrated in chronic alcoholics (Thomas et al., 1982; Agarwal et al 1983; Jenkins et al., 1984; Lin et al., 1984; Harada et al. 1985; Mathewson and Record, 1986; Towell et al. 1986). While the latter researchers found a gradual return of the initially low cytosolic ALDH levels to normal following periods of abstinence ranging from 2 to 8 weeks, suggesting that ALDH could serve as a marker of state, Thomas et al (1982) found persistently low ALDH levels even after prolonged abstinence. Their finding raises the possibility that low cytosolic ALDH activity may represent a primary abnormality predisposing to alcoholism. Thus, it is unclear whether ALDH is a marker of state or trait. One way to resolve this controversy is to study cytosolic activity in relatives of alcoholics who do not drink alcohol excessively (Mathewson and Record, 1986).

The present study was addressed towards answering this question by determining whether erythrocyte (cytosolic) ALDH levels in alcoholics and their first degree relatives differed from nonalcoholic controls; whether abstinence caused reversibility of erythrocyte ALDH, and whether erythrocyte ALDH levels correlated with the commonly employed indices of

hepatic dysfunction or quantity of alcohol consumption.

## MATERIALS AND METHODS

### Sample Characteristics:

The study group comprised of 57 male inpatient alcoholics (mean age 38.91, SD = 8.3) satisfying DSM-111 criteria for alcohol dependence (Diagnostic and statistical Manual, APA, 1980) screened for the absence of exclusion criteria (viz., anaemia, severe malnutrition, concurrent dependence on other drugs, consumption in the prior 3 months of drugs known to alter ALDH activity (viz., Disulfiram, sulfonyleureas, cephalosporins and nitrate antianginals). Details of alcohol consumption, alcohol related problems and family history were obtained by the use of a semistructured questionnaire.

The first blood sample in the alcoholic group was drawn from the antecubital vein after overnight fasting within 24 to 72 hours after the last drink, before any treatment was instituted. Following this, the patients were detoxified with chlordiazepoxide (40-170) mg/day) or diazepam (10-40 mg/day). They were also given thiamine and other B complex supplements. Detoxification was usually completed within 2 weeks. Following 4 weeks of supervised abstinence (i.e. 2 weeks after stopping benzodiazepines) 39 patients were reassessed for erythrocyte ALDH levels and liver functions tests. A third sample was analysed in 12 patients started on disulfiram in order to determine the effects of disulfiram on ALDH activity. In vitro effects, if any of drugs used in detoxification i.e., benzodiazepines and thiamine, on ALDH were also studied.

The first degree relatives consisted of 26 healthy males (mean age 28.92 years SD = 9.45). of this sample, 19 had never consumed alcohol, and 7 drank only on social occasions, but had not consumed alcohol in the prior 2 weeks. 16 were brothers of patients, 9 were sons and 1 a father.

The controls comprised 40 healthy male volunteers with no family history of alcohol dependence (mean age 28.92 years SD=4.3), of whom 33 did not consume alcohol, and 7 drank on social occasions. The same set of exclusion criteria as that for alcohol group applied to the controls and relatives.

A heparinised blood sample was drawn from

the antecubital vein after overnight fasting. Cells were separated by centrifugation (3000 rpm/15 mins) and the plasma was utilised for tests of liver function, in a Hitachi 705 autoanalyser (Boehringer Knoll). RBC's were lysed, rendered haemoglobin free by CM-Sephadex c-50 column elution and ALDH activity of the Hb-free lysate was estimated spectro photometrically at 340 nm, using propionaldehyde as substrate, at pH 7.4, by employing the method of Guru and Shetty (1990). ALDH activity was expressed as 'n' moles of NADH formed/min/mg protein. Protein estimation of the Hb free lysate was carried out by the dye binding method (Sedmak et al., 1977).

Data was statistically analysed using student's 't' test, paired 't' test, analysis of variance, median test and chi-square test. Correlation between various indices of hepatic dysfunction were examined using ePearson's correlation coefficient.

## RESULTS

Mean duration of alcohol consumption in the study group was 14.57 years (SD = 7.86) and quantity frequency index was 170.64g (SD = 128.3), 26 patients had a family history of alcoholism.

Erythrocyte ALDH was significantly lower in the alcoholic patients ( $3.381 \pm 1.70$  mU,  $p < 0.001$ ) and in their first degree relatives ( $4.035 \pm 1.55$  mU,  $p < 0.05$ ) as compared with the control group ( $5.056 \pm 1.57$  mU).

Following 4 weeks of abstinence there was no significant change in erythrocyte ALDH values. Disulfiram completely inhibited ALDH activity

TABLE -1

Changes in erythrocyte ALDH values after 4 weeks of abstinence and after treatment with disulfiram.

	N	Erythrocyte ALDH activity (mU)	
Alcoholics (Sample I)	39	$3.20 \pm 1.70$	
Alcoholics (Sample II)	39	$3.30 \pm 1.78$	$t=0.34$ , $df=38$ n.s
Alcoholics (Sample III) (on disulfiram)	12	(no activity)	

values are mean + sd.

In vitro studies of the effect of drugs commonly used in detoxification indicated that benzodiazepines activated erythrocyte ALDH activity, but thiamine did not alter ALDH activity.

Serum bilirubin, total cholesterol, plasma triglycerides and plasma magnesium did not differ significantly across the 3 groups. Mean Aspartate Aminotransferase (AST) values were significantly higher in the patient group (Table 2)

TABLE 2

## Comparison of plasma enzymes of liver function

ALCOHOLICS	FIRST DEGREE RELATIVES	CONTROLS	CHI*	P
AST	96.9 $\pm$ 121.9	23.08 $\pm$ 8.6	19.54 $\pm$ 9.83	48.64 >0.0001
ALT	64.42 $\pm$ 95.80	20.95 $\pm$ 11.0	18.59 $\pm$ 17.19	21.98 <0.05
GGT	102.26 $\pm$ 99.20	34.04 $\pm$ 14.3	37.03 $\pm$ 18.77	18.59 <0.001.

Values are mean + sd.

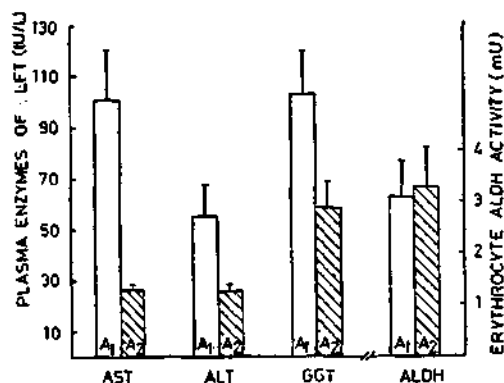
\* extension of Median test

TABLE-3

## Liver function tests at intake (alcohol group) and following 4 weeks of abstinence.

n	ALCOHOLICS SAMPLE	ALCOHOLICS SAMPLE 2	T	p
ast	100.6 $\pm$ 124.90	25.9 $\pm$ 8.32	48.64	<0.001
ALT	54.79 $\pm$ 69.30	25.56 $\pm$ 15.20	2.60	<0.05
ALP	117.07 $\pm$ 50.50	92.68 $\pm$ 32.80	24.10	<0.001
GGT	101.83 $\pm$ 100.70	58.19 $\pm$ 59.48	18.6	<0.001

paired 't' test was used for comparison and decline significantly after abstinence (Table 3) Similar trends were noted with Alanine Aminotransferase (ALT) and Gamma Glutamy Transpeptidase (GGT). The improvements in the parameters of liver function (viz AST, ALT, GGT) were not associated with any concomitant improvement in erythrocyte ALDH activity ( Fig-1)



Relationship between Erythrocyte ALDH (Mean $\pm$ SD) and Plasma LFT enzymes (Mean $\pm$ SEM) of Alcoholics (1st and 2nd samples). Aspartate Aminotransferase (AST), Alanine Amino Transferase (ALT), Gamma Glutamyl Transpeptidase (GGT), A1 = Alcoholic First Sample, A2 = Alcoholic Second Sample.

## DISCUSSION

Erythrocytes offer a suitable easily accessible peripheral source for monitoring cytosolic ALDH activity in alcoholics. The methodological aspects of ALDH assay has been discussed earlier (Guru et al., 1990).

This study examined inpatient alcoholics to ensure supervised abstinence. Patients who had recently been on drugs known to inhibit ALDH (Asad and Clarke 1976, Towell et al., 1985, Kitson 1986, Agarwal et al., 1987) were excluded from the study. The second sample following abstinence was collected after 2 weeks of cessation of detoxification with benzodiazepines, since in vitro experiments had shown increased ALDH activity with benzodiazepines (Murthy et al., 1991).

This study found significantly low levels of cytosolic ALDH in chronic alcoholics, which persisted following abstinence, in concurrence with one earlier work (Thomas et al 1982).

While the initial abnormal liver function tests showed a definite improvement after 4 weeks of abstinence, with recovery of hepatic function, in agreement with earlier reports (Sherlock 1982), erythrocyte ALDH levels remained low. This suggests that low

ALDH activity is not an epiphenomenon of nonspecific hepatic damage, as suggested by Mathewson and Record (1986), nor is a direct consequence of alcohol consumption as earlier reported (Jenkins et al., 1984). This point is further substantiated by a lack of correlation between ALDH, indices of hepatic dysfunction and quantity frequency index.

These observations were further corroborated by the finding of significantly lower ALDH values in first degree relatives of alcoholics as compared with controls.

In conclusion, this study found significantly lower erythrocyte ALDH in alcoholics and their first degree relatives. The low levels of the enzyme persisted despite abstinence. ALDH values did not correlate with indices of hepatic dysfunction or severity of alcoholism. These findings suggest that the observed low erythrocyte ALDH in alcoholics and their first degree relatives could be a trait marker for alcoholism.

Could erythrocyte ALDH help to identify a high risk, genetically vulnerable group? would the individuals with low ALDH levels be more prone to becoming alcohol dependent? These issues could be addressed by longitudinal studies. Findings from such a study would probably help in counselling such individuals, in whom social drinking may not be a feasible alternative to complete abstinence.

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