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ORIGINAL CONTRIBUTIONS

BIOCHEMICAL MEASURES IN THE DIAGNOSIS OF ALCOHOL DEPENDENCE USING DISCRIMINANT ANALYSIS

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ABSTRACT

BACKGROUND: Alcohol dependence often cannot be diagnosed based on self-report alone. Various biochemical and haematological parameters have been used to screen alcohol use disorders. AIM: To develop discriminant equations based on lipid and liver measures independently for identifying alcohol dependent and non-dependent subjects. SETTINGS AND DESIGN: Case control study in a tertiary care hospital. METHODS AND MATERIAL: One hundred subjects fulfilling the criteria of alcohol dependence and seventy healthy controls were included. The socio-demographic details, caloric intake, height, weight and blood pressure were recorded. Samples were analysed for various lipid measures as well as liver function.

Statistical analysis used: Diagnostic values such as sensitivity, specificity, positive predictive value (PV+), negative predictive value (PV-) and discriminant analysis. RESULTS: Using discriminant analysis, two equations were constructed based on liver and lipid measures independently. 84.7% of the subjects on the basis of total cholesterol (TC), apolipoprotein B (ApoB) and low density lipoprotein /high density lipoprotein-cholesterol (LDL/HDL-c and 89.1% on the basis of aspartate amino transferase (AST) and gamma glutamyl transferase (GGT) were correctly classified into their respective groups. CONCLUSIONS: This study demonstrates the ability of TC, ApoB and LDL/ HDL-c (among lipid measures) and AST and GGT (among liver measures) in discriminating alcohol dependents from non-dependent subjects.

KEY WORDS: lipid profile, liver enzymes, alcohol dependence.

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INTRODUCTION

In the health care delivery system the physician is strategically placed to encounter patients with health-related problems associated with alcohol. Alcohol use is related to morbidity affecting various systems in the human body. Significant morbidity is related to chronic heavy alcohol use. However, these patients seek treatment only when complications of drinking have set in.[1] Studies have suggested that only 20% of problem drinkers are recognized in routine clinical practice. Quite often, the diagnosis occurs when the health effects are already obvious and recognizable. This late diagnosis is of particular concern because effective and low cost methods are now available for treating alcohol addiction at an early stage.[2, 3]

Heavy alcohol consumption for prolonged periods results in marked perturbation of the lipid transport system, reflecting both effects of alcohol on lipid metabolism in hepatic and extra hepatic tissue as well as its marked toxic effects on liver function.[4] Therefore, it is important that the morbid condition, for which alcohol may be a risk factor, is identified and appropriate intervention is planned.

Various biochemical and heamatological parameters have assumed significance in alcohol use disorders. Some of the commonly studied parameters such as aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (GGT) and mean corpuscular volume (MCV) have been used both the assessment of alcoholism as well as external diagnostic markers validating excessive alcohol consumption.[5,6,7] Combination of more than one marker has been reported to give better sensitivity and diagnostic accuracy in alcohol dependence.[8,9] It is therefore important to detect alcohol use in its early stages so that interventions can be planned effectively.

Rationale

In view of the fact that many coronary care units and Drug Dependence Treatment Centres routinely perform lipid, lipoprotein and liver function assessments, it would be interesting to evaluate their contribution to diagnose Alcohol use disorders. On the basis of lipid profile and liver enzymes independently, an attempt has been made to identify Alcohol Dependents and non-dependents so that appropriate interventions can be made.

OBJECTIVES

The present study aimed to develop two discriminant equations based on lipid and liver measures independently for classification of alcohol dependents and non-dependents in their respective groups.

MATERIAL AND METHODS

Subjects in both the study and control group were informed about the nature of study. Information about demographic profile was obtained after taking informed consent.

Study group

One hundred male patients in the age range of 18-45 years admitted in drug dependence treatment center (DDTC) of our institute

fulfilling the DSM-1V criteria of alcohol dependence syndrome were included in the study group. Patients with co morbid medical illness such as diabetes mellitus, pre-existing hypertension, renal failure or with psychiatric illness and those on any medication except multivitamins were excluded.

Control group

Seventy healthy, age matched males (hospital employees with no current/ life time history of regular drinking and no family history of alcoholism) were included in the control group after applying the same exclusion criteria as in the study group.

Laboratory parameters

Blood sample (5-ml) was drawn from the median cubital vein after an overnight fast for all the subjects. The caloric intake, height, weight and blood pressure (BP) were recorded in both groups. A single point estimation of lipid profile that is total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), and very low density lipoprotein cholesterol (VLDL-c), apolipoprotiens (ApoA1and ApoB) and liver enzymes that is AST, ALT, GGT and alcohol dehydrogenase (ADH) was carried out.

HDL-c and LDL-c fractions were chemically separated from serum by the method of Burstein et al and Wilson and Spiger, and cholesterol content was estimated from the supernatant.[10,11] ApoA1 and ApoB were measured by immuno-turbidimetric method. Serum ADH activity was measured by enzymatic method.[12] Other lipid profile parameters and liver enzymes were estimated on an autoanalyser using commercial kits of Boehringer-Mannheim.

Data analysis

Sensitivity, specificity, predictive value and diagnostic accuracy were calculated using Epi Info 6.0. Sensitivity was defined as percentage of patients of alcohol dependence correctly identified in the study group and specificity was defined as percentage of normal subjects correctly identified in the control group. Positive predictive value (PV+) represents the true positives in study group & negative predictive value (PV-) represents the true negatives in control group. Discriminant analysis is essentially an adaptation of regression analysis and was done using the BMDP statistical software. It provides a means to classify any subject into the group it closely resembled. Discriminant analysis was undertaken to assess the power of individual lipid / lipoproteins parameters and liver enzymes to distinguish alcohol dependents from non-dependents. A stepwise discriminant analysis using Wilk's step-wise procedure with a minimum tolerance of 0.001 and *F* to enter or remove 4 (indicating that a variable would be entered if the ratio between group variance to within group variance for that variable was >4) was used.

RESULTS

Sample description

The study had a case-control design with one hundred patients of alcohol dependence referred to as alcohol dependents (cases), and seventy healthy subjects referred to as non-dependents (controls). The mean age of alcohol dependents and non-dependents

was 42.1 ± 8.2 and 43.5 ± 7.5 yr. respectively. The mean alcohol consumption in the last month before admission was 300 gms/day (60-590 gms/day). Most subjects were married, unskilled and uneducated in both groups. The two groups did not differ significantly on any of the socio-demographic and clinical variables except that blood pressure was higher in alcohol dependents as compared to non-dependents.

Descriptive data

All variables (TC, HDL-c, VLDL-c, TG, LDL/HDL-c, ApoA1, ApoA1/ApoB, AST, ALT, GGT, and ADH) except ApoB and LDL-c were significantly higher ($P<0.001$) in the alcohol dependents as compared to non-dependent subjects.

Diagnostic value

Sensitivity was highest for LDL-c at 94.6 % at which level the specificity was 46%. TC, VLDL-c, LDL/HDL-c, ApoA1 and ApoA1/ApoB had sensitivity exceeding 80%, whereas the specificity was in the range of 25 to 45.8%. Range of PV (+) and PV (-) was 39.6% to 94.7% and 52% to 73.7% respectively. The diagnostic accuracy varied from 44.4% (ApoB) to 69.4% (TC). Among the liver enzymes, the sensitivity was highest for AST (75.3%) followed by GGT (74.2%) at which level the specificity was 88 and 100% respectively. Sensitivity of ADH and ALT was 61% and 67% whereas specificity was 50% and 76% respectively. PV (+) and PV (-) were in the range of 66% to 100% and 51% to 56% respectively. The diagnostic accuracy of all the four liver enzymes ranged from 56 to 85.3%.

Discriminant analysis

Discriminant analysis was carried out separately for the lipid/lipoprotein variables and liver enzymes to assess the proportion of correct classification. Among lipids and apolipoproteins, TC emerged as the first variable to discriminate alcohol dependents from nondependents significantly. In order to see the classification ability of other variables when TC was removed as a candidate, ApoB emerged as the second variable to significantly discriminate alcohol dependents from nondependents. When ApoB was forced out, ratio of LDL to HDL-c contributed significantly beyond which none of the other variables could contribute significantly. When all the three variables (TC, ApoB, and LDL/HDL-c) were subjected together for classification, 84.7% of total subjects were classified into correct groups (94.7% of nondependents in control group and 81.1% of alcohol dependents in the study group) [Table 1].

Among the liver enzymes, AST emerged as the first variable to discriminate alcohol dependents from nondependents. When AST was removed as a candidate, GGT emerged as the second variable to discriminate alcohol dependents and nondependents beyond which none of the other variable could contribute significantly. When both AST and GGT were subjected together for classification, 89.1% could be classified into correct groups (92.3% as nondependents in control group and 85% as alcohol user in study group) [Table 1b].

On the basis of discriminant analysis (TC, ApoB, and LDL/HDL-c among lipid measures and AST and GGT among liver enzymes),

two different equations [Table 1a and 1b] were derived.

DISCUSSION

Alcohol use is fairly widespread all over the world. It has been estimated approximately 5% of Indian population (of adult males) fulfil the criteria of alcohol dependence syndrome. Although reliable estimates of nondependent users of alcohol such as hazardous or harmful users are not available, proportion of the population at risk for alcohol-related morbidity increases if these groups are also taken into account.

Alcohol use predisposes subjects to increased risk of coronary disease and changes in lipid profile are associated with coronary risk. In understanding the management of atherosclerosis, there has been an increasing interest in measurement of lipoproteins and lipid moieties. The use of lipids and lipoproteins as diagnostic tests revealed high sensitivity for some of the measures including TC, HDL-c, LDL-c, VLDL-c, HDL-c/TC, and ApoA1/ApoB, but the corresponding specificity was low. This would enable a high-positive pick-up rate but also a low true-negative rate, which is acceptable if these tests are used for screening. This is being tested in an ongoing community-based study.

The complex relation between alcohol use, liver function tests and lipid profile has been documented. Prabhakaran et al. in a community-based survey on the risk factors for coronary heart disease (CHD) in North Indian male population reported the cut-off levels of lipids (TC 200 mg%; HDL-c 40 mg%; TG 150 mg%), which are the same as ours.[13] Lower levels of LDL-c and ApoB in alcohol dependents as compared to nondependents in the present study are similar to those reported earlier.[4],[14] Low or subnormal LDL-c has been a consistent finding in chronic alcoholics. In parallel with LDL-c, the ApoB levels are also reduced in alcohol dependents as compared to nondependents indicating the direct effect of alcohol on LDL metabolism.[14] High levels of ApoA1 and low levels of ApoB along with significantly raised ratio of ApoA1 to B in our study group suggests that apolipoproteins may be better correlates of cardiovascular risk in alcoholics. This is in complete agreement with our earlier findings.[15],[16] Duhamel et al., while speculating the potential role of alcohol to act as inducer of ApoA1 biosynthesis, suggested that distribution of various apolipoproteins especially ApoA1 remains indeterminate.[4]

Serum cholesterol has been widely accepted as a risk factor for ischemic heart disease and its value in prevention has been strongly advocated.[17] On step-wise discriminative analysis, emergence of total cholesterol, as the first variable to discriminate alcohol dependents from non dependents in the present study shows that influence of alcohol on lipid metabolism opens the possibility that the protective effects of moderate alcohol consumption against development of coronary heart disease are to be attributed to transient changes in the lipid metabolism, and that the benefits in alcohol consumption needs to be weighed carefully against its considerable risk in the Indian population.[16] ApoB emerging as the second variable to discriminate alcoholics and nonalcoholics is in agreement with Durrington et al. who (in a

case–control study) found that ApoB is more closely associated with ischemic heart disease than any other lipid or lipoprotein variable.[18] However, the same group of researchers subsequently suggested that much, if not all of the genetic component of cardiac ischemia that is not expressed through ApoB or any of the established risk factors, operates through Apo(A).[17]

The role of lipoproteins and lipid profile in defining the alcoholic status of individual has not been extensively explored. In the present study, discriminant analysis using lipoproteins and lipid measures has been used to provide a way to classify subjects into alcohol dependents and nondependents. It was found that levels of TC, ApoB, and LDL to HDL-c ratio contributed significantly resulting in correct classification in 84.7% cases.

Liver is the prime target organ for alcohol-induced diseases. Liver enzymes are also important indicators of liver dysfunction, possibly as markers of alcohol dependence. The critical dose at which adverse effects of alcohol emerge differs in the target organ. Recently Dakeishi et al. reported that hepatocellular injury, as indicated by elevation of AST could emerge only when the alcohol intake is >50 gm/day.[19] This concurs well with our findings where the mean alcohol consumption was 300 g/day and AST levels were also elevated significantly. The AST appears to be the primary marker of hepatocellular injury because it is more specific than other liver enzymes for detecting alcohol induced diseases. Although some information has been developed about alcohol consumption and AST, the threshold of alcohol-associated elevation remains controversial.[20,21] The GGT is the most sensitive indicator of alcohol dependence/ hazardous drinking and is the first enzyme to be elevated. The GGT has also been reported to be more sensitive and is more likely to be elevated in regular than in episodic drinkers.[6] The increase in GGT, AST, ALT levels in the study group is in agreement with earlier reports.[22]

On step-wise discriminant analysis, emergence of AST as the first variable in correctly identifying alcohol dependents and nondependents with good diagnostic accuracy is in agreement with the literature.[23–25] However, Sorenson et al. suggested that AST has long-term prognostic value.[23] The GGT levels are elevated in approximately 80% of persons with established alcohol dependence, whereas it is increased in as few as 30% of hazardous drinkers.[6] The GGT emerging as the second variable to discriminate alcoholics and nonalcoholics is in agreement with earlier studies. Elevated GGT levels could be in response to hepatocellular damage due to long-term alcohol consumption, as well as its increased synthesis in the liver.[5,6,22]

It can thus be extrapolated that individuals can be classified with certainty on measures of TC, ApoB, and LDL/HDL-c (among lipid profile) and AST and GGT (among liver enzymes). While screening samples in a community, on the basis of equations derived (as seen under [Table 1a, and 1b]), if D (case) is more than D (control), it would be classified as case of alcohol dependence and vice versa. Accordingly, the subject may be referred to drug dependence treatment centre for

Table 1: The step-wise discriminant analysis

Classification	*Function*		*Classification*		*Matrix*	
				%	*N*	
Variable	*Controls*	*Case*	*Group*	*Classified*	*Controls*	*Case*
Step-wise discriminant analysis of lipid and lipoproteins						
T. Chol	0.069	0.122	Control	94.7	66	4
ApoB	0.128	0.053	Case	81.1	19	81
LDL-c	1.503	0.633	Total	84.7	85	85
HDL-c						
Constant	-13.07155	-15.31605				
Step-wise discriminant analysis of liver enzymes						
AST	0.402	0.630	Control	92.3	65	5
GGT	0.014	0.034	Case	85.0	15	85
Constant	-6.01	-14.49	Total	89.1	80	90

T. Chol, total cholesterol; ApoB, apolipoprotein B; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; AST, aspartate amino transaminase; GGT, gamma glutamyl transferase.

Table 1 gives the details of the step-wise discriminant analysis of lipid and lipoproteins and liver enzymes. Based on the analysis, the discriminant equation for the case/control is as follows

D(Case) = 0.12(TC) + 0.05 (ApoB) + 0.63 (LDL-c/HDL-c) - 15.31 (constant)
D(Control) = 0.07 (TC) + 0.13 (ApoB) + 1.50 (LDL-c/HDL-c) - 15 (constant)
} (for lipid and lipoproteins)

D(Case) = constant (- 14.5) + 0.63 (AST) + 0.034 (GGT)
D(Control) = constant (-6.01) + 0.40 (AST) + 0.014 (GGT)
} (for liver enzymes)

detailed alcohol use-related evaluation.

The study however, has some limitations: (1) The equations have not been tested independently in this study group. (2) It is a clinic-based study, which impairs the generalizability. (3) The study also did not include individuals with alcohol abuse, which could have resulted in better discrimination. The study may be viewed keeping in mind these limitations.

CONCLUSIONS

The study has documented the efficiency of TC, ApoB, and ratio of LDL to HDL-c (amongst lipid variables) and AST, GGT (amongst liver enzymes) in discriminating alcohol dependents from nondependents. The results of the present study also confirms the assumption that alcohol abuse in all phases such as alcohol dependence or heavy drinking may not be detected optimally with the use of one test. These findings can be extrapolated to nonspecialized zsettings in identifying alcohol-dependent and nondependent subjects by using limited number of tests. This is being tested in prospective community-based studies as screening tests.

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