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Original Article

Biochemistry Section

Serum Carbohydrate Deficient Transferrin as A Sensitive Marker in Diagnosing Alcohol Abuse: A Case – Control Study

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ABSTRACT

Background: Alcoholism is a major problem in India thereby causing a heavy toll on the health related expenditure of the country. Detection of alcohol abuse rely mainly on clinical details which is sometimes inaccurate or unreliable and hence using a specific diagnostic parameter might be of immense use not only for early diagnosis but also during follow up of the cases.

Aims and Objectives: This case control study aimed at evaluating the usefulness of Carbohydrate Deficient Transferrin (CDT) as a sensitive marker to diagnose alcohol abuse.

Materials and Methods: The study was approved by Institutional research and ethical committee. Twenty five known male alcoholics who attended to the OPD (Out Patient Department) of Alcohol de-addiction centre of a tertiary care hospital were selected as cases. All of them were diagnosed to have a strong likely hood of hazardous alcohol consumption based on 'Alcohol Use Disorders Identification Test" (AUDIT) questionnaire. Twenty five age matched, gender matched healthy individuals who were teetotalers were selected as controls. They scored zero in AUDIT questionnaire. Informed consent was obtained from all the cases and controls. The following tests were done: Liver function tests including Serum Bilirubin, Total Proteins, Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT) and Blood glucose levels were estimated using a fully automated biochemistry analyser, XL – 300 (Trans Asia Biomedical systems) and Mean Corpuscular Volume (MCV) was done using an automated hematology analyser Sysmex KX-21. Percentage of Serum Carbohydrate Deficient Transferrin (%CDT) was assessed using immuno Turbidimetric assay, ELISA method (iMark, Bio-Rad Laboratories,).

Statistical analysis of the data obtained was done using SPSS 16.0.

Results: There was a statistically significant difference in values of AST, ALT, ALP, MCV, GGT and % CDT in cases as compared to controls. ROC curves drawn to assess the sensitivity and specificity of each parameter showed that %CDT has the highest sensitivity and specificity (84% and 92% respectively) and MCV (48% and 52% respectively) had the least. GGT when compared to % CDT had a lower sensitivity and specificity (64% and 72% respectively).

Conclusion: % CDT is a sensitive biomarker which can be used to diagnose alcohol abuse and is superior to GGT in terms of sensitivity as well as specificity.

Key Words: GGT, % CDT, Diagnostic marker, Alcohol abuse

INTRODUCTION

Alcoholism represents a serious issue in India with an average 12 months prevalence of 19-34% [1]. According to National Survey of Drug Abuse, 2004, prevalence of alcohol use in adult men in Chennai ranges from 16.7%-34.4% [2]. Early detection and proper medication with counseling can restore the alcoholics to normalcy but most of the time alcoholics ingeniously hide their disease state and present to the physician very late [3] which will lead to major socio economic consequences. Moreover, the questionnaires that are routinely used to diagnose alcohol abuse may be subjected to un truthful responses [4]. So, there is a need for a specific assay procedure to detect alcoholics early, so that proper therapy can be instituted.

Alcoholism induced changes in the liver enzymes (viz. AST, ALT, & ALP) and in MCV is well known, but an increase in the serum levels of these parameters is not specific for the condition alone [5]. GGT is a popular test and has been established as a marker for alcohol abuse, but studies have pointed out the lack of its specificity in diagnosing alcoholism because it is increased in other conditions like hepatocellular carcinoma and Phenytoin intake [6-9]. Few studies have observed a low sensitivity with GGT [6, 10].

Serum Carbohydrate Deficient Transferrin percentage (%CDT) has been found to be specific for alcohol abuse [11-12]. The transferrins are a class of single chain iron binding glycoproteins. They consist of three sub-structural domains- a single polypeptide chain, 2 iron binding sites and 2 N linked complex glycan chains. The N glycan chains terminate with a negatively charged sialic acid molecule.

The number of sialic acid molecules in a transferrin chain can range from nil to 8 giving rise to various iso forms of transferrin. In normal conditions, transferrin iso forms range from di-sialo to hepta-sialo forms whereas, Asialo and mono sialo forms are not detected. In alcoholics, Ethanol and acetaldehyde suppresses the activity of glycosyl transferase and increases the activity of sialidase. Hence, transferrins which have a low degree of bond with carbohydrates, namely, Asialo, Mono sialo and di sialo forms of transferrins, collectively called as CDT are increased in alcoholics [13]. Percentage of these forms to total transferrin is percentage CDT (% CDT).

American Psychiatric Association has recommended % CDT as a marker for monitoring recurrence of alcoholic disease [12]. Moreover, % CDT levels are not influenced by drugs and so it is found to be more sensitive and specific than GGT in diagnosing alcohol abuse [14, 8], but not many studies have been done in our population.

AIMS AND OBJECTIVES

We aimed to perform a case control study to assess the clinical usefulness of % CDT in comparison with the other biological markers implicated in alcohol abuse. We also aimed to find out the sensitivity of %CDT as a marker to diagnose alcohol abuse.

MATERIALS AND METHODS

The study was approved by the Institutional research and ethical committee of Sree Balaji Medical College and Hospital, Chennai. Twenty five males in the age group of 20-60, who had consented for the study, formed the study group. They were known alcoholics, reported or brought to the OPD of the De addiction centre of Our Institution. Diagnosis of Alcohol abuse was established using AUDIT questionnaire [15]. They underwent a base line evaluation including a clinical history and structured interview on the quantity of alcohol consumption [16] followed by Complete Physical examination and Psychological evaluation. All of them had scored above 8 by AUDIT questionnaire [15]. They met the inclusion criteria listed below:

Inclusion Criteria:

- Age 20-60 years
- History of Chronic alcoholism
- A score of more than 8 in AUDIT questionnaire

The Control group had twenty five age matched apparently healthy males who were drawn from the Staff of our institution. An informed consent was obtained from them. They were teetotalers and were not ex drinkers. They scored zero by AUDIT questionnaire. They too underwent a complete physical and psychological examination. The following exclusion criteria were applied for both the groups.

Exclusion Criteria For Study Group And Control Group

History of

1. Hypertension
2. Diabetes mellitus
3. Co-existing drug dependence
4. Psychiatric illness
5. Tuberculosis
6. Malignancy
7. Liver disease

Sample Collection: Three ml of whole blood samples, 1 ml of Sodium fluoride sample and 2ml of EDTA samples were collected from both the groups using vacutainer system.

Tests: All the tests were done within 4 hours of blood collection. Liver function tests including GGT and blood glucose tests were performed using fully automated analyzer, Trans Asia XL-300. Percentage Carbohydrate Deficient Transferrin (%CDT) was estimated using Turbidimetric Immuno Assay (TIA) with column separation followed by Turbidimetric measurement by ELISA method [17]. It measured the relative quantity of %CDT in proportion to the total transferrin in un- fractionated serum (% CDT). (Anion exchange chromatography separation makes the investigation specific for %CDT and the estimation of %CDT to total transferrin by immuno turbidimetry makes it all the more sensitive also.). MCV was estimated using automated hematology analyser Sysmex KX-21. Dedicated reagents and standard methodologies were used for both the machines. The two-level quality controls were run every day and the analyzers were maintained according to the manufacturer's instructions during the entire period of study.

STATISTICAL ANALYSIS

Data were collected and analysed using SPSS 16.0. The inferential statistics student t-test was done for the difference of all the parameters between Study Group and Control Group with 5% level of significance. Sensitivity, Specificity and the Positive and Negative Predictive Values (PPV, NPV) of %CDT, GGT, AST, ALT and MCV were arrived at using Receiver Operating Characteristics (ROC) curves.

RESULTS

[Table/Fig-1] shows the baseline characteristics of the both the groups. The calculated mean and standard deviation for each parameter have been shown.

* One unit of drink is considered to contain 8 g of absolute alcohol. (according to guidelines drawn by Royal college of Physicians, U.K [15].

Variables	Mean ± SD		P value
	Group A (Cases) n=25	Group B (Controls) n=25	
Age (yr)	38.66 ± 10.2	38.12 ± 9.8	0.962
Units of Drinks/ Week*	44.2 ± 12.2	-	0.000 **
Plasma Glucose (mg/dl)	103.8 ± 13.2	106.3±14.4	0.062
AST (IU/L)	41.0 ± 13.9	31.1 ± 10.4	<0.01 **
ALT (IU/L)	29.9 ± 12.1	21.0 ± 8.7	<0.01 **
ALP (IU/L)	151.3 ± 26.4	140.3 ± 25.8	0.05 **
T.PROTEINS (g%)	6.6 ± 0.5	6.7 ± 0.5	0.452
Albumin (g%)	3.4 ± 0.4	3.5 ± 0.3	0.251
Total Bilirubin mg/dl	0.7 ± 0.1	0.7 ± 0.1	0.984
MCV fL	105.0 ± 11.6	93.9 ± 4.3	0.02 **
GGT (U/L)	59.9 ± 41.0	24.5 ± 10.7	<0.001 **
CDT(%)	5.1 ± 3.6	1.9 ± 0.9	<0.001 **

[Table/Fig-1]: Baseline Characteristics of the subjects.

SD, standard deviation; BMI, body mass index; CDT, carbohydrate deficient transferrin; GGT, gamma glutamyl transferase; AST, aspartate amino transferase; ALT, alanine amino transferase.

**Statistical significance < 0.05

The mean (±SD) age of Study Group was 38.66 ± 10.2 and Control Group was 38.12 ± 9.8. Since the controls were age matched with that of the cases there was no significant difference (p=0.962) in the age among the groups. Study Group had a mean (±SD) of 44.2 ± 12.2 drinks /week while Control Group had none. There is

a statistically significant difference in between both the groups in AST (IU/L) levels (p<0.01) with 41.0 ± 13.9 for Study Group and 31.1 ± 10.4 for Control Group, in ALT (IU/L) levels (p<0.01) with 29.9 ± 12.1 for Study Group and 21.0 ± 8.7 for Control Group, in ALP (IU/L) levels (p=0.05) with 151.3 ± 26.4 for Study Group and 140.3 ± 25.8 for Control Group, in MCV (fL) values (p =0.02) with 105.0 ± 11.6 for Study Group and 93.9 ± 4.3 for Control Group, in GGT levels (U/L) (p<0.001) with 59.9 ± 41.0 for Study Group and 24.5 ± 10.7 for Control Group, in CDT (%) (p<0.001) with 5.1 ± 3.6 for Study Group and 1.9 ± 0.9 for Control Group. There were no statistically significant differences among the groups for the biochemical parameters like Blood Glucose, Total Proteins, Albumin and Total Bilirubin.

[Table/Fig-2] shows the Sensitivity, Specificity, PPV and NPV of the study parameters in Study group. The optimal cut off point of % CDT to Study Group was found to be 2.4% at which the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 84%, 92%, 91.3 and 85.2 respectively. The optimal cut off point of GGT to Study Group was found to be 30 U/L at which the sensitivity, specificity, PPV and NPV were 64%, 72%, 69.6 and 66.7 respectively. The optimal cut off point of AST to Study Group was found to be 35 IU/L at which the sensitivity, specificity, PPV and NPV were 68%, 80%, 77.3 and 71.4 respectively. Sensitivity of the other two study parameters, ALT and MCV were very low at 32% and 48% respectively.

DISCUSSION

The present study selected 25 study subjects (Study Group) who were diagnosed to have harmful alcohol consumption according to AUDIT scoring and 25 healthy subjects (Control Group) in the comparable age group. The study population comprised fully of males which may be attributed to the cultural background of the local population. However, this is not a limitation of the study since no gender variation has been found in the level of %CDT among alcoholics [18] and hence the conclusion of the present study may be extended to the female alcoholics also.

Variables	CDT (2.4%)	GGT (30 U/L)	AST (35 IU/L)	ALT (35 IU/L)	MCV (100 fL)
Sensitivity %	84	64	68	32	48
Specificity %	92	72	80	92	52
PPV %	91.3	69.6	77.3	80	50
NPV %	85.2	66.7	71.4	67.5	50

[Table/Fig-2]: Sensitivity, specificity, PPV and NPV of study parameters in study group.

The Mean units of drinks / week was 34.2 ± 12.2 in Study Group [Table/Fig-1] and 80% of the subjects in Study Group were Problem drinkers with harmful levels of alcohol drinking [15] who consumed more than 49 units of alcohol per week.

From [Table/Fig-1] it is evident that there is a statistically significant difference between the cases and controls for the parameters namely %CDT, GGT, AST, ALT, ALP and MCV. Furthermore, it is observed that GGT and % CDT showed a highly significant difference among the groups when compared to the other parameters like AST, ALT, ALP and MCV.

Plasma glucose, Serum Total proteins, Albumin and Total Bilirubin did not show statistically significant differences among the groups.

GGT is a well established marker of liver disease and has been extensively studied in alcoholism. In the present study GGT was found to be significantly increased in the study group when compared to controls. The mean values (U/L) were 24.5 and 59.9 for control group and study groups respectively. Similar highly significant results have been reported earlier [8, 19]. At 30 U/L, which is the upper limit of normal range for GGT, the sensitivity was 64% and specificity was 72%. Increased sensitivity of 84% could be obtained at a cut off value of 22 U/L with trade off for a specificity of mere 52%. In our study, 28% of the study population had a serum GGT of less than30 U/L, so the sensitivity is only 64%.

For % CDT, the reference range suggested by the manufacturers is <2.6%. However, in the present study cut off value of 2.4% CDT had a sensitivity of 84% and specificity of 92%. These findings of the present study are consistent with the other studies [20-22]. The remarkable specificity of % CDT when compared to GGT makes it the most specific marker for alcoholism.

The moderate significance found in the level of AST is in agreement with that of few other authors [7]. At a cut off level of 35 IU/L it has a sensitivity of 68% and a specificity of 80%. We observed poor sensitivity with ALT and so it cannot be used for screening alcoholism.

The MCV values though showed a statistically significant difference among the groups, lack in sensitivity and specificity and so it cannot be considered as a marker for alcoholism.

Finally, in the present study, while serum % CDT was elevated in 92% of the study population, GGT was above the reference range in 72% of the study population. However, when the two markers are used in combination, 96% of those in the study group were found to have fallen in that group. This suggests that when used together GGT and % CDT make a better diagnostic tool than used alone.

CONCLUSIONS

To conclude Serum %CDT is a better marker both in terms of sensitivity and specificity when compared to conventional markers of alcoholism like GGT, AST, ALT, and MCV. Hence, it may be used as a tool to monitor therapy and for early identification of relapses in alcoholics during treatment.

Lack of sensitivity and specificity of GGT makes it a poor marker for alcohol abuse than serum % CDT. Moreover, Serum % CDT levels can be used to identify alcohol abuse in cases with normal GGT levels also. When % CDT is used as an adjunct with GGT, it improves the diagnostic accuracy.

SCOPE FOR FURTHER STUDY

Serial monitoring serum %CDT levels in alcohol abusers before and during therapy will throw more light on the usefulness of this marker in a better way.

REFERENCES

[1] Source: H. K. Sharma, *National Drug Dependence Treatment Centre AIIMS, New Delhi*, 2003 (statistics).
[2] Ray R, Mandal AB, Gupta K, Chatterjee A, Bajaji P. 2004. The Extent, Pattern and Trends of Drug Abuse in India: National Survey New Delhi: United Nations Office in Drugs and Crimes and Ministry of Social Justice and Empowerment, Government of India.
[3] Schuckit MA. Why don't we diagnose alcoholism in our patients? *J Fam Pract*. 1987; 25: 225-26.
[4] Bradley KA, Debenedetti AF, Volk RJ, Williams EC, Frank D, Kivlahan

DR. AUDIT-C as a brief screen for alcohol misuse in primary care. *Alcohol Clin Exp Res.* 2007;31:1208–17.
[5] Peter C Sharpe. Biochemical detection and monitoring of alcohol abuse and abstinence. *Annals of Clinical Biochemistry*.2001;38:652-64.
[6] Helander A. Biological markers in alcoholism. *J Neura Transm Suppl.* 2003; 66: 15-32.
[7] Reynaud M, Hourcade F, Planche F, Albuisson E,Meunier MN, Planche R; Usefulness of Carbohydrate deficient Transferrin in Alcoholic patients with normal γ Glutamyl transpeptidase. *Alcohol Clinical & experimental Research.* 1998; 22(3);615-18.
[8] Allen JP, Sillanaukee P, Anton R. Contribution of carbohydrate deficient transferrin to gamma glutamyl transpeptidase in evaluating progress of patients in treatment for alcoholism. *Alcohol Clin Exp Res.* 1999; 23: 115-20.
[9] Torsten Arndt. Carbohydrate-deficient Transferrin as a marker of chronic alcohol abuse: A critical review of pre analysis, analysis and interpretation. *Clinical Chemistry*. 2001; 47(1); 13-27
[10] De Feo TM, Fargion S, Duca L, Mattioci M, Chappellini MD, Sampietro M. Carbohydrate Deficient Transferrin, a sensitive marker of Chronic alcohol abuse, is highly influenced by body iron. *Hepatology*. 1999; 29: 658-63.
[11] Idun.Marette, Mikkelsen, Rolf-Dieterkanity, Odd Nilssen, Nils Erik Huseby. CDT: Marker of actual alcohol consumption or Chronic alcohol misuse? *Alcohol & Alcoholism*.1998;33(6) ;646-50.
[12] American Psychiatric Association. Diagnostic and statistical manual manual of mental disorders, (4th edition), text revision. Washington DC:*American Psychiatric Association*. 2000.
[13] Stibler H. Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed. *Clin Chem.* 1991;37: 2029-37.
[14] Stibler H, Borg S, Joustra M. Micro anion exchange chromatography of carbohydrate-deficient transferrin in serum in relation to alcohol consumption (Swedish patent 8400585-5). *Alcohol Clin Exp Res.* 1986;10: 535-44.
[15] Saunders JB, Aasland OG, Babor TF et al. Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption—II. *Addiction.* 1993, 88: 791–803.
[16] Royal College of Physicians. A Great and Growing Evil ± The Medical Consequences of Alcohol Abuse.Report of a Working Party. London: *Tavistock*,1987.
[17] Bio-rad. Instruction manual for %CDT TIA. USA: Bio-rad, 2002; 1-32.
[18] Martensson O, Harlin A, Brandt R, Seppa K, Sillanaukee P. Transferrin isorform distribution: Gender and alcohol consumption. *Alcohol Clin*. Exp.Res. 1997;21 (9);1710-15.
[19] Aithal PG, Thornes H, Dwarakanath AD, Tanner A R. Measurement of CDT in a general medical clinic: Is this test useful in assessing alcohol consumption? *Alcohol & Alcoholism;* 1998; 33 (3); 304-09.
[20] Anton RF, Dominick C, Bigelo M, West C, in collaboration with CDTect Research Group; Comparison of BioRad % CDT TIA and CDTect as laboratory markers of heavy alcohol use and their relationship with γ Glutamyl Transferase. *Clinical Chemistry.* 2001. 47:10;1769-75.
[21] Hackler R, Arndt T, Helwig-Rolig A, kropf J, Steinmetz A, Schaefer JR. Investigation by iso electric focusing of the initial CDT and Non CDT transferrin isoform fractionation step involved in determination of CDT by the chron Alcohol.D.Assay. *Clinical Chemistry.* 2000;46:4; 483-92.
[22] Salaspuro M (56) Research Unit of Alcohol Diseases, University of Helsinki, Finland. Carbohydrate deficient Transferrin as compared to other markers of Alcoholism: A systematic Review; Alcohol Vol.19, No.3 pp 261-271,1999.

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