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RESEARCH ARTICLE

Serum CDT (Carbohydrate Deficient Transferrin) levels among Indian Adult Alcoholic males with Alcoholic liver disease spectrum- An Observational Case Control study.

J Rathi Roopavathy[1], and K Chandramouleeswari[2]*.

[1]Assistant Professor, Department of Biochemistry, Central Laboratory, Tamil Nadu Govt. Multi Super Speciality Hospital, Omandurar Estate, Chennai - 02, Tamil Nadu, India.
[2]Professor &HOD, Department of Pathology, Central Laboratory, Tamil Nadu Govt. Multi Super Speciality Hospital, Omandurar Estate, Chennai - 02, Tamil Nadu, India.

ABSTRACT

Alcoholic liver disease comprises of spectrum of manifestations ranging from asymptomatic Fatty liver to symptomatic Hepatitis, progressing to severely symptomatic Cirrhosis. The Study was aimed at estimating the serum CDT levels among patients with Alcoholic liver disease spectrum to establish its association. An Observational type Case Control study, with 120 study participants aged from 20-60 years includes [90 Cases attending General OPD [30 cases in each of the three category of alcoholic liver disease spectrum (Category I: Alcoholic fatty liver, Category II : Alcoholic hepatitis, Category III : Alcoholic cirrhosis)] & 30 healthy age matched Controls of Tamil Nadu Government Multi Super Speciality Hospital, Chennai-2, conducted during the period between March – June 2020. There was significant difference between four groups [controls and case groups] for serum CDT levels by one way ANOVA (F ratio = 1050.609) ($p=0.0001$) even after multiple comparison by Tukey's test showed significant difference existed in each pairwise comparison ($p<0.01$). The measurement of serum CDT levels is imperative for finding its association and in identifying the progression of the Alcoholic liver disease patients and improving the Quality of their life.

Keywords: Alcoholic liver disease, Carbohydrate Deficient Transferrin, Progression.

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Corresponding author

INTRODUCTION

According to Global status report on Alcohol and health 2018 by WHO, Alcohol per capita consumption (APC) in the global population 15 years of age or older is 6.4 liters of pure alcohol per year [1]. Globally 3 million deaths occur every year [5.3% of all deaths] because of alcohol abuse. Alcohol abuse has a causal role in more than 200 disease conditions and contributes to 5.1% of global burden of disease as measured in Disability-Adjusted Life Years [DALYs] [2], according to WHO-GISAH (Global Information System on Alcohol and Health).

In India, Alcohol per capita consumption is continuously on the rise for the past 50 years. The Total Alcohol Per Capita (APC) consumption of pure alcohol is 5.7 liters for the year 2016 according to WHO report [3] in 2018. Alcohol abuse contributes to Alcoholic liver disease which comprises a spectrum of manifestations ranging from asymptomatic hepatic steatosis to symptomatic hepatic involvement which includes hepatitis, followed by cirrhosis leading sometimes even to hepatocellular carcinoma [4]. The laboratory tests that assess liver function though has its significant role in management of alcoholic liver disease patients, yet none appears to be specific to severity impact of alcohol abuse on liver dysfunction. Furthermore many physicians rely only on questionnaire to identify abuse. So there is always a need to objectively provide evidence on the effects of alcohol abuse by biochemical tests towards progression of alcoholic liver disease. CDT [Carbohydrate Deficient Transferrin] are Transferrin isoforms deficient in sialic acid residues. CDT has been implicated as a biomarker for heavy alcohol consumption by various studies for the past 25 years.

Many International Studies like Stibler et al.1991, Bell et al. 1993, Yamauchi et al.1993, Niemela et al.1995, Rublo et al.1997, Lieber et al.1999, Salaspro et al.1999, Hellander et al.2001, Golka et al.2004 in the past and recent National studies like Madhubala et al [5], Nandeesha et al [6] have showed positive associations on the use of CDT as a biomarker for alcohol consumption and alcoholic liver disease.

However some International studies like Behrens et al.1988, Defao et al.1999, Mere galli et al. have questioned the usefulness of CDT as a biochemical marker for heavy alcohol consumption and its association with alcoholic liver disease. Some Studies like Faganet al. [7] has suggested to exercise caution while interpreting CDT as a biomarker for alcohol abuse and alcoholic liver disease.

As these studies have opposing and cautious conclusions on the role of CDT as a biomarker in alcohol abuse and alcohol liver disease, and as there are no credible impact studies among alcoholic patients in Metropolitan South Indian population, there is always a need for further research into the role of CDT among Alcoholic liver disease patients in Chennai.

The present study was conducted with the aim of estimating the levels of serum CDT (Carbohydrate Deficient Transferrin) among patients with varying stages of Alcoholic liver disease and to find its significance in association with Alcoholic liver disease spectrum.

MATERIALS METHODS

A Formal Approval was obtained from Institutional Ethics Committee for Human Research, Tamilnadu Government Multi Super Speciality Hospital, Omandurar Estate, Chennai-02, in accordance with ICMR guidelines.

The Study Design is an Observational type Case Control study. The Sample size for each group was taken as 30, considering the minimum sample requirement with ($p<0.05$) as the level of significance. The Study was conducted in Tamilnadu Government Multi Super Speciality Hospital, Omandurar Estate, Chennai-2 during the period between March – June 2020. The Study population which includes 120 Adult male participants includes [90 Cases attending General Medicine OPD [30 cases in each of the three category of alcoholic liver disease spectrum (Category I : Alcoholic fatty liver, Category II : Alcoholic hepatitis, Category III : Alcoholic cirrhosis)] & 30 healthy age matched Controls (volunteers from Master health check-up) of Tamilnadu Government Multi Super Speciality Hospital, Omandurar Estate, Chennai-2]. An Informed Consent is obtained from all the participants in English and Regional language. All the participants of the study were questioned at the baseline for a general medical history and specific history relating to alcohol use including their drinking patterns, type of drinks, number of years of

drinking, and last drink date. They were also examined for a general medical examination, prior USG examination records, nutritional status with specific emphasis towards signs of liver disease.

Inclusion Criterion

Case Definition – Criterion [8]

Alcohol Abuse Criterion

Alcohol abuse / Alcohol use disorder being diagnosed using AUDIT (Alcohol use disorder identification test) questionnaire. A total score of 8 or more is considered positive for abuse.

Alcoholic Fatty Liver Criterion

The Criterion for categorizing Alcoholic fatty liver includes Patients with alcohol abuse: AUDIT score > 8, with hepatic steatosis on USG- Liver and /or, elevation in liver enzymes (AST>ALT, serum bilirubin < 3 mg %), and absence of other causes of liver disease.

Alcoholic Hepatitis Criterion

The Criterion for categorizing Alcoholic Hepatitis includes Patients with alcohol abuse > 5 years and AUDIT score > 8, hepatic steatosis, elevation of liver enzymes (ALT and AST × 1.5 times but < 400 U/L and AST/ALT > 1.5) , sudden or worsening jaundice (serum bilirubin > 3 mg %)and absence of other causes of liver disease.

Alcoholic Liver Cirrhosis Criterion

The Criterion for categorizing Alcoholic Cirrhosis includes Patients with alcohol abuse : AUDIT score > 8, clinical features of liver disease (ascites, jaundice, fluid retention,),USG evidence of fibrosis and classified according to Child Pugh criteria for severity.

Exclusion Criterion

Patients with associated features of Diabetes mellitus, Thyroid disease, Renal disease, on Hepatotoxic drugs, Cholestatic disease, Cardiac failure, Anemia and any Bleeding diathesis were excluded from the study participation.

Sample Collection

Venous Blood sample [10 ml] collected at random by Vene puncture under Strict Aseptic precautions, after obtaining verbal and written informed consent from the study participants (both cases and controls). The collected blood sample is processed for Serum separation [Allowed serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes] and then sent for Laboratory analysis.

Sample Analysis

The Laboratory Investigations done for statistical analysisincludes :Serum CDT (Carbohydrate Deficient Transferrin) levels by ELISA kit,Serum Bilirubin (Total) levels by Di Azo method,Serum Bilirubin (Direct) levels by Di Azo method,Serum ALT(Alanine transaminase) levels by Modified IFCC Method-Kinetic assay, Serum AST(Aspartate transaminase) levels by Modified IFCC Method- Kinetic assay, Serum ALP(Alkaline phosphatase) levels by IFCC Enzymatic method.

Statistics Analysis

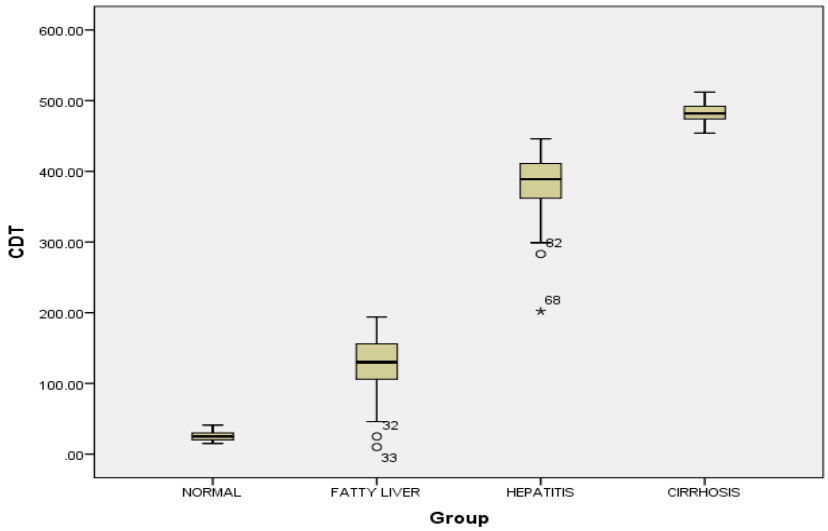
The laboratory data results of all the participants were tabulated using MS Excel sheet version 2007 and analysed for Measures of Central tendency (Mean) and Measures of Deviation (Standard deviation) using SPSS (Statistical package for social sciences) software version 18 along with construction of Descriptive Box Plot comparisons for Cases and Controls. The mean values of all the groups were were

analysed for tests of significance (F ratio calculation) using one-way ANOVA (analysis of variance) with $p<0.05$ as the level of significance and multiple comparisons were done using Tukey's test.

RESULTS

Results Of Serum CDT Levels

Mean Serum levels of CDT (Carbohydrate Deficient Transferrin) in the Control (Normal healthy volunteers) Group (n=30) is estimated to be 26.2±6.93ng/ml. Mean Serum levels of CDT (Carbohydrate Deficient Transferrin) in the Case Group – Category I (Alcoholic fatty liver) (n=30), Category II (Alcoholic hepatitis) (n=30), Category III (Alcoholic cirrhosis) (n=30) is estimated to be 125.53±46.05ng/ml, 379.6±52.12ng/ml, 482.86±17.70ng/ml respectively.The results show that serum CDT levels of all the case groups are significantly higher when compared to the control group and the levels are progressively higher for three categories as compared using Box Plot Graph Comparison shown below.



Graph 1: Box Plot Comparison of Mean CDT levels among the Control and Case groups.

There was significant difference between four groups [controls and case groups] for serum CDT levels by one way ANOVA (F ratio = 1050.609) (p=0.0001) even after multiple comparison by Tukeystest showed significant difference existed in each pairwise comparison ($p<0.01$).

Results of other parameters

Mean Serum levels of Bilirubin (Total) in the Control group is estimated to be 0.77±0.17 mg/dl, while that in the Case Group – Category I (Alcoholic fatty liver), Category II (Alcoholic hepatitis), Category III (Alcoholic cirrhosis) is estimated to be 1.36±0.32 mg/dl, 2.21±0.79 mg/dl, 4.04±0.88 mg/dl respectively. Mean Serum levels of AST (Aspartate Transaminase) in the Control Group is estimated to be 19.83±3.16 IU/L while that in the Case Group – Category I (Alcoholic fatty liver), Category II (Alcoholic hepatitis), Category III (Alcoholic cirrhosis) is estimated to be 19.2±5.04 IU/L, 50.2±5.28 IU/L, 92.6±22.47 IU/L respectively. Similar to CDT, there was significant difference between the four groups for Bilirubin (Total) AST (Aspartate Transaminase) by one way ANOVA (F ratio = 157.291& 252.624 respectively) (p=0.0001), While calculated multiple comparison by Tukey's test also showed significant difference existed in each pairwise comparison ($p<0.01$).

Mean Serum levels of Bilirubin (Direct) in the Control Group is estimated to be 0.39±0.14 mg/dl, while that in the Case Group – Category I (Alcoholic fatty liver), Category II (Alcoholic hepatitis), Category III (Alcoholic cirrhosis) is estimated to be0.72±0.18 mg/dl, 0.93±0.20 mg/dl, 1.43±0.39 mg/dl respectively. Mean Serum levels of ALT (Alanine Transaminase) in the Control Group is estimated to be 12.33±1.74 IU/L while that in the Case Group – Category I (Alcoholic fatty liver), Category II (Alcoholic hepatitis), Category III (Alcoholic cirrhosis) is estimated to be 41.86±5.49 IU/L, 32.76±6.11 IU/L, 60.06±5.94 IU/L respectively. There was significant difference between the four groups for Bilirubin (Direct) and ALT (Alanine Transaminase) by one way ANOVA (F ratio = 88.886 & 446.378) respectively

(p=0.0001). When evaluated multiple comparison test, it showed significant difference existed in each pairwise comparison (p<0.01) but Control group and Category I (Alcoholic fatty liver)case group was not significant (p=0.9).

Mean Serum levels of ALP (Alkaline Phosphatase) in the Control Group is estimated to be 63.83±15.91 IU/L, while that in the Case Group – Category I (Alcoholic fatty liver), Category II (Alcoholic hepatitis), Category III (Alcoholic cirrhosis) is estimated to be 66.86±10.53 IU/L, 112.33±22.20 IU/L, 109.36±22.68 IU/Lrespectively.There was significant difference between among groups for ALP(Alkaline Phosphatase) by ANOVA (F ratio =60.615)(p=0.0001). In multiple comparison by Tukey's test significant difference existed in each pairwise comparison (p<0.01) except Category II (Alcoholic hepatitis) with Category III (Alcoholic Cirrhosis) group (p=0.9).

DISCUSSION

Alcoholic Liver Disease

Alcoholic liver disease spectrum includes Steatosis, Hepatitis, Cirrhosis, and sometimes progressing even to Hepatocellular carcinoma [9]. Steatosis is characterized by the accumulation of triglycerides, phospholipids, and cholesterol esters in hepatocytes. Further alcohol consumption, leads to Hepatitis, which involves inflammatory damage to the liver. Cirrhosis represents the next stage damage that leads to fibrotic changes along with regenerative nodules eventually leading to shrinkage of liver.

Ethanol oxidation to Acetic acid is a two-step process carried out by the enzymes Alcohol dehydrogenase and Aldehyde dehydrogenase. Alcohol dehydrogenase first oxidizes Ethanol to Acetaldehyde, which is then further oxidized to Acetate by Aldehyde dehydrogenase.

As a result of its electrophilic nature, Acetaldehyde can bind and form covalent chemical adducts with proteins, lipids and DNA. These adducts are broadly pathogenic because they alter cell homeostasis, changing protein structure and promoting DNA damage and mutation [10].

The Pathogenesis of Alcohol mediated Liver injury is mediated through several mechanisms which includes acetaldehyde mediated damage, DAMP (damage associated molecular patterns), steatosis, inflammatory immune response to injury, impaired regeneration, intestinal permeability changes and microbiome, epigenetic changes and several other mechanisms.

Carbohydrate Deficient Transferrin

The Transferrins are monomeric, iron binding glycoproteins which are synthesized in the liver. It contains two Carbohydrate chains with sialic acid end groups. It comprises atleast six different isoforms, with respect to the number of sialic acid side chains :penta-, tetra-, tri-, di-, mono- and asialo- Transferrins (Wong 1977) as it shows microheterogeneity both in its amino acid compositionand in its iron and carbohydrate content. The normal main isoform of Transferrin contains four terminal sialic acid residues, two in each of the bifurcated chains consisting of varying amounts of four carbohydrates : N-acetyl glucosamine, Mannose, Galactose, Sialic acid.

Transferrin isoform with 0-3 sialo residues or the deglycosylatedtransferrins that lack sialic acid residues are designated as CDT(Carbohydrate Deficient Transferrin). The half life of serum CDT is 2 weeks.

Review of Literature

CDT levels in alcohol abuse

Many Studies have established the role of serum CDT levels as a biomarker of alcohol abuse during the past 25 years. The studies includes Hellander et al., Lieber et al., Stibler et al., Salaspro et al., Bell et al., Stauber et al., Cesk et al.,. Hellanderet al [11]. has showed that CDT is a more specific marker for identifying excessive alcohol consumption. Lieberet al [12]. showed that CDT is now considered to be the most sensitive and specific biological marker of alcohol abuse. Stibleret al [13] showed that 87% of current alcohol abuse patients showed elevated CDT levels than with controls and concluded that CDT

can be used as a marker of present alcohol abuse. Salasproet al [14]. made a systematic review on the role of CDT and has pointed that CDT was slightly a better marker than conventional laboratory markers in identifying alcohol abuse.

Bell et al [15] and Sharpe et al. showed that none of the newer markers offers significant advantage although CDT seems to be better at monitoring patients for alcohol abuse. Stauberet al [16]. showed that CDT had a sensitivity of 70% and a specificity of 84% for detection of alcohol abuse. The sensitivity and specificity of CDT was superior to two other markers of chronic ethanol consumption, serum gamma-glutamyltransferase and mean cell volume, and thus proved to be the best single laboratory test for the detection of alcohol abuse. Cesket al [17] showed that the specificity and cumulative sensitivity of laboratory markers can be elevated by CDT evaluation. CDT can be the only detectable abnormality in some patients of chronic alcohol consumption. In men the study proved a comparatively high diagnostic efficiency of CDT (AUC 0.94, sensitivity 82.6%, specificity 96.7%) for chronic alcohol consumption.

Other studies have found association of serum CDT levels with alcoholic liver disease. It includes studies like Niemela et al., Frankel et al.,Yamauchi et al., Seitz et al., Rublo et al., Ohtsuka et al., Liu et al., Liang et al., . Niemelaet al [18] showed that CDT levels was found to be elevated in those with early stage of alcoholic liver disease. Frankel et al [19]. and Dimartini et al. showed that CDT levels are elevated in alcoholic liver disease and has hypothesised that CDT can be used as a marker of liver impairment in these subjects. Yamauchi et al [20] showed that serum CDT levels is a useful marker of alcoholic liver disease and that the serum levels was significantly higher in patients with alcoholic liver disease (38.9+/-2.8U/L) compared to normal subjects (18.9+/-0.2U/L). Seitz et al [21] showed that CDT has sensitivity of 57% and specificity of 100% with mean CDT concentration in male alcoholics with liver damage (53.4±9 U/l) compared to male alcoholics without liver damage (35.5±5 U/l).

Rubloet al [22] showed that serum CDT is a good marker of alcoholism and is less influenced than the currently used biochemical markers for associated liver disease with a sensitivity of 64% and specificity of 82%. The Study showed that the serum CDT in active alcoholics was 37.5 +/- 3.6 units/liter, being significantly higher than that of abstinent alcoholics (20.3 +/- 1.5 units/liter), patients with nonalcoholic liver disease (18.1 +/- 1.1 units/liter), and controls (13.1 +/- 0.8 units/liter). Ohtsukaet al [23] showed that the serum CDT values of all patients with NASH were lower than the cutoff value, 2.66%, and those of all patients with alcoholic hepatitis were higher than the cut-off value.

You Shi Liu et al [24] showed that the positive rate of CDT in the patients with ALD was 93.4%(71/76), which was higher than that in those with alcoholism (52.7%, 29/55, P<0.001), in those with NALD(9.4%, 3/32, P<0.001), and in healthy controls, respectively. The sensitivity and specificity of CDT for ALD was 93.4% and 71.9%, respectively and even concluded that the CDT levels may help diagnose Alcoholic liver disease. Liang et al [25] showed that The CDT level in the alcoholic group was significantly higher than that of the non-alcoholic liver disease and healthy control groups (P < .05). The area under the curve for alcoholism diagnosis was the highest for CDT, at 0.922, whereas those for gamma-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, and mean corpuscular volume were 0.860, 0.744, 0.615, and 0.754, respectively. When the cutoff value of CDT was set at 1.25%, the sensitivity and specificity were 85.5% and 89.6%, respectively. However, the correlation between CDT and daily alcohol consumption was weak (r = 0.175; P = .16). Compared with the other parameters evaluated, CDT was a better indicator of alcoholism.

While most studies have found serum CDT as a positive biomarker for alcohol abuse and alcoholic liver disease, some studies either exercised caution or found no significant association for serum CDT levels in alcohol abuse and alcoholic liver disease. It includes Golka et al., Bovim et al., Neumann et al., Stadheim et al., Behrens et al., Defao et al., Golkaet al [26] showed that the study CDT has high specificity as a biomarker for chronic alcohol intake, yet the disadvantage mentioned by the study is its relatively low sensitivity. Bovimet al [27] showed that a single test or even repeated tests of CDT may give a wrong conclusion on the status of alcohol abuse. Neumann et al [28] showed that More research is needed to determine the value of markers (single or combined, [GGT, MCV, CDT] with questionnaires) in the context of clinical decision-making algorithms in defined settings and with defined dichotomous outcome variables.

Stadheimet al [29] showed that Alcohol as a cause of liver disease is not perfectly established by CDT analysis, although a high total CDT value favors ALD over NASH. The ROC-AUC values for individual transferrin isoforms in ALD vs NASH for pentasialo, tetrasialo, trisialo, disialo, monosialo, and asialo were 0.806, 0.917, 0.885, 0.933, 0.804, and 0.785, respectively. Only 58% of patients with ALD were detected at a specificity that excluded ALD in 84% of those who did not have it. Behrens et al [30] showed that some alcoholic patients even with heavy daily intake fail to manifest elevated CDT levels. Defaoet al [31]. and Mere galli et al. has concluded that false positive results have been reported in severe non alcohol related liver failure.

Mechanism behind CDT elevation in study result

The results of our Study showed that Mean Serum levels of CDT (Carbohydrate Deficient Transferrin) is significantly higher among case groups (Category I : 125.53±46.05) (Category II : 379.6±52.12) (Category III : 482.86±17.70) compared to the control groups (26.2±6.93). F ratio was found to be 1050.609 (p value is 0.0001) with the mean comparison results significant at 5% significance level. This is in line with many previous studies conducted all around the globe as in the literature review.

The exact mechanism by which chronic alcohol consumption induces CDT formation has remained unclear. Studies have indicated that transferring synthesis is accelerated in patients with fatty liver (Potter et al.,1985). Other mechanisms postulated for increased CDT levels in alcoholics include disturbed glycoprotein synthesis in the hepatocytes.

Investigations into hereditary carbohydrate deficient glycoprotein syndromes have suggested defects in N linked oligosaccharide processing or attachment of the sugar chains to the protein, although defective synthesis and transfer of nascent dolichol linked oligosaccharide precursors has been documented.

The newly formed Transferrin present in alcoholics during abstinence seems to have a higher sialic acid content than most of the transferrin already present in blood, suggesting impaired uptake of sialic acid deficient transferrin by the hepatocytes in alcoholics, due to membrane dysfunction, rather than a defect in the sialylation process. Other mechanisms suggested by many studies like Enzymes which glycosylate transferrin are inhibited by ethanol metabolites. Enhanced loss of sialic acid groups occurs . Receptor mediated CDT uptake might be inhibited. Acetaldehyde mediated inhibition of post translational protein glycosylation. Increased Desialylation of completely glycosylated transferrin molecule. Impaired hepatic binding of asialoglycoproteins. Transferrin is glycosylated by specific enzymes called glycosyltransferases which are inhibited by acetaldehyde, a metabolite of ethanol. The exact mechanism by which alcohol intake elevates CDT is not yet exactly known and seems to be a multi step process. To Summarize, several mechanisms appear to play a cumulative role for increased serum CDT levels in Alcohol abuse patients suffering from Alcoholic liver disease.

CONCLUSION

Alcohol abuse has been continuously on the rise which has contributed significantly to the morbidity and mortality of the Alcoholic liver disease patients in the Society. The Study concludes that there is significant rise in serum CDT (Carbohydrate Deficient Transferrin) levels among Indian Adult Alcoholic males suffering from Alcoholic liver disease along with proportionate increase in levels as the severity of alcohol abuse progresses through all three Categories including Fatty liver, Hepatitis and Cirrhosis. Molecular basis for the rise has been supported through many mechanisms including direct effects of alcoholic metabolites on the Transferrin synthesis and glycosylation abnormalities in several studies worldwide.

The Strengths of the study includes the design of categorical grouping among cases with measurements of CDT levels among different stages of the Alcholic liver disease spectrum and the statistical analysis which allowed one way ANOVA and multiple comparisons using Tukeys test, as similar research studies are lacking in the past. The Limitation of the study includes small sample size which limits the generalizations of the results on larger populations. The other limitation is the use of ELISA for CDT estimation, which is not a standard reference method. But this offers the scope for future research prospects into CDT estimation using standard reference measurement methods like HPLC and Capillary Electrophoresis, with improved analytical precision.

But on the Whole, the measurement of serum CDT levels is imperative for finding the association and in identifying the progression of the Alcholic liver disease patients and future research studies are needed for further validation among different study populations of Alcoholic liver disease patients including the prognostic value in progression of Alcoholic liver disease and improving the Quality of their life.

Conflict of Interest

The Authors declare no conflict of interest.

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