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Serum Protein and Its Fractions in Patients of Alcohol Dependence and Healthy Individuals – A Comparative Study

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Abstract
Introduction: *Heavy consumption of alcohol can cause untold misery to the individual, who is usually affected by other physical, psychological and social disabilities as well. In chronic alcoholism the liver cells are damaged and since albumin is solely synthesized in the liver there is hypoalbuminemia. On the other hand serum globulin levels are raised leading to altered Albumin/Globulin ratio. The study was conducted with the objective to assess and compare the levels of Serum Protein and its fractions in patients of alcohol dependence with equal number of age and sex matched healthy controls.*
Materials and Methods: *This was a hospital based case control study conducted on 100 inpatients of alcohol dependence and equal number of age and sex matched controls. Blood samples were collected from the patients on the first day of admission and, Serum Protein, Albumin were measured using Biuret Reaction and Bromocresol Green method respectively. The results were analysed using SPSS Version 16.0 setting the significance threshold at p=0.05.*
Results: *There was significant elevation of Serum Globulin in patients of alcohol dependence when compared to controls. On the other hand Serum Protein and Albumin were significantly lower in alcohol dependent cases compared to healthy controls.*
Conclusion: *Alcohol induced hepatic damage and poor nutrition may be some of the causes behind the low protein levels in alcoholics. Ethanol consumption slows down the rate of hepatic protein catabolism and changes may be related to the degree of ethanol-induced oxidative stress.*
Keywords: *Albumin, Alcohol Dependence, Globulin.*

Introduction

The problem of alcohol consumption is a major cause of public health concern in many countries of the world today. Heavy consumption of alcohol can cause untold misery to the individual, who is usually affected by other physical, psychological and social disabilities as well. In 1976 Edwards and Gross proposed the

existence of alcohol dependence within a syndrome model. Their description was based on the clinical observation that certain heavy drinkers manifested an interrelated clustering of signs and symptoms. [1] It is a cluster of physiological, behavioural and cognitive phenomena in which the use of alcohol takes on a much higher priority for a given individual than other behaviors that once had greater value. A central descriptive characteristic of the dependence syndrome is the desire (often strong, sometimes overpowering) to take alcohol. There may be evidence that return to alcohol use after a period of abstinence leads to a more rapid reappearance of other features of the syndrome than occurs with nondependent individuals. [2]

According to ICD-10[2] alcohol dependence can be diagnosed if 3 or more of the following are experienced or exhibited at some time during the last year –

1) A strong desire or sense of compulsion to take the substance.
2) Difficulties in controlling substance taking behavior in terms of its onset, termination or levels of use.
3) Physiological withdrawal state when alcohol use has ceased or been reduced.
4) Evidence of tolerance such that increased doses of the psychoactive substance are required in order to achieve effects originally produced by lower doses.
5) Progressive neglect of alternative pleasures or interests because of psychoactive substance use and increased amount of time necessary to obtain or take the substance or to recover from its effects.
6) Persisting with alcohol use despite clear evidence of overtly harmful consequences.

Narrowing of the personal repertoire of patterns of alcohol use is also a characteristic feature (e.g. a tendency to drink alcoholic drinks in the same way on weekdays or weekends, regardless of social constraints that determine appropriate drinking behaviour).[2]

Serum protein and its fractions

The liver is the major source of most of the serum proteins. The parenchymal cells are responsible for synthesis of albumin and most of the alpha and beta globulins.

Albumin: Albumin is considered quantitatively the most important protein in plasma synthesized by the liver and is also a useful indicator of hepatic function. Since the half life of albumin in serum is around 20 days, the serum albumin level is considered not a reliable indicator of hepatic protein synthesis in acute liver disease. Albumin synthesis is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure. Liver is the only site of synthesis of albumin. The serum albumin levels tend to be normal in diseases like acute viral hepatitis, drug related hepatotoxicity and obstructive jaundice. Albumin levels below 3g/dl in hepatitis should raise the suspicion of chronic liver disease like cirrhosis which usually reflects decreased albumin synthesis. In ascitis there may be normal synthesis but the levels may appear reduced because of increased volume of distribution. Hypoalbuminemia is not specific for liver disease and may occur in protein malnutrition, Nephrotic syndrome and chronic protein losing enteropathies. [3, 4, 5]

Globulin: Globulin is the second type of serum protein, the first being albumin. There are four major groups that can be identified: gamma globulins, beta globulins, alpha-2 globulins, and alpha-1 globulins. The globulin level is elevated in chronic infections, Liver disease (biliary cirrhosis, obstructive jaundice), Carcinoid syndrome, Rheumatoid arthritis, Ulcerative colitis, Multiple myelomas, leukemias, Waldenstrom's macrogl-obulinnemia, Autoimmunity (Systemic lupus, collagen diseases) and Kidney dysfunction. Since the gamma fraction usually makes up the largest portion of the globulins, antibody deficiency should always come be considered when the globulin level is low. Antibodies are produced by mature B lymphocytes called plasma cells, while

most of the other proteins in the alpha and beta fractions are made in the liver. [6]

In chronic alcoholism the liver cells are damaged and since albumin is solely synthesized in the liver there is hypoalbuminemia. On the other hand serum globulin levels are raised leading to altered A/G ratio. [6]

Limited research and dearth of study on the level of serum protein and its fractions in alcohol dependent patients in the North-Eastern part of India was one of the main motives behind this study. A higher prevalence of alcohol use among youth working in the tea industry in Assam is present compared to other communities in this part of the country (Chaturvedi et al, 1998, 2003, 2004; Hazarika et al, 2000). This added further significance to the study as a good proportion of the Dibrugarh (Assam) Population is comprised of the tea community.[7]The study was conducted with the objective to assess and compare the levels of Serum Protein and its fractions in patients of alcohol dependence with equal number of age and sex matched healthy controls.

Materials and Methods

This was a hospital based case control study carried out in a tertiary medical institution located in the upper part of Assam, India. The study duration was one year (2016- 2017). The study received the ethical approval from the institutional review board. An informed written consent was obtained from every participant and they were free to withdraw their consent at any point of time. The total sample size was 200 (100 cases and 100 controls). The cases were selected from patients, admitted in the institution between August 2016 and July 2017, who were diagnosed as Alcohol Dependence Syndrome or Alcohol withdrawal state with or without Delirium Tremens as per ICD-10, who fulfilled the inclusion and exclusion criteria and gave an informed written consent for participating in the study. In patients of Delirium Tremens written consent was taken from one adult family member (spouse/son/daughter) accompanying the patient. It was seen from previous admission registers of the institution that on an average around 100 patients of alcohol dependence were admitted in one year in the last 5 years (2011-2016). Hence the size of the study group (or case group) was taken to be 100. An equal number of age and sex matched people from healthy population were selected as controls, fulfilling the inclusion and exclusion criteria. The control population comprised of adult family members accompanying the patient and staff members working in the same institution. They did not have any history of alcohol intake in their lifetime. Informed written consent was taken from each of the subjects and they were free to withdraw their consent at any point of time.

Inclusion criteria

Study Group -

1) Patients in the age group of 18 to 65 years.
2) Patients of both the sexes.
3) Cases of Alcohol dependence, Alcohol withdrawal state with or without delirium tremens diagnosed as per ICD-10 and confirmed by Consultant, Department of Psychiatry.
4) Patients giving informed written consent for the study.

Control Group

1) Age and sex matched controls from healthy population who do not consume alcohol.
2) Persons giving informed written consent for the study.

Exclusion criteria

Study Group –

1) Those with co morbid systemic illness.
2) Those with co morbid mental illness.
3) Those with co morbid other substance abuse.

Control Group -

1) Those with history of hepatitis.
2) Those with any systemic illness or mental illness.
3) Those with history of any kind of substance abuse

Assessment tools –

- Informed consent form
- The ICD-10 classification of Mental and Behavioural disorders
- Estimation of Serum Protein by Biuret Reaction [8]
- Bromocresol Green Method for estimation of Serum Albumin [8]
- SPSS version 16.0 for statistical analysis of obtained data

Procedure – Inpatients in the age group of 18 -65 years, within the time period of August 2016 to July 2017, and diagnosed as Alcohol dependence (or alcohol withdrawal state with or without delirium tremens) as per ICD-10, confirmed by the consultant and fulfilling the inclusion criteria and exclusion criteria were included in study or case group. Every consecutive case admitted in the study period was selected in the study group till the total sample size was reached. An equal sex and age matched control group was selected from normal healthy population who did not consume alcohol. Informed written consent was taken from each participant of both the study and control group. They were free to withdraw their consent at any given point of time. Serum Protein and its fractions (Albumin and Globulin) were measured from all the participants of both the groups. From study group, blood samples were collected on the very first day of admission for the sake of uniformity. The blood investigations of both the groups were done in the Laboratory of Department of Biochemistry of the same institution. Reference interval for the measured parameters were used as followed in Laboratory of Department of Biochemistry. Analysis of the observed data was done using tests like Chi square test and unpaired sample t-test in SPSS windows version 16.0. The significance threshold for the tests were set at $p<0.05$. Pie chart and bar diagrams were used for graphical representation of the data.

Results

In both the study and control group most people were in the middle age group of between 30 and 53 years. In both the study group and the control group, 59 were in the age group of 30-41 years and 26 were in the age group of 42-53 years out of the total sample size of 100 each. Chi Square test was applied to look for significant difference between the age distributions of the two groups. The test result showed a p-value of 0.910 which was statistically insignificant. The study group had a mean age of 40.47 whereas the control group had a mean age of 38.69. Unpaired sample t-test was applied to look for any significant difference between the mean ages of the two groups The test result showed a p value of 0.1425 which denotes that that there was no significant difference in age between the two groups.

Table 1: Distribution of Case and Control on the basis of age

Age (in years)	Case		Control		X^2	DF	p-value
	no	(%)	no	(%)			
18-29	7	7	9	9	0.5357	3	.910
30-41	59	59	59	59			
42-53	26	26	26	26			
54-65	8	8	6	6			

*p-value significant at < 0.05, DF – Degree of Freedom, X^2 – Pearson Coefficient

Table 2: Mean age distribution of case and control

	Case		Control		p-value
Age (in years)	Mean ± S.D	Range	Mean ± S.D	Range	0.1425
	40.47±8.456	20-60	38.69±8.640	22-60	

*p value significant at <0.05

Both study and control group comprised of 98 males and 2 females respectively. On applying Chi Square no significant difference was found in the distribution of participants in both the groups on the basis of gender.

Table 3: Distribution of case and control according to Gender

Gender	Case		Control		X^2	DF	p-value
	no	%	no	%			
Male	98	98	98	98	0.000	1	1.000
Female	2	2	2	2			

*p value significant at <0.05

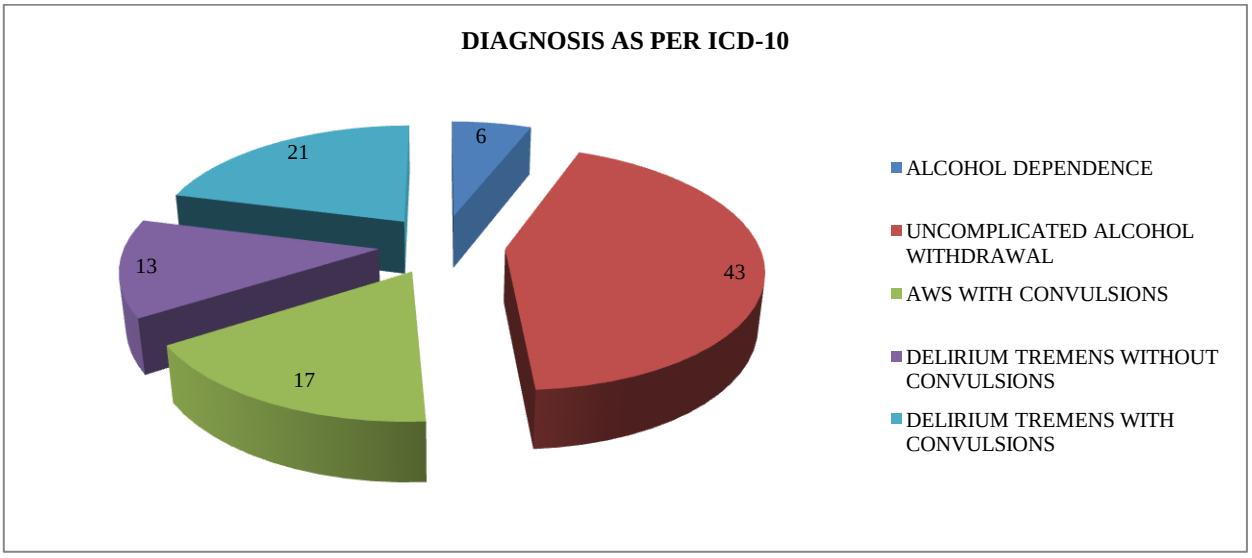

Figure 1: Pie Diagram showing the diagnosis of cases as per ICD-10

Table 4: Distribution of Case and Control according to Total Serum Protein level

Total Serum Protein (6.4-8.2 g/dl)	Case		Control		p-value
	no	(%)	no	(%)	0.0003*
Decreased	9		0		
Normal	85		93		
Elevated	6		7		
Mean ± SD	7.24 ± 0.66		7.54 ± 0.46		

*p value significant at <0.05

Table 5.14 shows that the mean Serum Protein value in the study group was 7.24 with a standard deviation of 0.66 whereas the mean Serum Protein value in the control group was 7.54 with a standard deviation of 0.46. On applying unpaired sample t-test, the p value was found to be 0.0003 which denotes that Total Serum Protein was significantly higher in the control group than the study group.

Table 5: Distribution of Case and Control according to Serum Albumin level

Serum Albumin (3.4-5.0 g/dl)	Case		Control		p-value
	no	(%)	no	(%)	
Decreased	34		0		
Normal	66		90		
Elevated	0		10		<0.0001*
Mean ± SD	3.50 ± 0.47		4.40 ± 0.43		

*p value significant at <0.05

Table 5.15 shows that the mean Serum Albumin value in the study group was 3.50 with a standard deviation of 0.47 whereas the mean Serum Albumin value in the control group was 4.40 with a standard deviation of 0.43. On applying unpaired sample t-test, the p value was found to be <0.0001 which denotes that Serum Albumin was significantly higher in the control group than the study group.

Table 6: Distribution of Case and Control according to Serum Globulin level

Serum Globulin (2.5-3.5 g/dl)	Case		Control		p-value
	no	(%)	no	(%)	
Decreased	1	1	1	1	<0.0001*
Normal	32	32	81	81	
Elevated	67	67	18	18	
Mean ± SD	3.75 ± 0.53		3.19 ± 0.36		

*p value significant at <0.05

Table 5.16 shows that the mean Serum Globulin level in the study group was 3.75 with a standard deviation of 0.53 whereas the mean Serum Globulin level in the control group was 3.19 with a standard deviation of 0.36. On applying unpaired sample t-test, the p value was found to be <0.0001 which denotes that Serum Globulin was significantly higher in the case group than the control group.

Table 7: Distribution of Case and Control according to Albumin Globulin Ratio

AG Ratio (1.5-2.5 g/dl)	Case		Control		p-value
	no	(%)	no	(%)	
Decreased	100		62		<0.0001*
Normal	0		38		
Elevated	0		0		
Mean ± SD	0.96 ± 0.18		1.40 ± 0.24		

*p value significant at <0.05

Table 5.17 shows that the mean Albumin Globulin ratio in the study group was 0.96 with a standard deviation of 0.18 whereas the mean Albumin Globulin ratio in the control group was 1.40 with a standard deviation of 0.24. On applying unpaired sample t-test, the p value was found to be <0.0001 which denotes that Albumin Globulin ratio was significantly higher in the control group than the study group.

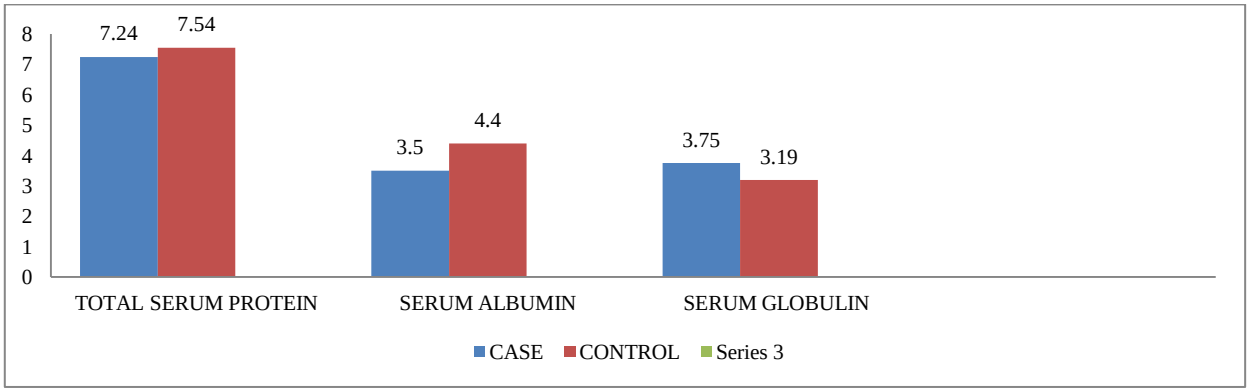

Figure 2: Bar Diagram showing the Mean Serum Protein, Mean Serum Albumin and Mean Serum Globulin levels in case and control group

Discussion

Most of the subjects in both the study and control group belonged to the middle age group. The mean age for the study group was 40.47 years whereas the mean age for the control group was 38.69 years. There was no significant difference in age between the two groups. Majority of subjects in both the study and control groups were males (98% in both groups). There was no significant difference when it came to distribution of subjects in both the groups on the basis of gender. This was an expected finding as an age and sex matched control population was selected for the study. Our findings were in accordance with the findings of Pitkänen et al. [9] who found, that level of alcohol use was significantly higher in men; Jean H. Kim et al. [10] who reported, that prevalence of alcohol abuse and alcohol dependence were higher among men than women and Juliana Gabrielle Martins-Oliveira et al. [11] who found, that male adolescents were more likely to develop alcohol dependence in comparison to females.

In this study it was seen that mean Serum Globulin in the study group was significantly higher than in the control group whereas the Total Serum Protein, Serum Albumin and Albumin Globulin ratio were significantly lower in the study group than the control group. Some of our findings were in line with the findings of Osaretin Albert Taiwo Ebuchi et al. [12] who reported serum protein and serum albumin levels were impaired by alcohol consumption in both males and females, Kyosola.K et al. [13] who found that the most consistent finding in liver function tests in chronic alcoholics was hypoalbuminemia and N. Priya et al. [14] who reported serum globulin was raised in alcoholics whereas total protein and albumin were decreased. However our finding was contradictory to the finding of –Gloria Luisa et al. [15] who reported normal Serum Albumin values in all 33 chronic alcoholics in his study with no evidence of liver disease and Joanna Danielsson [16] who found that serum albumin values were higher in heavy drinkers compared to moderate drinkers or abstainers.

Conclusion

Total Serum Protein and Serum Albumin were low among alcohol dependent patients whereas Serum Globulin and Albumin Globulin Ratio were raised in these patients compared to the controls. Poor nutrition and Ethanol induced hepatic damage may among the causes behind the low protein levels in alcoholics. Ethanol consumption slows down the rate of hepatic protein catabolism and changes may be related to the degree of ethanol-induced oxidative stress.

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