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Original Article

Observation on Ceruloplasmin Activity in Viral Hepatitis

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Abstract
Objective: *The present study was undertaken to the estimation of serum ceruloplasmin in cases of viral hepatitis and its comparative study with other liver function tests (Serum Bilirubin, serum alkaline phosphatase and S.G.P.T).*
Materials and Methods: *A total of 60 patients of viral hepatitis diagnosed clinico-biochemically were included from different units of inpatient medical ward. The estimation of serum ceruloplasmin activity was done by ELISA methods (modified methods of Rabin).*
Results: *Out of 60 patients, 43 (71.66%) were male and 17 (28.33%) were female and male to female ratio was 2.52:1. In control series 19 cases (63.33%) were male and 11 cases (36.67%) were female. Serum ceruloplasmin level in viral hepatitis was ranges from 40.25 to 70 mg% (mean 55.87% + 8.22), serum bilirubin ranges 3 to 15 mg% (mean 7.83% + 2.81), alkaline phosphtase 14 to 36 IU/L units (mean 24.93 + 6.35) and SGPT were ranges 42 to 170 IU/L (mean 98.37 + 35.90). On comparision of ceruloplasmin level with control it was found highly significant. Serum ceruloplasmin in viral hepatitis in relation to serum Bilirubin and SGPT are not highly significant but serum alkaline Phosphatase are highly significant. It is observed that mean serum ceruloplasmin were different in different weeks according to the duration of appearance of clinical Jaundice. It was evident that maximum rise occurred in the first week, and values gradually started falling in subsequent week, and mean values were highly significant statistically.*
Conclusion: *High serum ceruloplasmin value was confirmed in viral hepatitis. The possible cause of rise of serum caruloplasmin in viral hepatitis may be due to its acute phase reactant activity possibly because of biliary obstruction and also assess its significance in diagnosis severity and prognosis of the disease.*
Keywords: *Ceruloplasmin, Hepatitis, Jaundice, SGPT.*

Introduction

The Term **'Viral Hepatitis'** refers to a Primary infection of the liver by any one of the hetrogeneous group of hepatitis virus, which consist of Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C Virus (HCV), Hepatitis D Virus (HDV) Hepatitis E Virus (HEV) and Hepatitis G Virus. Hepatitis may occur incidently during many other viral infections such as yellow fever, Lass fever, marburg, EB, CMV, Herpes simplex, varicella zoster, measles, Rubella or coxsackie virus but these are not included in the

category of viral hepatitis. Hepatitis virus are taxonomically unrelated, Except for HBV which is a DNA virus, all the others are RNA viruses. The features common to them are their hepatotropism and ability to cause a similar icteric illness, ranging in severity from unapparent to the fulminant fatal forms.

Hepatitis B occurs throughout the word having massive morbidity and mortality and transmitted mainly by blood and blood product through carriers. There are over 350 million carrier now world-wide of them, about 45 million are in India which has the second largest carrier pool, next only to Chiana.

Inspite of various tests and elaborate laboratory techniques evolved in recent years, many cases of viral hepatitis still poses problems in diagnosis as well as in the evaluation of prognosis. Any simple test like serum ceruloplasmin estimation, if of any value in these respects will definitely go a long way in helping the clinician to arrive at a correct diagnosis specially in an early case of foresee a definite prognosis of such a case.

Clinically liver function tests are used chiefly to detect involvement of liver by disease, to evaluate the degree and type of functional impairment of liver. These tests are supposed to render valuable aid in the differential diagnosis and to follow up the course of the disease but their limitation in permitting accurate diagnosis are increasingly recognised. None of the test is specific and affords direct evaluation of the integrity of liver. The result of each tests are dependent in part on other factors and may be altered by the diseases other than primary liver disease. These tests do not effectively measure status or reserve function of liver. The separation or differentiation of various liver diseases by these tests is based more on empiricism than on any inherent properties of these tests.

Ceruloplasmin (or **caeruloplasmin**) is a ferroxidase enzyme that in humans is encoded by the *CP* gene. Ceruloplasmin is the major copper-carrying protein in the blood, and in addition plays a role in iron metabolism. It was first described in 1948. Another protein, hephaestin, is noted for its homology to ceruloplasmin, and also participates in iron and probably copper metabolism.

Ceruloplasmin is an enzyme synthesized in the liver containing 6 atoms of copper in its structure. Ceruloplasmin carries more than 95% of the total copper in healthy human plasma. The rest is accounted for by macroglobulins. Ceruloplasmin exhibits a copper-dependent oxidase activity, which is associated with possible oxidation of Fe^{2+} (ferrous iron) into Fe^{3+} (ferric iron), therefore assisting in its transport in the plasma in association with transferrin, which can carry iron only in the ferric state. The molecular weight of human ceruloplasmin is reported to be 151kDa.

Like any other plasma protein, levels drop in patients with hepatic disease due to reduced synthesizing capabilities.

Ceruloplasmin is blue coloured alpha-2 globulin and constitutes about 0.5% of total plasma protein. It is multicopper serum metalloenzyme with very strong absorbance at 610 mm (Homberg and Laurell, 1948). At a moleoular weight of 132,000 it contains 5 copper atoms per molecule and serves as an indispensable protein for copper transport and incorporation of ferrous iron into apoferritin.

In 1975 Goswami and Bhattacharya carried out studies on serum copper and ceruloplasmin level in liver diseases. In their series of cases ceruloplasmin was high and there was significant correlation between the severity of liver function and the level of ceruloplasmin. They proposed that high values of serum ceruloplasmin alongwith abnormal liver function tests might help in the diagnosis of liver diseases.

In the light of the conflicting reports, the present work "Significance of serum ceruloplasmin activity in viral hepatitis" aims at exploring the possibilities of:-

The serum ceruloplasmin activity in the diagnosis of viral hepatitis, measurement of hepatic dysfunction and hepatocellular damage, help in assessing the prognosis of viral hepatitis, relation with other liver function tests like serum bilirubin,

alkaline phosphatase and SGPT with ceruloplasmin.

Materials and Methods

The present study was conducted in the Department of Pathology, and with the help of Department of Microbiology, Medicine and Paediatrics, Sri Krishna Medical College, Muzaffarpur during the period of October 2017 to March 2018. A total of 60 cases of viral hepatitis diagnosed on the basis of classical clinical symptomatology, Routine Laboratory investigations and Liver function test (ceruloplasmin, serum Bilirubin, Alkaline Phosphatase and SGPT), Were selected from the different units of inpatient medical ward. The control group consisted of 30 healthy individuals of both sexes were included without any evidence of liver disease and female taking OCP were not included. Samples were collected, Estimation of serum ceruloplasmin were done by modified methods of Ravin by ELISA reader and LFT was done on automated system supplied by Roche Pharma.

Principle

The test is based on enzymatic oxidation of p-phenylenediamine dihydrochloride to a lavender coloured compound. The rate of formation of colour product is directly proportional to concentration of the enzymes over a wide range. The concentration of the enzyme is estimated from optical density after one hour incubation. A conversion factor to change optical density to milligram of the enzyme per 100 millilitre has been determined.

Reagents

(1) P-phenylenediamine dihydrochloride 0.5% solution- p-phenylenediamine dihydrochloride minimum amount of hot distilled water was dissolved in and decolourised with activated charcoal, filtered while hot and crystallized. The purified crystal was kept in sealed vials and before each experiment 0.5% fresh solution was prepared. (2)Acetate buffer, 0.4 M, pH-5.5 About 12 ml of M-acetic acid was added to 200 ml of M-sodium acetate to adjust the pH at 5.5. It was stored at 40C. (3) Sodium fluoride-2% aqueous solution.

Procedure

0.1 ml of serum preferably fresh and free from haemolysis and turbidity was measured into three 15 ml test tubes. One test tube was marked as control and the other two tubes were labeled as Test-1 and Test-II. 1 ml of 2% aqueous solution of sodium fluoride was added into the test tube marked as control. Now 8 ml of acetate buffer was added to all the three test tubes. Then 1 ml of 0.5% aqueous solution of p-Phenylenediamine hydrochloride was added to each test tube in the same order of as acetate buffer was added. The tubes were well shaken to mix all the reagents properly. Now all the test tubes were arranged on a rack and kept in water bath at 370C for one hour. After one hour of incubation tubes were removed from the water bath and 1 ml of 2% sodium fluoride was added to both test tubes marked as Test- 1 and Test-II. All the tubes were shaken well to ensure thorough mixing and kept in the refrigerator at 40C to 100C for 30 minutes exactly.

At the end of 30 minutes tubes were taken out and optical density was measured at 530 nm or using a yellow green filter with control tube as blank without any delay. Normal sera give a final colour which was dark lavender and most sera fall between an optical density of 0.2 to 0.7. Solution with high readings were diluted with buffer solution and readings were corrected on the basis of dilutions made.

Calculation

Average of absorbance (O.D.) of unknown solution-Absorbance of blank x87.5=mg of ceruloplasmin/ 100 ml of serum.

Results

Out of 60 patients, 43 (71.66%) were males and 17 (28.33%) were females. Male to female ratio was 2.52:1. Out of 30 individuals of control series, 19 cases (63.33%) were male and 11(36.67%) cases were female. The value of serum

ceruloplasmin obtained in the control group was 18.3 to 36.2 mg/100 ml. (Mean 25.09 + 4.16 mg/100ml).

It was observed that serum ceruloplasmin in viral hepatitis was 40.25mg/100ml to 70mg/100 ml (mean value 55.87 + 8.22 mg/100 ml). The rise in ceruloplasmin value was highly significant when compared with the control value.

The normal ceruloplasmin level shows slight variation with sex. In 19 males the mean value was 25.98 + 4.22 mg/100 ml. In 11 females the range was 18.3 to 30.0 mg/100 ml (mean 23.55 + 3.73). In viral hepatitis mean ceruloplasmin level in 43 male were 55.72 + 8.22 and in 17 female mean value was 56.23 + 8.14mg/100mg. The value was insignificant.

The normal serum ceruloplasmin showed insignificant variation with age.

It was observed that mean serum ceruloplasmin value were different in different weeks. The value were 65.50 + 2.68, 54.81 + 2.82 and 45.95 + 2.80 mg/100 ml in different weeks respectively. It was evident that maximum rise occurred in the first week and values gradually started falling in subsequent weeks. The mean values were highly significant.

It was observed that serum bilirubin has no relation with the elevation of serum ceruloplasmin and it was insignificant.

Positive correlation was found with serum alkaline phosphtase. The mean value of serum ceruplasmin were 46.87 + 3.70, 56.91 + 5.22 and 66.49 + 2.48 IU/L in 18 patients, 30 patients and 12 patients respectively. When compared with each other in relation to S. alkaline Phosphatase it was found highly significant and bearing a direct relationship with serum ceruloplasmin level.

On correlating value of SGPT with serum ceruloplasmin level, mean value of 56.55 + 9.0, 54.21 + 8.45 and 50.85 + 6.02 mg/100ml were found in 20 patients, 28 patients and 12 patients respectively on comparison of different number of patients with each other observation were non significant.

Table 1 Showing the range, mean, standard deviation and standard error of mean of Ceruloplasmin level in 30 normal cases of control series

Sex group	No. of cases	Ceruloplasmin level in mg/ 100ml				't' value	'p' value	Remark
		Range	Mean	±SD	±SEM			
Male	19	20-36.2	25.98	4.22	0.97			
Female	11	18.3-30	23.55	3.73	1.12			
Both Sexes	30	18.3-36.2	25.09	4.16	0.76	1.59	>0.05	Insignificant

Table 1 shows that the mean value of serum ceruloplasmin in both sexes was found to be 25.09±4.16 mg% with a range of 18.3 to 36.2 mg%. In 19 male cases the mean value of serum ceruloplasmin was 25.98±4.22 mg% with a range of 20 to 36.2 mg%. In 11 female cases the mean value of serum ceruloplasmin was 23.55±3.73 mg% with a range of 18.3 to 40 mg%. The ceruloplasmin value in normal females was slightly lower than the male group and was statistically insignificant (`p'>0.05).

Histogram showing serum ceruloplasmin in control group

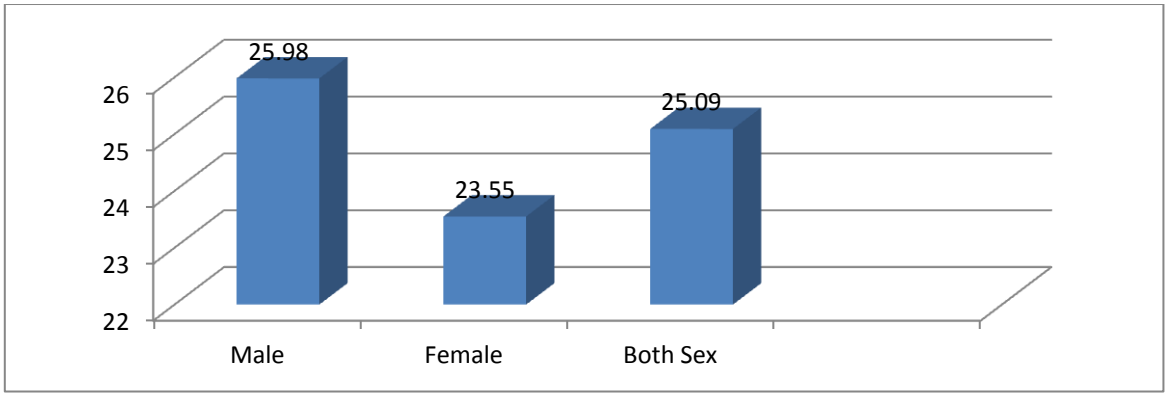


Table – 2 Showing the range, mean, SD and SEM of serum ceruloplasmin, serum bilirubin, alkaline phosphatase and SGPT in control series of cases

	Range	Mean	±SD	±SEM
Serum ceruloplasmin in mg / 100ml	18.3-36.2	25.09	4.16	0.76
Serum bilirubin in mg / 100ml	0.2-0.8	0.48	0.20	0.04
Alkaline phosphatase in K.A. unit	4-12	8.7	2.13	0.39
SGPT in IU	12-32	20.47	5.30	0.97

Table – 3 Showing Serum Ceruloplasmin level according to sex in viral hepatitis cases with statistical value

Sex group	No. of cases	Ceruloplasmin level in mg/ 100ml				't' value	'p' value	Remark
		Range	Mean	±SD	±SEM			
Male	43	40.25-70	55-72	8.22	1.06			
Female	17	46.37-69-12	56.23	8.14	1.97			Insignificant
Both Sexes	60	40.25-70	55.87	8.22	1.06	0.23	>0.05	

Table – 4 Showing the range, mean, SD and SEM of serum ceruloplasmin, serum bilirubin, alkaline phosphatase and SGPT in viral hepatitis

	Range	Mean	±SD	±SEM
Serum ceruloplasmin in mg / 100ml	40.25-70	55.87	8.22	1.06
Serum bilirubin in mg / 100ml	3-15	7.83	2.84	0.37
Alkaline phosphatase in K.A. unit	14-36	24.93	6.15	7.75
SGPT in IU	42.170	98.37	35.90	4.63

Table – 5 Comparative statistical analysis showing range, mean, SD, SEM and 'p' value of serum cerulopasmin in viral hepatitis and control group

Group	No. of cases	Range	Mean	±SD	±SEM	'p' value	Significance comment
Study	60	40.25-70	55.87	8.22	1.06		Highly Significant
Control	30	18.3-36.2	25.09	4.16	0.76	>0.001	

On comparison of cerulopasmin level in viral hepatitis with control, it was found statistically highly significant.

Pie Chart Showing Serum ceruloplasmin level in study and control group

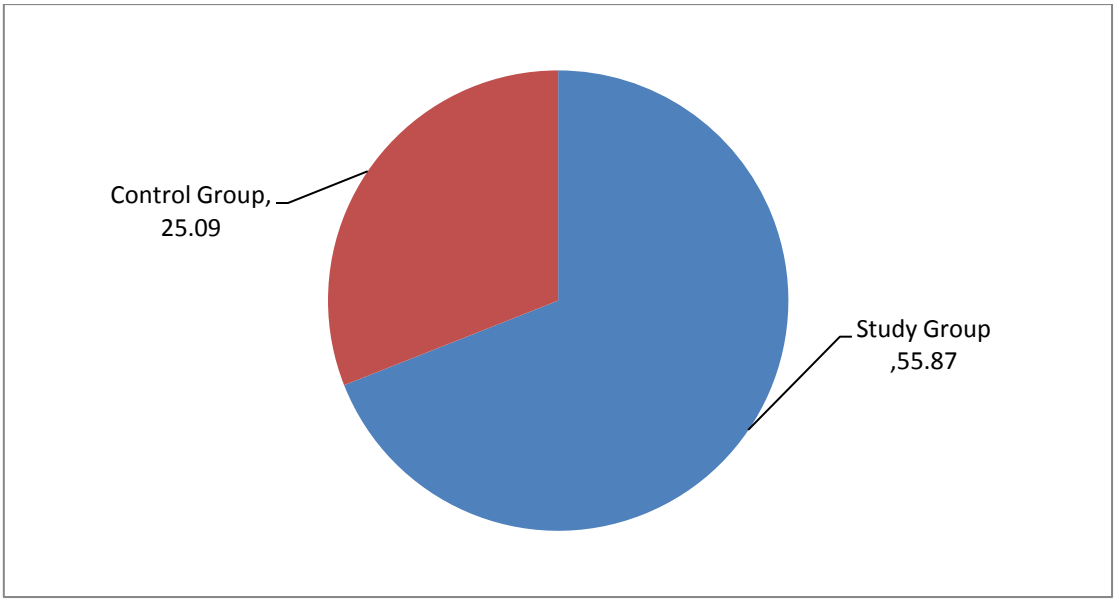


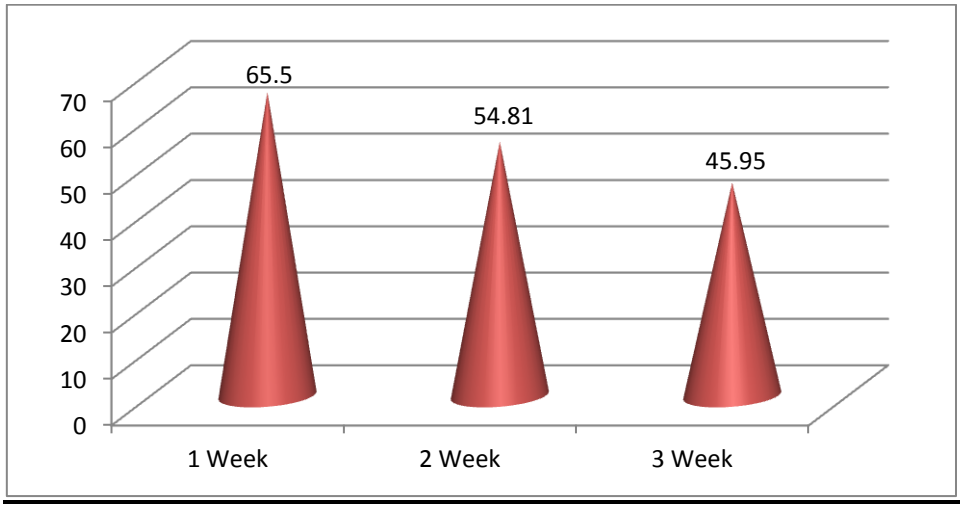
Table – 6 Serum ceruloplasmin in viral hepatitis showing relation with duration of jaundice

Duration of Jaundice	No. of cases	Serum ceruloplasmin in mg%	
		Mean	±SD
A. 1st week	20	65.50	2.68
B. 2nd week	23	54.81	2.82
C. 3rd week	17	45.95	2.80

Remarks:

1. Individually A,B and C significantly high above the control value ($p<0.001$).
2. Significantly high when compared with each other ($p<0.001$).

Histogram Showing serum ceruloplasmin in viral Hepatitis in relation to duration of jaundice



Duration of Jaundice

Table – 7 Serum ceruloplasmin in viral hepatitis in relation to serum bilirubin

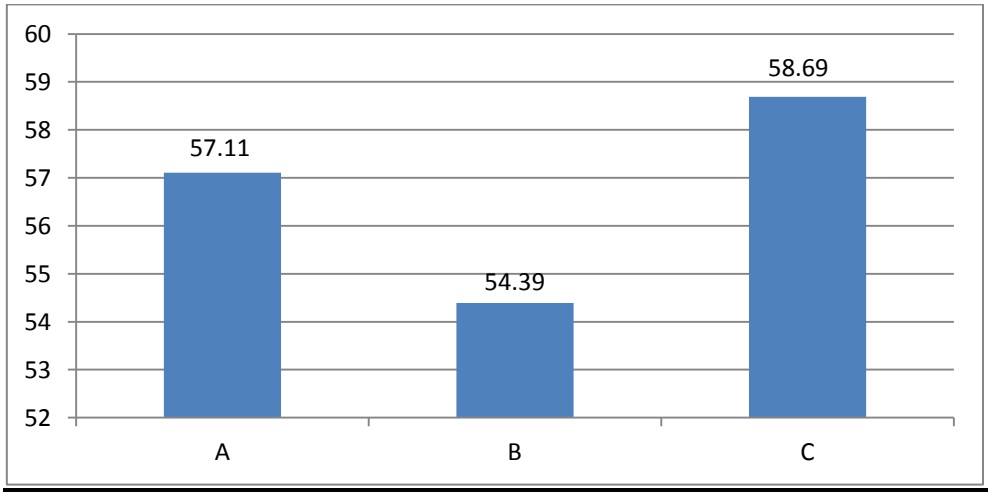
Serum Bilirubin	No. of cases	Serum ceruloplasmin in mg%	
		Mean	±SD
A. 2.0-5.0	16	57.11	8.97
B. 5.1-10.0	34	54.39	8.25
C. >10	10	58.59	5.91

Remarks

1.Individually A,B and C significantly high above the control value ($p<0.05$).

2.The difference among A, B and C are not significant statistically ($p>0.05$).

Histogram Showing serum ceruloplasmin level in Viral Hepatitis in Relation to Serum Bilirubin



Serum Bilirubin in mg%

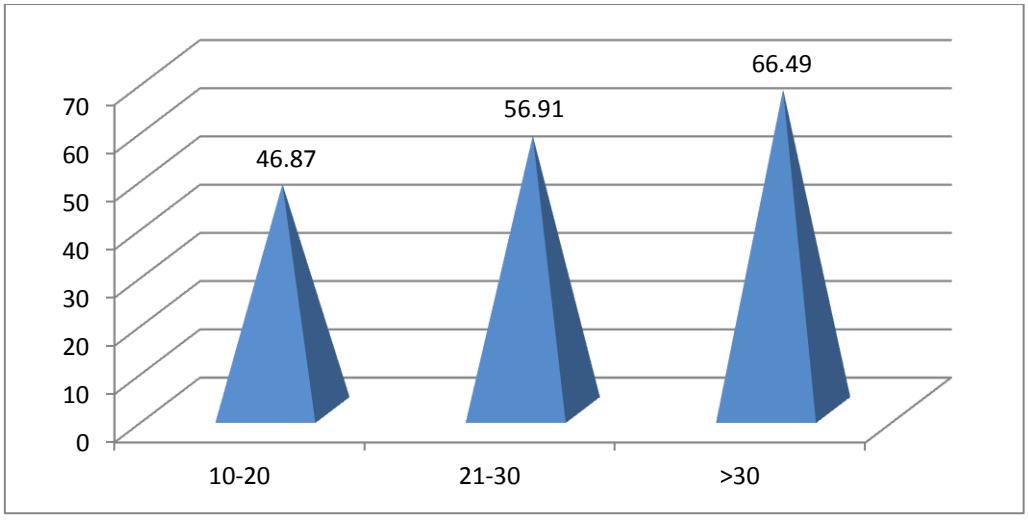
Table – 8 Serum ceruloplasmin in viral hepatitis in relation to serum Alkaline phosphatase

Serum Alkaline phosphatase	No. of cases	Serum ceruloplasmin in mg%	
		Mean	±SD
A. 10-20	18	46.87	3.70
B. 20-30	30	56.91	5.22
C. >30	12	66.49	2.48

Remarks:

1. Individually A,B and C significantly high above the control value ($p<0.05$).
2. The difference among A, B and C are not significant statistically ($p>0.05$).

Histogram Showing serum ceruloplasmin level in Viral Hepatitis in Relation to Serum Alkaline phosphatase



Serum Alkaline phosphatase (K.A. Units)

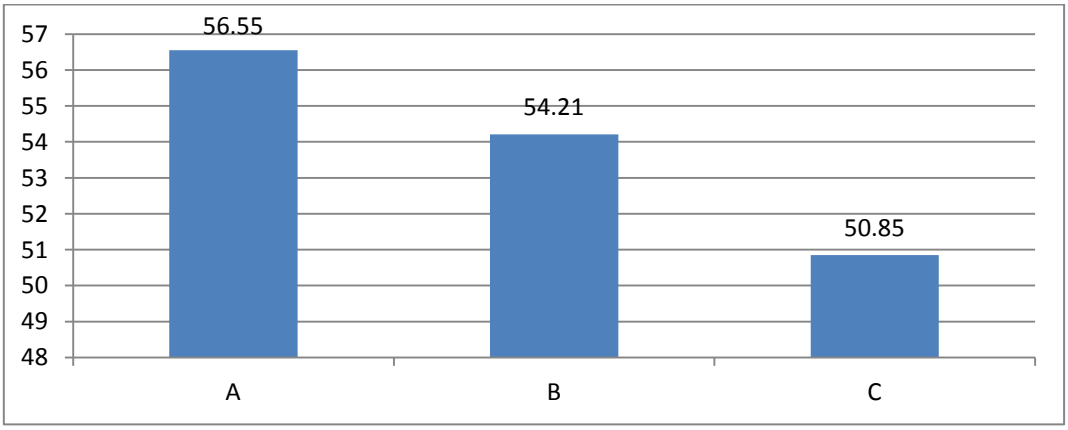
Table – 9 Serum ceruloplasmin in viral hepatitis in relation to serum SGPT

SGPT (in IU)	No. of cases	Serum ceruloplasmin in mg%	
		Mean	±SD
A. 40-80	20	56.55	9.0
B. 80-120	28	54.21	8.45
C. 120 and above	12	50.85	6.02

Remarks :

1. Individually A, B and C significantly high above the control value ($p<0.05$).
2. No. significant different among A, B and C ($p>0.05$)

Histogram Showing serum ceruloplasmin level in Viral Hepatitis in Relation to SGPT



S.G.P.T (in I.U.)

Discussion

An attempt was made to correlate serum ceruloplasmin level with viral hepatitis as evidenced by clinical findings and various liver function tests with ultimate aim in view whether serum ceruloplasmin has any value in diagnosis or in prognosis or in assessing the hepatic dysfunction and hepatocellular damage in viral hepatitis.

30 healthy normal cases served as the control group. There were 19 males (63.33%) and 11 females (36.67%) in this group. The value of ceruloplasmin obtained in the control group was 18.3 to 36.2 mg per 100 ml with a mean value of 25.09 + 4.16 mg per 100 ml. The values of serum ceruloplasmin obtained in the control group corresponds well with the values obtained by various workers- (Putamen, 30-35 mg%; Ravin, 17.6-47 mg%; - Walshe and Briggs, 19-49 mg%; Gault & Aronoff, 24.4-47 mg%; Goswami and Bhattacharya, 30.4 mg% and Hasan et al, 24.3-39.9 mg%).

The incidence of viral hepatitis was found to be higher in males as compared to females. The overall higher incidence in male patients could well be due to the fact that they were more exposed to infection due to their field work and because of the fact that more males seek admission to the hospital than females who are paid less attention and care in our society.

It was observed that serum ceruloplasmin in viral hepatitis was 40.25 to 70 mg per 100 ml with a mean value of 55.87 + 8.22 mg per 100 ml. The rise in ceruloplasmin value was highly significant when compared with the control value. The present observation of increased serum ceruloplasmin in cases of viral hepatitis was in tune with the findings of the following workers:- Pineda et al (1962) – Who found raised level of ceruloplasmin in 4 cases out of 11 cases of acute viral hepatitis. Gault et al (1966) – Who showed raised level of ceruloplasmin in 9 cases, normal in 4 and reduced in 1 case out of 14 cases of viral hepatitis. Goswami and Bhattacharya (1974) – Who found significantly high level in all the 20 cases of viral hepatitis. Hassan et al (1985) – Who found high level of ceruloplasmin in 64 cases of infective hepatitis out of 68 cases they studies.

These cases belonged to various stages of the disease. On separating these cases according to the duration of appearance of clinical jaundice it was observed that mean serum ceruloplasmin value were different in different weeks. The values were 65.50 + 2.68, 54.81 + 2.82 and 45.95 + 2.80 mg per 100 ml in 20 patients, 23 patients and 17 patients respectively. It was evident that maximum rise occurred in the first week and the values gradually started falling in subsequent weeks. The mean values of these groups were found highly significant when compared with each other statistically. This finding was in confirmity with the observation of following workers:- Hauftova et al (1967) noted that high values of serum ceruloplasmin returns to normal after 5 weeks. Hasan et al (1985) reported that maximum rise occurs in 1st week which falls with duration of jaundice.

The present observation was also a strong evidence in favour of the cause of rise of serum ceruloplasmin in viral hepatitis which may be due to its acute phase reactant activity to which partial contribution occurs by biliary obstruction.

Conclusion

High serum ceruloplasmin value was confirmed in viral hepatitis. The rise of serum ceruloplasmin was maximum in first week than in the subsequent weeks. There was no direct correlation with serum bilirubin and SGPT with the rise in the ceruloplasmin value. However, a positive correlation was found to exist with alkaline phosphatase. The possible cause of rise of serum ceruloplasmin in viral hepatitis may be due to its acute phase reactant activity. Since the ceruloplasmin was believed to be excreted through bile, it rises along with alkaline phosphatase possibly because of biliary obstruction in viral hepatitis.

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