Study of serum amylase in patients with infective hepatitis and its comparison in patients without infective hepatitis

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Abstract
Introduction: Patients with infective hepatitis are faced with danger of pancreas getting affected. This can be reflected as increased serum amylase values.

Objective: To study serum amylase in patients with infective hepatitis.

Materials and Methods: Present study was cross sectional study. Two groups of patients were compared. One group was with infective hepatitis and the other group was without infective hepatitis. This was a hospital based comparative study. Serum amylase was determined using modified Huggins and Russel method.

Results: The mean serum amylase value was 70.4 units among cases and it was 22.8 among the controls. The difference in the values was found to be statistically significant. It was found that the mean serum amylase value was 22.8 in the age group of 20-34 years which slightly increased in the age group of 35-49 years and again decreased slightly above the age of 50 years. Hence the difference was not found to be statistically significant.

Conclusion: There is a significant increase in the serum amylase levels among patients with infective hepatitis.

Keywords: Amylase, Hepatitis, Comparison, Cases, Controls.

Introduction
Amylases are a group of enzymes that split complex carbohydrates constituted of α-D glucose units linked through carbon atoms 1 and 4 located on adjacent glucose residues.1

Two types of amylases are recognized. Beta amylase or exo amylase e.g. plant and bacterial amylase. This enzyme acts upon glucosidic linkages only at the terminal end of polysaccharide chain. Alpha amylases or endo amylases e.g. animal amylases including those present in humans. This enzyme acts upon alpha 1-4 glucosidic linkages randomly anywhere along the polysaccharide chain. Since the first recognition of the presence of amylase in blood by Magendie in the year, its value has been increasingly recognized in various clinical disorders. Though normally the blood level of the enzyme varies widely but still the alteration of its level has been shown to be of immense clinical significance.2

In the human body, amylase is present in number of organs and tissues. Various sources being pancreas, salivary glands, ovaries, fallopian tubes, lungs, testes, semen, striated muscle, liver, adipose tissue, colostrums, tears and milk. Liver is a major if not only the source of amylase found in the serum under normal physiologic conditions. Serum amylase level increases in hepatitis, pancreatitis, perforated peptic ulcers, peritonitis, and ruptured ectopic pregnancy etc.3

Liver and pancreas are very close to each other. Both the organs are connected via various ducts, blood vessels. Hence if one is diseased, the chances of other getting damaged are more. It has been said that in the pancreas disease, there is obstruction of the bile flow and it can lead to liver disease. But data and literature on whether in liver disease there is disturbance in the function of the pancreas is not clear. Various reports have suggested that patients who developed acute hepatitis are at risk of developing acute pancreatitis.4 5

In cases with liver disease of chronic nature, exocrine function of the pancreas gets affected. Serum amylase value increases in such cases.6 But one such study has reported the reports on the other way. That is the report says that enzymes of the pancreases are elevated only in liver disease of chronic nature. Hence it is not clear whether pancreas is affected or not in liver disease of chronic nature.7

It was since 1944 known that there is association between viral hepatitis and acute pancreatitis. It is well recognized that fulminant viral hepatitis gets complicated in acute pancreatitis. But there are few reports on the fact that non fulminant hepatitis leads to acute pancreatitis. Hepatitis A and B are known to cause but hepatitis E related cases are also reported. Once the patient develops viral hepatitis, there is chance of developing the acute pancreatitis 2-3 weeks after.8

The present study is an attempt to know the variations of serum amylase level in acute infective hepatitis, which is a common systemic disease affecting the liver predominantly and also affecting other organs to some extent.

Materials and Methods
Present study was cross sectional study. Two groups of patients were compared. One group was with infective hepatitis and the other group was without infective hepatitis.
This was a hospital based comparative study. These two groups were compared in terms of serum amylase levels to know the effect of infective hepatitis on the function of pancreas. JJM Medical College and hospital was the study place for the present study. Over a period of one year from January 2002 to December 2002 it was possible to complete the present study.

Ethical approval from the Institutional Ethics Committee was obtained. Informed consent was obtained from all study subjects who were willing to participate in the present study.

Out of 112 study subjects, 69 were found to have infective hepatitis and they were grouped in one group. 43 were normal healthy and they were grouped as controls.

Only adults in the age group of 21-60 years were included in the present study. Those who do not gave their consent, were excluded from the study. Cases were those with infective hepatitis and controls were those who do not have infective hepatitis.

Serum amylase was determined using modified Huggins and Russel method.²

**Principle:** The diminution in blue color produced when serum starch substrate mixture taken before and after digestion are added to iodine is measured at 680 nm.

**Reagents**

1. Phosphate buffer: 1.735 gm of disodium hydrogen orthophosphate and 1.009 gm of potassium dihydrogen phosphate in a liter of solution.
2. Starch 0.5% solution prepared freshly once in 2-3 days,
3. Iodine reagent: 0.1 N containing 0.3% K. I. dissolve 30 gm of K. I in 250 ml water weigh 13 gm of iodine in a weighing bottle and transfer quantitatively to a 1 liter volumetric flask with the iodine solution. Shake well to dissolve and make to the mark with water. Standardize against thiosulphate.
4. Iodine solution: prepared from the iodine reagent by diluting 1-10 with water.

**Procedure:** Measure 5 ml of phosphate buffer and 4 ml of 0.5% starch solution into a test tube (or 9 ml of mixture of 5 volume of phosphate buffer and 4 volume of 0.5% starch solution) and bring to 37°C. Add 1 ml of serum diluted (1-5 with 0.9% NaCl). Mix and remove immediately 0.5 ml into a 25 ml stoppered measuring cylinder containing about 20 ml of water and 1 ml of 0.01 N iodine (control). Note the time and replace the serum starch mixture into the water bath at 37°C.

At the end of 15 min remove 0.5 ml into 25 ml stoppered measuring cylinder containing about 20 ml of water and 1 ml of 0.01 N iodine (test). Make both the measuring cylinders up to the 25 ml mark with water. Then read optical density at 680 nm or using a red filter against water as blank.

**Calculation:** Units of amylase activity per ml serum. (Starch in 0.5 ml of control – mg starch in 0.5 ml of unknown) x 100.

**Statistical Analysis**

The data was expressed as mean values of serum amylase in cases and controls. Difference was tested using t test. Significance was known by p value of < 0.05.

**Results**

Table 1 shows age distribution of cases and controls. Overall the study subjects in the age group form 21-50 years were almost equally distributed but after the age of 50 years, there were only 16.1% of the study subjects. Age wise comparison of cases and controls also showed the similar trend across all age groups. Numerically there was not much difference in the age wise distribution between cases and controls.

Table 2 shows comparison of mean serum amylase value between cases and controls. The mean serum amylase value was 70.4 units among cases and it was 22.8 among the controls. The difference in the values was found to be statistically significant.

Table 3 shows comparison of mean serum amylase value according to age in controls. It was found that the mean serum amylase value was 70.4 units among cases and it was 22.8 among the controls. The difference in the values was found to be statistically significant.

Table 1: Age distribution of cases and controls

| Age (years) | Controls | Cases | Total |
|-------------|----------|-------|-------|
|             | Number   | %     | Number | %     |
| 21-30       | 14       | 42.4  | 19     | 57.6  |
| 31-40       | 08       | 27.6  | 21     | 72.4  |
| 41-50       | 11       | 34.4  | 21     | 65.6  |
| > 50        | 10       | 55.6  | 08     | 44.4  |
| Total       | 43       | 38.4  | 69     | 61.6  |

Table 2: Comparison of mean serum amylase value between cases and controls

| Groups | Serum amylase | P value |
|--------|---------------|---------|
|        | Mean ±2SD     |         |
| Controls | 22.8 ± 5.7 | < 0.0001 |
| Cases   | 70.4 ± 23.5 |         |

Table 3: Comparison of mean serum amylase value according to age in controls

| Age (years) | Serum amylase |
|-------------|---------------|
|             | Mean ±2SD     |
| 21-30       | 70.4 ± 23.5   |
| 31-40       | 70.4 ± 23.5   |
| 41-50       | 70.4 ± 23.5   |
| > 50        | 70.4 ± 23.5   |
Table 3: Comparison of mean serum amylase value according to age in controls

| Groups | Age (years) | Number | Serum amylase Mean ±2SD |
|--------|-------------|--------|-------------------------|
| Controls | 20-34       | 19     | 22.8 ± 5.6              |
|         | 35-49       | 14     | 23 ± 55                 |
|         | > 50        | 10     | 22.6 ± 6.9              |
| P value |             |        | 0.99 Not significant    |

Table 4 shows comparison of mean serum amylase value according to sex in controls. The mean value of serum amylase was 22.6 in males. It was slightly higher i.e. 23 in females. Hence the difference was not found to be statistically significant.

Table 4: Comparison of mean serum amylase value according to sex in controls

| Groups | Sex | Number | Serum amylase Mean ±2SD |
|--------|-----|--------|-------------------------|
| Controls | Male | 20     | 22.6 ± 6.1              |
|         | Female | 23     | 23 ± 5.5                |
| P value |       | 0.80   | Not significant         |

Table 5 shows comparison of mean serum amylase value according to sex in cases. The mean value of serum amylase was 67.98 in males and it was higher in females i.e. 78.2. But this difference was not found to be statistically significant.

Table 5: Comparison of mean serum amylase value according to sex in cases

| Groups | Sex | Number | Serum amylase Mean ±2SD |
|--------|-----|--------|-------------------------|
| Cases | Male | 52     | 67.98 ± 24.5            |
|         | Female | 17     | 78.2 ± 18.4            |
| P value |       | 0.12   | Not significant         |

**Discussion**

In the present study we found that the mean serum amylase value was 70.4 units among cases and it was 22.8 among the controls. The difference in the values was found to be statistically significant.

Lechi A et al\textsuperscript{10} studied 167 patients who were having acute viral hepatitis. They investigated patients for serum as well as urinary amylase. They observed that there was significant increase in serum amylase in all groups of patients. This finding was in accordance with finding of the present study. Hepatitis A patients were showing excretion of amylase in the urine more significantly than any other groups of patients in their study. The authors concluded that in cases of acute viral hepatitis the chances of pancreatic injury is not uncommon but it may not be so severe.

Singhal M et al\textsuperscript{11} in their study investigated 20 cases of viral hepatitis and 10 controls that were healthy for serum amylase and HSF. They found that the cases of viral hepatitis exhibited very high levels of serum amylase which was consistent with the present study findings. HSF was also found to be significantly elevated. Thus the authors concluded that there is not only diagnostic importance of these markers but also prognosis can be predicted.

Katakura Y et al\textsuperscript{12} observed that amylase from serum, saliva, and pancreas and also the serum lipase levels were more in patients with hepatitis than the controls that were healthy. This difference they found that was statistically significant. This finding is in accordance with the present study finding. The authors based on their study findings concluded that as the liver disease progresses, the levels of enzymes of the pancreas also increase.

Jain A et al\textsuperscript{13} studied 124 cases of acute viral hepatitis. Among them 24 had severe pain in the abdomen. The incidence of acute pancreatitis was 5.65%. Out of these seven patients, four had concurrent hepatitis E, two had hepatitis A and one had hepatitis B. The authors concluded that acute pancreatitis do occur in cases who first developed acute viral hepatitis but it is mild and can be easily managed conservatively.

Raj M et al\textsuperscript{14} studied 790 patients with acute pancreatitis. Out of them 16 (2.1%) were found to have hepatitis E. Two were found to have hepatitis A and hepatitis B was seen in one patient. They concluded that there is association between acute pancreatitis and hepatitis E. This association is not uncommon but this condition would have a very good prognosis.

**Conclusion**

There is a significant increase in the serum amylase levels values among patients with infective hepatitis. This may be due to hepatocellular damage. Serum amylase values can indicate liver as well as pancreas damage.

**Conflict of Interest:** None.

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