SPECIAL ARTICLE

THE ROLE OF SERUM ALDOLASE ACTIVITY IN THE DIAGNOSIS OF HEPATIC DISEASE

I. Ethem Erinç, M.D.*

Aldolase was discovered in 1934 by Meyerhof and Lohman; later Warburg and Christian isolated this enzyme in crystallized form from muscle and yeasts. Aldolase catalyzes the splitting of fructose 1, 6 - diphosphate into two molecules of acid triosephosphate (dihydroxyacetone phosphate and glyceraldehyde phosphate). They are then transferred to the pyruvic acid cycle.

Since this enzyme was isolated, aldolase has been shown to be present in all tissue : particularly in skeletal and cardiac muscle, but also in skin, liver, kidney, brain, and cerebrospinal fluid. These findings are obtained from animal as well as human tissue.

Aldolase has also been found in blood serum, although in small quantities. Rymenant and Tagnon have recently demonstrated that serum aldolase levels are subject to wide fluctuations.[1] For instance, in hemoconcentration caused by hemorrhage the serum aldolase levels are increased; in hemodilution secondary to parenteral fluid therapy they are decreased. Serum aldolase activity is elevated in various diseases,[2-6] particularly in hepatic disorders: hepatitis, carbon tetrachloride poisoning, infectious mononucleosis, obstructive jaundice, cirrhosis, metastatic tumors of the liver, and all pathological conditions which result in hepatic necrosis.

The following conditions are also known to have elevated aldolase activity: severe hemolytic anemia, megaloblastic anemia, leukemia, pneumonia, myocardial infarction, acute pancreatitis, pulmonary infarction, diseases associated with skeletal muscle necrosis, muscular dystrophies, myxedema, and peripheral gangrene. Other factors, such as foods, stress, age, sex, have been shown not to influence the levels.[2]

From Hacettepe Medical School, Ankara University.

* Department of Physiology.

* All the reagents were obtained from C. F. Boehringer's «Biochemica test combination» and prepared according to the accompanying instructions.

The relationship between serum aldolase activity and hepatic disease has been studied by several authors. However, the attempts at using the measurement of aldolase as a diagnostic tool in hepatic disease have not given conclusive results,[1, 8-13] and we have therefore studied serum aldolase activity in a series of liver diseases.

Methods

The method we used for determination of serum aldolase activity is identical to that described by Bruns.[8, 9, 11] The reagents included: 1. collidine (2, 4, 6, trimethyl-pyridine); 2. sodium salt of fructose 1, 6 - diphosphoric acid; 3. hydrazine sulfate; and 4. 2, 4, dinitrophenol hydrazine. Trichloracetic acid and sodium hydroxide were also needed.* Fresh and nonhemolyzed blood was used. One control determination was run for each serum aldolase determination.

For each determination two test tubes were used. Into one of these, two ml of collidine and one ml of patient serum were pipetted. The second tube comprised the control to which two ml of collidine were added. Both tubes were then stirred mildly and incubated for one hour in a water bath at 37 C. Two ml of trichloracetic acid were next added to the sample tube and two ml of trichloracetic acid and one ml of serum to the control tube.

The precipitate was then filtered and afterwards both test and control tubes underwent the same procedures: two ml of 1:1 mixture of the deproteinized filtrate and sodium hydroxide were added to each tube. The tubes were left at room temperature for 10 minutes, and one ml of chromogen was added to each. Then sodium hydroxide was added to both tubes to make the volume 10 ml. After leaving both tubes for 10 minutes at room temperature, they were placed in a spectrophotometer and, using the control tube for comparison, the measurements were carried out at a wave length of 350 millimicron.

Among the several ways of expressing the aldolase level,[3, 7] we preferred that of Bruns,[8, 9, 11] because we followed his method.

Aldolase activity is indicated in aldolase units. One unit of aldolase is the quantity of fructose 1, 6 - diphosphate which is decomposed in one hour by one ml of serum into glyceraldehyde phosphate and dihydroxyacetone phosphate and is expressed in cubic millimeters. According to Bruns, normal levels are within four to eight units.

Material

In order to compare our results with those in the literature, 10 normal controls were examined. Their serum aldolase activities ranged between 2.8 and 6.5 with an average of 4.9 units.

Our subjects were selected from inpatients with various hepatic diseases. They consisted of 17 male and two female patients. Ten of these 19 patients had cirrhosis and two had Wilson's disease (Table 1); six patients had viral hepatitis (Table 2); and three had obstructive jaundice, one case caused by a stone in the common bile duct, the other two caused by carcinoma of the pancreas (Table 3).

The following laboratory examinations were carried out routinely in all patients: urinalysis (for bilirubin, urobilin and urobilinogen), serum bilirubin (total, direct and indirect), serum protein electrophoresis, hepatic flocculation tests (formol-gel, thymol, zinc sulfate and cadmium sulfate turbidities). Some patients had levulose tolerance tests and serum alkaline phosphatase determinations. The conventional laboratory methods were used for these tests.[4, 6] Results of laboratory findings together with aldolase activity are shown in Tables 1, 2 and 3. Serum protein electrophoresis studies were found to be within normal limits.

Results and Discussion

The enzyme activity in three groups of patients was as follows:

1. *Patients with cirrhosis*: serum aldolase activity in these patients varied between 5.6 and 20.2 (with an average of 9.5 units). Only two cases showed remarkably increased values, two were slightly higher than normal, and six were within normal limits. It can be seen from Table 1 that the majority of cirrhotic patients (either compensated or decompensated) shows practically no increase in serum aldolase activity.

Rymenant and Tagnon found increased serum aldolase activity in only ten per cent of patients with cirrhosis of the liver.[1] Métais and Turner reported somethat higher levels in their cirrhotic patients, but the levels were never more than 20 units.[10] Bruns reported only three cases with slightly elevated levels among 17 patients.[8, 9]

2. *Patients with viral hepatitis*: serum aldolase activity of these patients varied between 10.8 - 40.3 units with an average of 20.9 units. All patients had their serum aldolase activities checked

TABLE 1

PATIENTS WITH CIRRHOSIS OF THE LIVER

Diagnosis	Urinary			Serum bilirubin (% mg)		Hepatic flocculation tests				Levulose tolerance test (mg)			Other tests	Serum aldolase (Bruns' units)
	Bilirubin	Urobilin	Urobilinogen	Direct	Total	Formolgel	Thymol turbidity (units)	Zn SO4 (units)	Cd SO4 (units)	1hr.	2hr.	3hr.		
Portal cirrhosis	+	++	+	0.1	0.9	—	34	32	++				Prothrombin time 27 sec. (Cont. 16 sec.)	7.8
" "	—	—	—	0.2	1.6	—	26	20	—	8	7.5	1		7.6
" "	+	+	—	1.0	1.8	—	28	20	+	13.2	10.3	7	Prothrombin time. 20 sec. (Cont. 16 sec.)	7.2
" "	+	—	—	0.2	1.6	—	40	15	+	25	20	4.5		20.2
" "	—	++	+	0.5	2.2	+	32	13	+	18.2	12	9		16.2
" "	—	++	+	0.1	0.7	+	42	60	+	17	16	15		6.5
" "	+	+++	++	1.0	1.9	—	31	32	+					8.4
Cholangiilitic cirrhosis	+	++	++	2.3	3.2	—	50	58	+					5.6
Wilson's dis.	—	—	—	0.1	0.8	—	33	30	+	32	21	12		8.6
" "	—	—	—	0.2	0.6	—	30	26	+	15	13	0		6.8

during the icteric phase. In one patient, whose serum aldolase level was also examined during the convalescent phase, it was seen that the aldolase activity was decreased along with an improvement in liver function tests (Table 2).

In 300 cases of viral hepatitis studied, Bruns found only one case with a normal serum aldolase activity. In all cases the levels were at least three or four times normal.[9] He observed that in uncomplicated cases of viral hepatitis, aldolase activity returned to normal levels within three weeks.[9] Rymenant and Tagnon had similar results.[1] Métais and Turner had an average of 27.4 units in 20 patients.[10]

3. *Patients with obstructive jaundice* : serum aldolase activity was elevated in two patients whose jaundice was caused by carcinoma of the head of the pancreas. One patient with a stone in the common bile duct showed normal levels (Table 3).

According to Bruns, aldolase is not excreted via the biliary tract as alkaline phosphatase is. Therefore a marked increase in serum aldolase levels cannot be expected in biliary obstruction. In nine of his 17 cases the aldolase activity was slightly elevated, but it was never higher than 14 units.[8, 9, 11] Rymenant and Tagnon found hyperaldolasemia in only three per cent of their patients.[1] Métais and Turner reported moderately elevated (less than 20 units) levels in eight cases with tumors and seven cases with common bile duct stone.[10] It is possible that some other factors might have been involved in increasing the serum aldolase levels in our two patients with pancreatic carcinoma; otherwise, our findings in patients with cirrhosis, viral hepatitis and obstructive jaundice are compatible with those previously reported in the literature.[1,8-11]

Summary and Conclusions

The serum aldolase activity was determined by the Bruns method in 10 normal subjects and in 19 patients with various hepatic diseases. In normal controls the aldolase activity was found to be 2.8 - 6.5 with an average of 4.9 units. Among 10 cirrhotic patients, six had normal aldolase levels; the levels of the others were within the upper limits of normal; only two patients with cirrhosis had elevated levels.

Increased aldolase activity was found in all of the six patients with viral hepatitis. In one of these patients serum aldolase deter-

TABLE 2
PATIENTS WITH VIRAL HEPATITIS

Diagnosis	Urinary			Serum bilirubin (% mg)		Hepatic flocculation tests				Levulose tolerance test (mg)			Other tests	Serum aldolase (Bruns' units)
	Bilirubin	Urobilin	Urobilinogen	Direct	Total	Formolgel	Thymol turbidity (units)	Zn SO4 (units)	Cd SO4 (units)	1hr.	2hr.	3hr.		
Viral hepatitis	+++	—	±	2	2.4	—	35	15	+					14.2
" "	±	+	++	1.7	2.4	—	41	48	—					10.8
" "	+	+	++	1.9	2.7	+	43	55	+					12.0
" "	+++	++	+++	2.3	3.3	—	40	23	+++					36.5
" "	++	+++	+	3.9	5.1	+	55	20	+++					40.3
" "	+++	+	+	12.5	18.7	+	57	48	+					11.8

TABLE 3

PATIENTS WITH OBSTRUCTIVE JAUNDICE

Diagnosis	Urinary			Serum bilirubin (% mg)		Hepatic flocculation tests				Levulose tolerance test (mg)			Other tests	Serum aldolase (Bruns' units)
	Bilirubin	Urobilin	Urobilinogen	Direct	Total	Formolgel	Thymol turbidity (units)	Zn SO4 (units)	Cd SO4 (units)	1hr.	2hr.	3hr.		
Carcinoma of head of pancreas	+++	++	±	18.7	23.4	—	40	15	+	9	6.8	0	Serum alk. phosphatase 29.7 B.U.	15.5
Common bile duct stone	+	—	+	3.4	5.3	—	27	25	+				Serum alk. phosphatase 5.7 B.U.	7.2
Carcinoma of head of pancreas	—	—	—	2.3	3.3	—	26	35	—	21	20	19	Serum alk. phosphatase 11.4 B.U.	25.9

minations were repeated at the end of the icteric phase or during convalescence and a marked decrease in aldolase activity was observed. It was high in two patients with obstructive jaundice.

We have concluded that the determination of serum aldolase activity is an important diagnostic tool in acute hepatitis, whereas in chronic hepatic disease the activity is increased only during acute attacks associated with parenchymal damage and therefore its diagnostic value is limited.

REFERENCES

1. Rymenant, M. V. and Tagnon, H. J.: Enzymes in clinical medicine, New Eng. J. Med. 261: 1325, 1959.
2. Berkol, S.: [On Aldolase], Türk Tıp Cem. 27: 133, 1961.
3. Bodansky, O.: Diagnostic applications of enzymes in medicine, Am. J. Med. 27: 861, 1959.
4. Page, L. B. and Culver, P. J.: Syllabus of Laboratory Examinations in Clinical Diagnosis, rev. ed., Harvard, 1960.
5. Cantarow, A. and Trumper, M.: Clinical Biochemistry, ed. 6, Philadelphia, W. B. Saunders, 1962.
6. Davidsohn, I. and Wells, B.: Todd-Sanford: Clinical Diagnosis by Laboratory Methods. ed. 13, Philadelphia, W. B. Saunders, 1962.
7. [Determination of the activity of FDP aldolase in the serum], Mannheim, C. F. Boehringer and Son, GmbH.
8. Bruns, F. and Puls, W.: Die activität der serumaldolase bei erkrankungen der leber. Ein neuer enzymatischer test, Klin. Wchnschr. 32: 656, 1954.
9. Bruns, F. and Jacob, W.: Studien über serumenzyme bei ekrankungen der leber. Die activität der phosphohexoseisomerase, aldolase und alkalischen phosphatase, Klin. Wchnschr. 32: 1040, 1954.
10. Métais, P. and Turner, J.: [Aldolasemia. Its variations in various pathological conditions, mainly hepato-biliary diseases and coronary disease], Path. Biol. (Par) 9: 57, 1961.
11. Bruns, F.: Über die aldolase der erythrocyten, Biochem. Ztschr. 325: 429, 1954.
12. Wroblewski, F.: Serum enzyme alterations in diseases of the liver and biliary tract, Med. Clin. N. Amer. 44: 699, 1960.
13. Mulay, A. S.: Tissue and serum aldolase of rats with primary hepatoma, Proc. Soc. Exper. Biol. and Med. 101: 763, 1959.