ENZYME CHANGES IN TWO GROUPS OF JAUNDICED PATIENTS (EXTRAHEPATIC OBSTRUCTION AND VIRAL HEPATITIS) COMPARED

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SYNOPSIS

From 1965 till 1970, a survey was carried out to assess the value of some of the enzyme tests available in the differential diagnosis of jaundice due to two common liver conditions, extrahepatic obstruction (EHO) and viral hepatitis (VH). Seventy-nine patients were studied, of which 41 had EHO and 38 had VH. Analysis of the results showed that no single enzyme test was outstanding in this respect. However, four enzymes, namely serum glutamic oxaloacetic transaminase (SGPT), serum isocitric dehydrogenase (SICD), serum alkaline phosphatase (SAP), and serum leucine aminopeptidase (SLAP) were found to have greater discriminatory value than the others. This capability was well shown when some selection was used. When patients with SICD levels more than 600 u and SLAP less than 400 u were considered, practically all were found to have VH. On the other hand, with patients having more than 30 u for SAP and less than 400 u for SGPT, the opposite was found. This kind of arbitrary enzyme selection was extremely useful in excluding one group of patients, but the separation of patients was not total, because a substantial number of patients from the other group would also be excluded.

A brief review of the literature shows that though the subject of jaundice and its diagnosis still sustains wide interest amongst clinicians and scientists alike, no simple and reliable test has yet been evolved that is accepted by most centres. Mention is made of some unusual tests reported elsewhere which may be of use in the occasional patient, and also of some recent innovations published from centres with special interest in this field.

INTRODUCTION

The differential diagnosis of jaundice is a perennial clinical problem in Singapore, just as it is in most countries. Amongst the hepatobiliary disorders, the commonest causes are viral hepatitis, acute cholecystitis and biliary obstruction due to gallstones. Although typical cases do not usually present any problem in diagnosis, this may not necessarily be true of the less typical examples. The patient with viral hepatitis may present predominantly with cholestatic features, with only minimal gastrointestinal symptoms. On the other hand, extrahepatic obstructive jaundice may manifest with hepatocellular dysfunction as its main feature, masking the usual symptoms of pain, rigors and high fever. The atypical case of viral hepatitis may experience more than the usual dull pain over the liver, which may be accompanied by a pronounced febrile response simulating acute cholecystitis or even obstructive jaundice.

For decades, various tests, from the simple to the very involved, have been devised in an attempt to distinguish between intrahepatic and extrahepatic cholestasis. Many were related directly to liver function, as exemplified by enzyme estimations (Henley 1966, Clermont 1967). Others include the tolerance tests, using various substances such as galactose and ammonium chloride (Giansiracusa 1947, Conn 1960). The estimation of cholesterol esters and the identification of the rheumatoid arthritis factor and various antibodies by immunological means have also been invoked, (Atwater 1963, Doniach 1972). So are the estimations of serum caeruloplasmin and haptoglobin levels, which are abnormally raised in obstructive jaundice (Prellwitz *et al*, 1969). Even the serum iron and blood sugar measurement has been reported as useful in the differential diagnosis of jaundice (Goldstein *et al*, 1958, Bolin 1968, Felig *et al*, 1970). Mention must also be made of the tests involving drug challenge or response, such as the steroid test, the response to vitamin K. and the effect of phenobarbital.

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More complicated procedures include percutaneous liver biopsy, upper gastrointestinal series with or without hypotonic duodenography, percutaneous cholangiography (Mujahed 1966, Yu and Oon 1973), examination of duodenal aspirates for cholesterol crystals and cytology, and peritoneoscopy.

The multitude of tests highlight the fact that there is as yet no single, simple and reliable answer to this recurrent problem. Recent innovations such as direct transhepatic cholangiography and cannulation of the ampulla of Vater via fiberoptic duodenoscopy have given encouraging results. All these procedures however require trained personnel and expensive equipment. In an attempt to look for simpler answers, a joint survey was carried out from 1965 till 1970 between the Medical Unit, Thomson Road General Hospital and the Biochemistry Department, Outram Road General Hospital, to assess various enzymes that have been known to be raised in liver diseases.

MATERIAL AND METHODS

Within this period, 41 patients with extrahepatic obstructive jaundice (EHO) and 38 patients with jaundice due to viral hepatitis (VH) were included. The diagnosis of obstruction was confirmed by biopsy, cholangiographic studies, laparotomy or post-mortem findings. In the case of viral hepatitis, many of the patients had histological diagnosis, and those without histology had typical clinical features, including contact with jaundiced persons. Drug associated hepatitis and gallstone disease were excluded by careful history and subsequent cholecystographic studies. These patients were followed for a minimum period of two years and no evidence of biliary tract infection was observed.

The following is a report on some of the enzyme changes found in these two groups of patients. Serum isocitric dehydrogenase (SICD) was estimated by the method of Lee and Tan (1965), and serum and urine leucine aminopeptidase (SLAP and ULAP) according to the method of Goldbarg and Rutenburg (1958). Urine collection for the ULAP measurement was uniform for all patients. The average serum bilirubin level at the time of the enzyme tests was 9·4 mg.% for patients with EHO and 8·3 mg.% for VH.

ANALYSIS OF RESULTS

Tables I to IV give a list of the changes in SICD, SGPT and SGOT. A rise in these enzymes generally reflects hepatocellular dysfunction, and not unexpectedly, they show greater changes in patients with VH. SICD, an enzyme which has been found to be extremely useful in the diagnosis of liver cancer (Seah *et al*, 1968, Chua and Seah 1973) is also altered considerably during the acute stage of VH, the average level being more than twice the upper normal range. However, it is also abnormally raised in a number of patients with EHO (24 out of 41), though the rise is slight or moderate, the average for the group being slightly above the normal range. If only high values were considered (more than 1200 u), then 97·6% of the EHO would be excluded, though it would also eliminate 84% of VH. Therefore, an SICD level of 1200 u practically rules out EHO, but only a small number (16%) of VH cases can be clearly separated out from the rest.

TABLE I

S.I.C.D. CHANGES IN EHO AND VH

	E.H.O.	V.H.
Normal range (60—360 u.)		
Number of patients tested	41	38
Average value	432	883
No. of pat. with more than 360 u.	24	31
No. of pat. more than 1200 u.	1	6
Percentage of pat. more than 1200 u	2·4	16

TABLE II

S.G.O.T. CHANGES IN EHO AND VH

	E.H.O.	V.H.
Normal range (35—125 King u.)		
Number of patients tested	34	34
Average value	244	415
Percentage with more than 400 u.	5·9	29·4

TABLE III

S.G.P.T. CHANGES IN EHO AND VH

	E.H.O.	V.H.
Normal range (30—110 King u.)		
Number of patients tested	33	33
Average value	327	783
Percentage with more than 400 u.	24·2	54·5
Percentage with more than 600 u.	6	36

TABLE IV(a)

COMBINED ENZYME STUDIES: SICD, SGPT AND SGOT

	E.H.O.	V.H.
Number of patients	33	33
No. of pat. with high values in at least one enzyme:	5	15
Percentage:	15	45

TABLE IV(b)

COMBINED ENZYME STUDIES: SICD AND SGPT

	E.H.O.	V.H.
Number of patients	33	33
Number of pat. with high values in at least one enzyme:	4	14
Percentage:	12	42

The same shortcomings are found in the other two enzyme tests (SGPT and SGOT). Generally, the changes in SGPT are greater for both groups of patients. This is in accord with the experience of others, namely, that the rise in SGPT is generally more marked than SGOT in acute liver conditions. It is worth noting that 24·2% of patients with EHO have SGPT levels greater than 400 u. A level of 400 u or more is regarded by some as indicating hepatocellular dysfunction, and this level has been used to separate hepatocellular jaundice from obstructive jaundice. The results here however, show that nearly one-quarter of the cases with EHO fall above this range. A higher level, such as 600 u, may be more discriminatory in this respect. Table III shows that only 6% of EHO are above 600 u compared with 36% for VH. Above this range, 94% of EHO will be ruled out. Comparing SGPT with SICD from the diagnosis point of view, there seems to be no significant difference. Using a three-fold increase above the normal range (600 u for SGPT and 1200 u for SICD), it is found that the proportion of EHO cases excluded is similar (94% for SGPT and 97·6% for SICD). However, the proportion of VH cases included in the high levels slightly favours SGPT (36% compared to 16% for SICD). This slight advantage of SGPT over SICD needs confirmation with more extensive studies.

If we consider a combined study of the 3 enzymes together, as shown in Table IV (a), only 15% of EHO will have at least one of the 3 enzyme levels in the high range, compared to 45% in the case of VH. Table IV (b) considers SICD and SGPT together, and respective percentages for those in the high range are similar to those utilising 3 enzymes. Hence, there is no advantage in adding SGOT estimation to the other two.

Table V to VIII list the enzyme results of SLAP, ULAP and SAP. These enzymes are known to be raised in obstructive jaundice. Not surprisingly, the average levels for EHO in all 3 enzyme tests are much higher. When high SLAP values are only considered, 94% of VH are excluded, in contrast to only 53·7% of EHO, as shown in Table V. The ULAP test, shown in Table VI, has less discriminating value than the SLAP test. The mean level for SAP is very high in EHO, which is twice that of VH patients. With levels greater than 30 u, 89% of VH and 45% of EHO are excluded. The differentiating value for the enzymes SAP and SLAP appears to be equal. The SLAP excludes a greater percentage of VH cases compared to SAP (94% against 89%). On the other hand, the SAP includes a greater proportion of EHO patients than the SLAP (55·2% against 46·3%). If they are studied together (Table VIII), it is found that 72·4% of EHO will have high levels in at least one of the tests, whereas only 22·2% of VH fall into this category. If all the 3 enzymes are considered, the corresponding percentages for EHO and VH are 80·8 and 40 respectively. As with SGOT, there seems to be no advantage in adding the urine LAP test.

TABLE V

S.L.A.P. CHANGES IN EHO AND VH

	E.H.O.	V.H.
Normal range (70—200 GR u)		
Number of patients tested	41	34
Average value	408	260
Percentage with more than 500 u.	46·3	6

TABLE VI

U.L.A.P. CHANGES IN EHO AND VH

	E.H.O.	V.H.
Normal range (30—200 GR u)		
Number of patients tested	31	18
Average value	462	232
Percentage with more than 500 u.	32·3	16·7

TABLE VII

S.A.P. CHANGES IN EHO AND VH

	E.H.O.	V.H.
Normal range (3—13 KA u)		
Number of patients tested	29	18
Average value	36·1	18·4
Percentage with more than 30 u.	55·2	11

TABLE VIII(a)

STUDIES IN TWO ENZYMES: SLAP AND SAP

	E.H.O.	V.H.
Number of patients	29	18
Number of pt. with high values in at least one enzyme:	21	4
Percentage:	72·4	22·2

TABLE VIII(b)

STUDIES IN THREE ENZYMES: SLAP, ULAP AND SAP

	E.H.O.	V.H.
Number of patients	26	15
Number of pt. with high values in at least one enzyme:	21	6
Percentage:	80·8	40

GRAPH I

EHO IN BLACK CIRCLES
VH IN OPEN CIRCLES

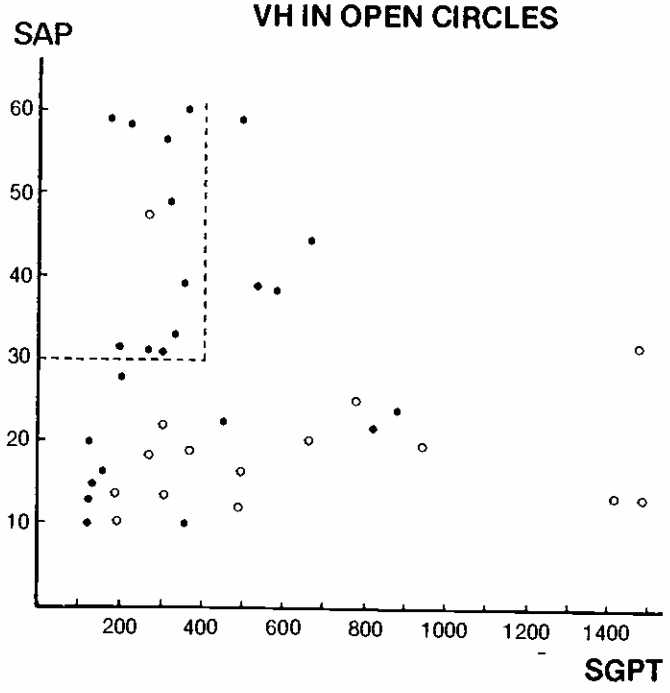

Table IX compares the two groups of patients with selected enzymes, SGPT and SAP. The data are also presented in graph I. It is shown that only 6·7% of VH patients have SAP greater than 30 u and SGPT less than 400 u at the same time. The corresponding percentage for EHO is much higher (41·7). Selected enzyme tests, therefore, can be extremely useful in excluding a high proportion of one group of patients (in this case 93·3% of VH), while retaining a fair percentage of the other (41·7% of EHO). A study of graph I also reveals that in the compartment enclosing 30 u or higher for SAP and 400 u or less for SGPT, practically all the data come from patients with EHO. It could then be said that any enzyme figures falling within this particular compartment would most probably indicate obstructive jaundice. Similar enzyme selection has been employed by others in dealing with the same problems. Clermont (1967), in reviewing a much larger collection of material, has shown that the vast majority of cases found to have high values for SAP (more than 25 K.A. u) and less than 250 Karmen u. for SGPT at the same time were suffering from EHO. Only less than 10% of this group of cases had VH. This seems to agree quite closely to the results here on a smaller number of cases.

TABLE IX

STUDIES IN TWO SELECTED ENZYMES: SGPT AND SAP

	E.H.O.	V.H.
Number of patients	24	15
No. with SGPT LESS than 400 u and SAP MORE than 30 u.	10	1
Percentage:	41·7	6·7

Table X considers two other selected enzymes, namely, SICD and SLAP. If we take levels greater than 600 u for SICD and less than 400 u for SLAP, then 97·6% of EHO patients are excluded, but half of the VH patients are included (graph II). Hence, combination of SICD with SLAP is very useful for excluding EHO cases, just as SGPT and SAP together are in eliminating VH patients.

DISCUSSION

These results reveal that none of the enzyme tests, whether single, combined or selected, will completely separate one group of patients from the other. There is always a certain degree of overlap. When the enzyme level is set high, it is useful in excluding a high percentage (usually

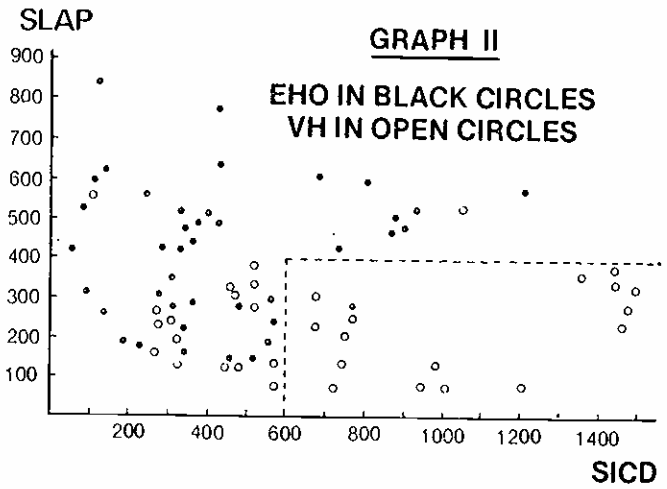

TABLE X

STUDIES IN TWO SELECTED ENZYMES: SICD AND SLAP

	E.H.O.	V.H.
Number of patients	41	33
No. with SICD MORE than 600 u. and SLAP LESS than 400 u.	1	16
Percentage:	2·4	48·8

more than 90%) of one group, but a substantial number from the other group would be eliminated too. This applies even to combination of enzymes. However, the separation of the patients is found to be significantly greater if selection of enzyme is employed, such as SGPT with SAP or SICD with SLAP. This device would exclude about 95% or more of one group while retaining half or more of the other. When isoenzymes of SAP and SLAP can be performed routinely, more patients could be separated. In the case of SAP, several papers have shown that the intestinal component of the isoenzyme is absent in patients with EHO, while this is present in other liver conditions, including intrahepatic cholestasis (Newton 1967, Chen 1972, Warnes *et al*, 1972).

Although lactic dehydrogenase (LDH) estimation is not reported here, this enzyme was estimated in the occasional case. As the number of tests performed in these two groups was too small, LDH was not assessed together with the rest. Reports elsewhere have shown that this enzyme is raised in EHO, especially when the range of SGOT or SGPT is high, so that a high SGOT or SGPT value accompanied by a high lactic dehydrogenase value points to EHO (Abbruzzese 1969, Ginsberg 1970). Similarly, glutamic dehydrogenase (GDH), which can also be estimated here, has been found by others to be raised in EHO compared with VH. Henley (1966) found that the ratio of SGPT/GDH was significantly greater in cases of VH when compared with that in EHO.

Apart from enzyme estimation, other simple investigations include the detection of the rheumatoid arthritis factor, the mitochondrial antibodies, and the estimation of fasting blood sugar and serum iron. The RA factor has been found to be present in a portion of patients suffering from various types of liver diseases apart from EHO (Caplan 1963, Chew 1966). A positive RA factor in a jaundiced patient would rule out practically all EHO of relatively recent onset. In the same way, the mitochondrial antibody and other tissue antibodies are present in significant proportions in many acute and chronic liver conditions, but they are rarely positive in EHO. Indirect fluorescein anubody studies show that the mitochondrial antibody is present in more than 90% of cases of primary biliary cirrhosis, and to a lesser degree in other chronic liver diseases. In one large series, the smooth muscle antibody, though in low titres, is present in 80% of acute viral hepatitis (Doniach 1972, Smith 1973).

Several papers have demonstrated that the serum iron level is raised so frequently as to be of value in the differential diagnosis between acute hepatitis (in which the level is raised) and obstructive jaundice (Goldstein *et al*, 1958, Bolin 1968, Schamroth *et al*, 1956). As for fasting blood sugar level in acute hepatitis, Felig *et al* (1970) found it to be valuable in differential diagnosis, as hypoglycaemic levels are frequently obtained even in cases of acute hepatitis uncomplicated by submassive hepatic necrosis. In these patients, the response to glucagon was also diminished. A modification of this, named the double glucagon test by the authors, was assessed by Verhagen *et al* (1971) in various liver disorders, and they concluded that it was of vaiue in distinguishing parenchymal liver disease from obstructive jaundice.

The thymol turbidity and the Jirgl's tests, though regarded by many as old-fashioned, are simple to perform and they still have a few proponents (Beveridge 1961, Rosenthal 1965). The prednisolone test has been used for a long time, and it is still regarded as valuable to date, though it is now greeted with less enthusiasm than in its inception in the early 1950s. A fall in serum bilirubin level of more than 40% after a 5-day course of prednisolone (30 mg. daily) strongly suggests hepatitis, especially when there is a definite response in the first 48 hours. Patients

with obstructive jaundice, whether mechanical or due to chlorpromazine, show little change (Sherlock 1963 and 1973, Schiff 1966). Phenobarbital too has been found to decrease serum bilirubin and serum bile salts and relieve pruritus in patients with intrahepatic cholestasis within 4 to 7 days, but not in patients with EHO (Stiehl *et al*, 1972).

A search of the literature has revealed some unusual but interesting tests. Weaver (1966) evaluated the Hepatitis-Infectious-Mononucleosis Test, and found this to be positive in 100% of cases of infectious hepatitis and in 88% of serum hepatitis, though it was also positive in 40% of controls. A negative test would therefore rule out acute hepatitis at once. In the field of isotope scanning, rose bengal labelled with I^{131} has been used to demonstrate the patency of the bile ducts (Shehadi 1966). In normal subjects the radioactivity will be detectable in the small and large intestines after an interval, whereas in patients with EHO no activity is found in the gastrointestinal tract as the isotope cannot be excreted via the obstructed biliary tree. Schaffner (1966) studied the morphology of the fat-storing mesenchymal cells ('lipocytes') in normal and diseased livers and found that the number of lipocytes were greatly increased in patients with EHO, while these cells were decreased in acute hepatitis. This histological difference should augment the value of liver biopsy in the differential diagnosis of jaundice.

Lately, Hellum (1973) evaluated the nitro-blue-tetrazolium test (NBT) in various liver conditions and found an increased NBT reduction by the neutrophils from a high percentage of cases of acute viral hepatitis (14 positive out of 16 cases), while 12 out of 13 patients with obstructive jaundice had negative NBT test. He concluded that the NBT test might be of some aid in the occasional difficult distinction between acute viral hepatitis and common-duct obstruction. Despite these encouraging findings, which should be confirmed by more extensive studies before they can be widely accepted, a single, simple and reliable test is yet to be found. This comes mainly from the fact that the aetiological agent for the commonest cause of intrahepatic cholestasis, namely viral hepatitis, still defies identification. The demonstration of the Australia antigen and antibody only partly solves the problem, as the antigen is found only in those suffering from the type B or long-incubation virus hepatitis (Sherlock 1973). Another limitation of this test is that the antigen is usually detectable only early in the course of the illness. Electron microscopic identification of particles in liver biopsy specimens offers morphological evidence of viral infection, but this necessitates the performance of liver biopsy, usually in deeply jaundiced patients, as well as involving a specialised service.

Finally, two rather involved procedures merit some mention. Reports from centres actively engaged in the cannulation of the ampulla of Vater via duodenoscopy to obtain duodenal aspirates for chemical and cytological studies as well as retrograde cholangiograms, have shown that the procedure is safe. The quality of the cholangiograms obtained is of a high standard in competent hands (Cotton 1972, Morrissey 1972). Strack *et al* (1971) innovated the other procedure whereby a direct transhepatic cholangiogram is obtained via a small epigastric incision made under local anaesthesia. In their hands, they found that this was a safe and reliable procedure, which was performed in the radiology room. Besides obtaining high quality films, an open liver biopsy could also be done without fear of bile peritonitis, and, whenever indicated, a portovenogram could be performed in the same sitting. All it requires is a team of enthusiastic surgeons and radiologists. Nevertheless, no matter how accurate and reliable these tests may be, the main aim of the clinician is to seek information from non-invasive investigations to aid him in his assessment of the problem. The more invasive procedures should only be resorted to if they are deemed absolutely necessary for the management of the patient under consideration.

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