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Fasting Serum Bile Acid Levels in Hepatitis B Carriers*

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= Abstract = Fasting serum bile acid levels have been investigated in 41 HBV carriers to evaluate its diagnostic usefulness in predicting the progression to chronic liver diseases. The level of bile acid in serum was measured enzymatically using a 3-hydroxysteroid dehydrogenase in a discrete chemistry analyzer. The results showed that there are two groups of carrier state showing different serum bile acid levels; high serum bile acid HBV carrier group (18 subjects, 44%, mean 16.6 μmol/l) and normal serum bile acid HBV carrier group (23 subjects, 56%, mean 6.1 μmol/l). The mean and range of serum bile acid level in control group (n=34) were 4.2 and 1~9.0 μmol/l, respectively. In conclusion, these results showed that increased levels of serum bile acid in high serum bile acid group of HBV carriers may indicate the presence of hepatocellular injury which could not be detected early in the course of illness by conventional liver function tests including serum transaminase measurement. However the diagnostic value of serum bile acid determinations in asymptomatic HBV carriers in distinguishing the heterogeneity and predicting the progression to chronic liver diseases are remained to be clarified through forthcoming prospective study.

Key Words: ***Fastng serum bile acid, Hepatitis B carriers, High serum bile acid HBV carrier group, Normal serum bile acid HBV carrier group, Liver function test, Chronic liver diseases***

INTRODUCTION

Since several investigators reported that the measurement of serum bile acid would be useful in indicating histopathological changes of liver, gradually it became accepted as one of valuable tests to assess hepatic function (Osuga *et al.* 1977). However, serum bile acid assay has been used mainly as an additive test and attempts to apply it to the specific clinical setting related to hepatic derangements have not been performed extensively.

In recent years the problem has attracted much interest in considering sustained HBV infection which occurs prevalently in world-wide endemic region (Nielson *et al.* 1971; Redeker 1975); that is how we identify early the patient with acute infection who is fated to develop chronic hepatitis (Perrillo and Aach 1981). Although various biochemical and serologic tests have been examined for their usefulness to predict transition from acute to chronic hepatitis B (Sherlock 1976), no single test or group of tests has been found to be highly predictive (Korman *et al.* 1974).

The purpose of present investigation was to determine whether serum bile acid assay is useful in predicting the progression to chronic liver diseases in Hepatitis B carriers.

MATERIALS AND METHODS

1. Subjects: Fourty-one Hepatitis B carriers, 9 to 59 years old, were included in the study who were confirmed to maintain serum HBsAg positivity for 6 months or longer without any abnormalities by standard liver function tests, *i.e.* total bilirubin (Bil), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT). Thirty-four healthy individuals were investigated their serum bile acid levels as control group. Eighteen chronic persistent hepatitis patients with sustained HBs antigenemia and moderate LFT abnormalities

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(defined as having elevations of AST and ALT levels below than 50 U/l) and thirty-two chronic active hepatitis patients with sustained HBs antigenemia and severe LFT abnormalities (defined as having elevations of AST and ALT levels above than 50 U/l) were also investigated their FBA levels as one of attempts to know how FBA levels correlates with standard LFT test results in patients with chronic persistent hepatitis (CPH) and chronic active hepatitis (CAH).

In all experimental and control group fasting serum specimens were analyzed (Table 1).

2. Serum bile acid determination: Total serum bile acid was determined by an enzymatic technique using "Enzabile" (Nyegaard) on the Impact 400 E (Gilford) discrete analyzer. Analytical factors applied on this instrument were 25 μl of serum with 250 μl of reagent, which was incubated 10 minutes at 37 °C. The formazan produced was measured at 540 nm which is its absorption maximum. The coefficient of variation with this technique was 7.2%, 5.4% and 4.0% at mean concentration of 5, 25, and 100 μmol/l respectively as shown in Table 2.

3. Liver function test: Determination of serum total bilirubin, ALP, AST, ALT was performed simultaneously by routine methods described elsewhere. The upper reference limits of them were 1.2 mg/100ml in total bilirubin, 115 U/l of adult, 300 U/l of children in ALP, 25 U/l in AST, and 29 U/l in ALT test respectively.

RESULTS

1. Fasting total serum bile acid in healthy individuals: In a control group of 34 subjects without clinical or biochemical evidence of hepatobiliary disease we found a fasting serum bile acid (FBA) of mean, 4.2 and SD, 2.4 μmol/l. Therefore our upper limit of FBA is 9.0 μmol/l. In Table 3, a variety of reference ranges of fasting serum bile acid by different methods were listed.

2. Fasting total serum bile acid in Hepatitis B carriers: At present we have investigated on 41 HBV carriers. The results showed that there were two groups of population having different FBA levels among HBV carriers judged by the upper reference limit (9 μmol/l). The one, tentatively named as high FBA group (HFBA Group) has their mean FBA level as 6.6 μmol/l with the SD of 3.4 μmol/l. The other one, named as normal FBA group (NFBA Group) has 6.1 μmol/l and 1.3 μmol/l as their mean and SD values. Among 41 HBV carriers, 18 subjects (44%) belonged to HFBA Group and 23 subjects (56%) belonged to NFBA Group as shown in Table 4. The HFBA Group has significantly increased FBA levels compared to those of control group ($p<0.01$, t-test).

3. Results of Liver Function Test in HBV carriers: Table 5 summarizes the results. It is seen that only the ALP levels were significantly higher within normal reference range in HFBA Group than

Table 1. Distribution of the subjects investigated in the study

Subjects	Number
HBV Carriers	41
Normal Healthy Control	34
CPH Group*	18
CAH Group**	32

* Chronic persistant hepatitis with elevations of serum AST and ALT levels below 50 U/l.

** Chronic active hepatitis with elevations of serum AST and ALT levels above 50 U/l.

Table 2. The coefficient of variation of serum bile acid determinations by direct enzymatic spectrophotometry

Concentrations (μmol/l)	C.V.(%)*
5	7.2
25	5.4
100	4.0

* Coefficient of variation

Table 3. The fasting serum bile acid levels in healthy individuals by different methods

Investigators	Range or Mean (S.D.), μmol/l	Methods
Mashige, 1976	0-7	ES*
Osuga, 1977	1.5-9.2	EF**
Pennington, 1977	2.11 (0.86)	GLC***
Skerede, 1978	2.5-6.8	ES
Uenoyama, 1981	2.88 (0.74)	HPLC
Alm, 1982	0-5	ES
Yi, 1983	0-11	ES
Author, 1985	1-9	ES

* Direct enzymatic spectrophotometry

** Enzymatic fluorimetry

*** Gas liquid chromatography

Table 4. The fasting serum bile acid levels in fourty-one asymptomatic Hepatitis B carriers

Subjects	No. (%)	Mean (S.D.), μmol/l	Range, μmol/l
HFBA Group*	18 (44%)	16.6 (3.4)	9.8-23.4
NFBA Group**	23 (56%)	6.1 (1.3)	3.5- 8.7

* The HBV carriers having the higher fasting serum bile acid levels compared to those of control group

** The HBV carriers having the fasting serum bile acid levels within the normal reference range

Talbe 5. The results of liver function tests in asymptomatic HBV carriers

Tests	HFBA Group Mean(SD)	NFBA Group Mean(SD)	Significance
T. Bilirubin	0.64 (0.25)	0.64 (0.23)	N.S.
ALP	109.2 (64.8)	74.3 (36.1)	$p<0.001$
AST	13.7 (4.8)	13.0 (4.8)	N.S.
ALT	13.6 (6.3)	13.7 (5.8)	N.S.

T. bilirubin; Total bilirubin, mg/100ml
ALP; Alkaline phosphatase, U/l, 37°C
AST; Aspartate aminotransferase, U/l, 37°C
ALT; Alanine aminotransferase, U/l, 37°C
N.S.; Not significant.

Table 6. The fasting serum bile acid levels in patients with chronic persistent hepatitis and chronic active hepatitis

Subject	No.	Mean (SD), μmol/l
CPH	18	27.6 (39.1)*
CAH	32	59.2 (89.7)*

* $P<0.05$, t-test.

NFBA Group ($p<0.001$, t-test).

4. FBA levels in patients with sustained HBS antigenemia and LFT abnormalities: The FBA levels between chronic persistent hepatitis and chronic active hepatitis were compared each other in Table 6. The group of CAH has more elevated FBA levels than those of CPH group. The difference of mean FBA levels between two groups was statistically significant ($p<0.05$, t-test).

DISCUSSION

This study shows that certain group of HBV carriers can have increased serum bile acid levels, and that the increase was statistically significant compared to healthy individuals. In HFBA Group, results have shown that only the serum ALP levels were higher than those of NFBA Group. These data supported that the synthesis of cholestatic ALP isoenzyme could be stimulated by the presence of bile acid (Hatoff and Hardison 1979). This finding also suggest that asymptomatic HBV carriers without serum transaminase elevations may have significant hepatic lesions (Korets *et al.* 1978), of which cholestatic liver injury appears to be very important (Kim and Kim 1985). The investigation of FBA levels on patients with chronic hepatitis revealed that FBA levels correlates well with the serum transaminase elevations. Therefore, serum bile acid assay was remained yet as one of additive test to support conventional liver function test in managing the patients with chronic liver diseases. There have been reports of appreciable hepatocellular damage in asymptomatic subjects with only mild elevation of the serum transaminase level (Hadziyannis 1974: Mihas and Conrad 1978). However, our results of serum transaminases determination revealed that there were no cases showing the elevation of serum transaminases beyond normal reference range among HBV carriers, and were not so significant differences between two groups of asymptomatic HBV carriers as shown in FBA's. So, it was suggested that bile acid measurement might be able to detect sensitively the minimal hepatocellular damage which is under the progression in asymptomatic HBV carriers.

Although still liver biopsy remains the most definitive procedure to characterize the nature and extent of hepatic diseases (Korets *et al.* 1978), it is regarded as too invasive to apply it serially on asymptomatic HBV carriers to predict their destination to chronic liver diseases. Furthermore, extensive laboratory work up is not very rewarding in the diagnostic setting of chronic hepatitits B and its antecedents (Perrillo and Aach 1981). The evidence from the present investigations suggest that we can identify, with the application of FBA assay, the group of asymptomatic HBV carriers early in their course of progression to chronic liver diseases which could not be detected sensitively by conventional LFT. Thus, we suggest serial check of fasting serum bile acd levels in asymptomatic HBV carriers as one of reliable diagnostic reasoning in prediction of their chronicity, which is summarized in Fig. 1. The clinical relevance of this suggestion requires further investigation to clarify the following two

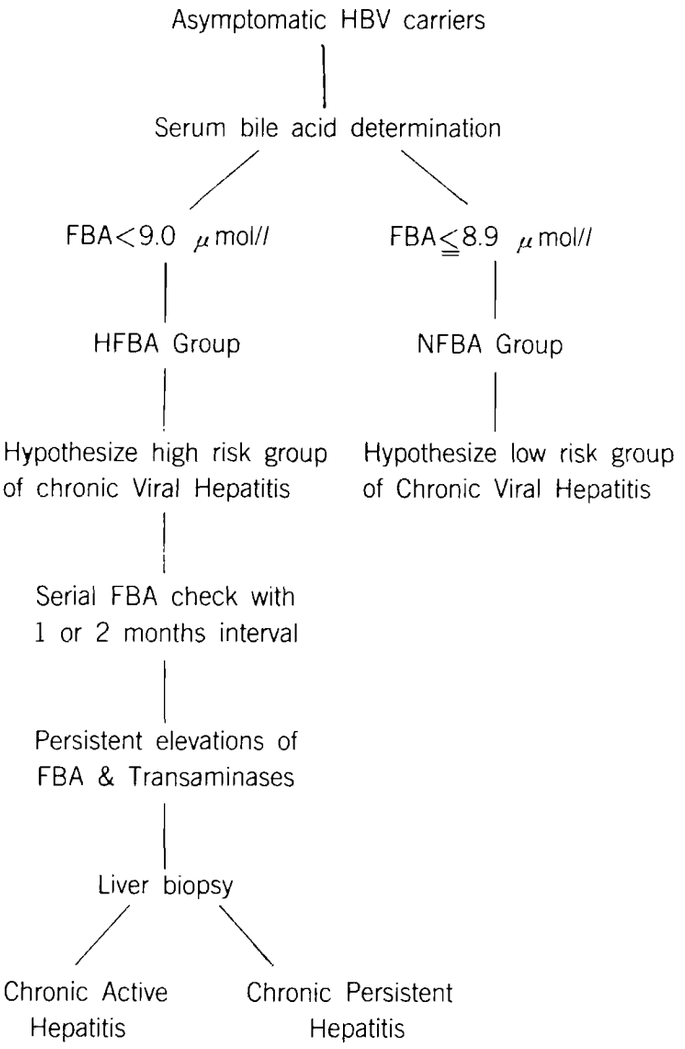

Fig. 1. Diagnostic reasoning for chronic viral hepatitis.

points, those are very important in elucidating the speculation; The one is to confirm the correlation of HFBA Group and their proneness to the progression to chronic liver diseases, and the other one is to evaluate the diagnostic relevance of 9.0 μmol/l of fasting serum bile acid, established as the cut-off value in predicting the high risk group of chronic hepatitis B among asymptomatic HBV carriers. Normal reference ranges of serum FBA have been reported by several investigators; 1.5-9.2 μmol/l by Osuga *et al.* (1977), 2.5-6.8 μmol/l by Skerede *et al.* (1978), 0-5 μmol/l by Aml *et al.* (1982) and 0-11 μmol/l by Yi *et al.* (1983).

In conclusion, followings are suggested from present investigation, although it has limited evidence, that serum bile acid determination might be used as the most valuable routine biochemical test having excellent diagnostic reliability for the early recognition and prediction of asymptomatic HBV carriers in their course of illness to chronic liver diseases.

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= 국문초록 =

B형 간염 보균자의 공복 혈청 담즙산치에 관한 연구

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B형 간염보균자가 만성 간염 등 만성 간질환으로 진행되는 데에 있어서 공복 혈청 담즙산의 측정이 그 진행의 조기 발견에 유용한 검사지표로서 이용될 수 있는지를 알고자 정상인 34예, 만성 B형 간염 보균자 41예, 만성간염 50예를 대상으로 공복 혈청 담즙산 및 aspartate aminotransferase 등의 기존 간기능검사를 실시하고 다음과 같은 결과를 얻었다.

1. 정상 대조군 34예의 공복혈청 담즙산의 평균은 4.2 μmol/l(S.D. 2.4 μmol/l)이었으며 2 S.D를 기준한 정상참고치의 상한은 9.0 μmon/l 이었다.

2. B형 간염 보균자 41예중 공복 혈청 담즙산이 증가된 (9.0 μmol/l 이상) 예는 18예 (44%)로서 그 평균은 16.6 μmol/l (S.D. 3.4 μmol/l)이었으며 정상 참고치 이내에 속하였던 예는 23예 (56%)로서 그 평균은 6.1 μmol/l (S.D. 1.3 μmol/l) 이었다.

3. B형 간염 보균자에서 혈청 총 빌리루빈, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase를 측정한 결과 alkaline phosphatase 에서만 고 공복 혈청 담즙산치를 갖는 B형 간염 보균자 군 (평균 109.2 U/l)과 정상 공복 혈청 담즙산 치를 갖는 군 (평균 74.3 U/l) 간에 유의한 차이를 나타내었다 ($p<0.001$).

4. 만성 간염 환자 50예를 혈청 transaminase치가 50 U/l이상으로 증가된 군(18예)과 50 U/l이하의 범위에서 정상보다 증가된 군(32예)으로 나누어 공복 혈청 담즙산을 측정하였는바 50 U/l 이상인 군의 공복 혈청 담즙산 (평균 59.2 μmol/l) 치가 50 U/l 이하인 군 (평균 27.6 μmol/l)의 그것보다 유의하게 높았다 ($p<0.05$).

이상의 연구 결과로 B형 간염 보균자중에 공복 혈청 담즙산치가 증가된 군이 있음을 알게 되었으며 이는 기존 간 기능 검사로 구별되진 않으나 미세한 담즙 울체성 간병변이 진행되고 있음을 시사하는 것이라고 생각되어 공복 혈청 담즙산 측정이 B형 간염 보균자가 만성 간 질환으로 진행됨에 있어 그 진행의 조기 발견과 예후 관찰에 유용한 진단적 지표가 될 수 있으리라 생각된다.