LIVER FUNCTION PROFILE AND BENZOM

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OBJECTIVE: To determine the outcome of hippuric acid test on both subjects and control used in order to evaluate the conjugating capacity of the liver in the two groups.

MATERIALS AND METHODS:-
34 patients with idiopathic hypertension and 18 normal subjected use as the control were subjected to different laboratory investigation of liver functions following an informal consent. The hippuric acid test was conducted in all of them to evaluate the conjugating capacity of the liver.

RESULTS: A marked derangement of hippuric acid test was seen in patients with idiopathic hypertension when compared with normal subjects used as control.

CONCLUSION:
The result of this preliminary investigations is no conclusive evidence that this defect bears any direct relationship to the hypertension seen in this group, they must however be considered significant. It is suggested that the disturbance of this mechanism which inevitablt would interfere with elimination of certain hypertension inducing hormones and their breakdown products may at least be partially contributory to the elevated blood pressure seen in this group.

KEY WORDS: Primary hypertension, Hippuric acid conjugation test, Nigerians.

INTRODUCTION.

In more than 95% of cases a specific underlying cause of hypertension is not found and such patients are said to have essential hypertension[1]. The aetiology or pathogenesis of essential hypertension is not known, but generally it is believed that the underlying defect is an increase in peripheral resistance. High blood pressure is in most cases a multifactorial condition. Essential hypertension is defined as a sustained high blood pressure not attributable to a single cause but reflecting the interaction of multiple genetic and environmental factors the diagnosis of which is one of exclusion[2]. The selection of criterion for hypertension is an arbitrary process and the factors that may influence development of essential hypertension includes the following:-Genetic and familial, socioeconomic, Dietary factors, Hormonal factors and Neurotransmitters. The glucocorticoids as well as the mineralocorticoids and the catecholamines of the adrenal are catabolized in the liver, where they undergo conjugation before excretion through the kidney in the urine. The

hypertensive effects of these substance as well as some of their break-down products are well recognized[3]. Liver dysfunction and in particular the derangement of its conjugating capacity will lead to the retention of these hormones together with their breakdown products in the body, with a likely positive effect on the blood pressure. The cause of idiopathic hypertension is yet to be established, I have therefore considered it necessary to assess the efficiency of the liver in this disease, and more especially its conjugating capacity. The account which follows is an assessment of *hippuric acid test* in thirty four patients with the disease and 18 normal subjects used as control.

MATERIAL AND METHODS.

A total of 52 subjects were investigated in this study, out of which 34 (male=26, female =8) age range 34-63 years were selected as having severe hypertension (B.P over 180/110 mm of Hg) and remaining 18 (male=12, female=6) age mean 30-55 years were taken as control from the normal group with normal blood pressure. The patients were selected and assessed at the consultants' clinic of Jos University Teaching Hospital, Jos after an informed consent between January-July 1995. The blood pressures were carefully monitored and patients with primary (idiopathic) hypertension based on criteria defined above were included in the present study. About 10-12 ml of blood were withdrawn from the antecubital vein of each subject in the fasting condition and the clear sera separated through centrifugation (x3000 rpm) for 10 minutes were subjected to the various liver function investigations using standard procedures, which included total protein, albumin, (globulin by derivation), total bilirubin, alkaline phosphatase, alanine transaminase and aspartate transaninase. These were routinely assayed in the Diagnostic Laboratory of the Hospital. The endogenous creatinine clearance test (C.C.T.)[3] was subsequently carried out in all the subjects to assess the integrity of their renal (glomerular) function. The Liver conjugating capacity test was then carried out in each of the subjects assessed, and a short description of the procedure adopted as described by varley[4] is given below.

Oral Hippuric Acid synthesis Test[4]

Subjects were asked to come 2 hours after their normal breakfast to the Research Laboratory of Chemical Pathology Department, University of Jos. After obtaining the necessary informed consent, each of them were fed orally 200ml of sodium benzoate solution prepared freshly by dissolving 6g of sodium benzoate in 200ml of clean water. They were then asked to empty the bladder and the urine was discarded. After this all the urine passed to the end of 4 hours was collected in a clean container and the concentration of the hippuric acid in the urine was determined.

This was done by the precipitation of hippuric acid crystal by adding 30g of sodium chloride (NaCl) and 3lml of sulphuric acid in 100ml of urine. The above mixture was stirred gently and left overnight for complete precipitation. The following day the precipitates formed in the flask was thoroughly washed with saturated (NaCl) solution until no further precipitation with (NaCl) solution occurred. Finally the precipitate was dissolved in hot water and the hippuric acid content titrated against sodium hydroxide (NaOH) 500 mmol/1) solution.

Calculation.

Amount of hippuric acid (g) in 4 hours urine collection

= (0.072 x ml NaOH + 0.1) x Urine Volume (ml) 100

The results of the above investigation were matched with a control group which consisted of eighteen healthy individuals of the similar age and sex group with normal blood pressure (less that 130/85mmHg) for statistical comparison. Student's (t) test was employed to calculate the degree of significance (p values) between the groups.

RESULTS

Tables 1, 2 and 3 show the results of the liver function profiles, endogenous creatinine clearance investigation and hepatic sodium benzoate conjugation tests observed in the subjects of control and hypertensive groups respectively.

There was no significant change seen in the mean values in any of the liver function parameters measured with both the groups, i.e. normal subjects and the patients with hypertension and compared with each other (Table 1). Similarly, no significant changes were seen in the mean values of creatinine clearance investigation between the control and the hypertensive group. The values in both the groups were found to be within the acceptable normal range (Table 2).

Table 3 shows the findings of the benzoic acid conjugation test. When compared to the normal group there was a marked decrease in the capacity of the liver in the hypertensive patients to synthesize hippuric acid from benzoic acid and glucoronic acid by conjugation. The mean value of the amount of hippuric acid synthesized from the orally fed benzoic acid as derived from the pooled 4 hour urine sample in the subjects with hypertension was 2.96g, compared with the mean value of 4.24g seen in normal group, and the difference was statistically significant. ($t = 6.7$, $df=50$, $p < 0.001$).

TABLE 1: Liver Function profile in Control and Hypertensive Subjects.

Liver function Investigations (serum	CONTROL (n = 18)			HYPERTENSION (n = 34)			Significance of difference between Mean values
	Mean	SD	Range	Mean	SD	Range	
Total protein (gm/100ml)	7.06	0.5	(6.06-8.06)	6.5	0.67	(5.16-7.84)	t = 3.40, df=50, p> 0.05
Albumin (gm/100ml)	4.27	0.39	(3.49-5.05)	3.86	0.71	(2.44-5.28)	t = 2.69, df=50, p> 0.05
Globulin (gm/100ml)	2.79	0.28	(2.23-3.35)	2.64	0.42	(1.80-3.48)	t = 0.21, df=50, p> 0.05
Bilirubin (gm/100ml)	0.4	0.13	(0.14-0.66)	0.4	0.13	(0.04-0.76)	t = 0.13, df=50, p> 0.05
Alkaline Phosphate (KA units)	7.6	2.65	(2.3-12.9)	7.74	2.67	(2.04-13.08)	t = 0.18, df=50, p> 0.05
Aspartate transaminase (I.U)	10.7	3.07	(4.56-16.84)	11.15	6.48	(4.67-17.68)	t = 0.34, df=50, p> 0.05
ALanine transaminase (I.U)	9.83	2.91	(4.01-15.66)	10.21	5.61	(4.6-15.82)	t = 0.32, df=50, p> 0.05

TABLE 2: Liver Function profile in Control and Hypertensive Subjects.

Renal function Test	CONTROL (n = 18)			PRIMARY HYPERTENSION (n = 34)			Significance of difference between Mean values
	Mean	SD	Range	Mean	SD	Range	
Endogenous creatinine clearance test (ml/minute	120.3	6.43	(107.44-133.16)	118.65	7.24	(104.17-133.13)	t = 0.52, df=50, p> 0.80

TABLE 3: Liver Function profile in Control and Hypertensive Subjects.

Renal conjugating capacity	CONTROL (n = 18)			IDIOPATHIC HYPERTENSION (n = 34)			Significance of difference between Mean values
	Mean	SD	Range	Mean	SD	Range	
Na-benozoate conjugating Test expressed in g per 4 hours Collection of urine of hippuric Acid synthesized from the Orally fed benzoic acid	120.3	6.43	(107.44-133.16)	118.65	7.24	(104.17-133.13)	t = 0.52, df=50, p> 0.80

DISCUSSION

One of the vital functions of the liver, among many others is to eliminate toxic substances generated in the body during various metabolic processes either through bile or urine. This is achieved by their degradation to simpler constituents prior to their conjugation with glucuronic acid or sulfate or glycine. The conjugation and subsequent excretion of these substance are therefore of vital importance to the body as may otherwise accumulate in the system with detrimental consequences from their toxic effects. The liver conjugating mechanism is thought to be very intricate and the events or physiological processes which occur during the various phases of the conjugation of a metabolite are still poorly understood. It is recognized that there are different mechanism that operate in the conjugation process in the liver for the different substances for final elimination from the body. This is against the original hypothesis suggesting a singular conjugating mechanism operating for all substances or metabolites, via conjugation with glucuronic acid.

The conjugating mechanism for bilirubin was first recognized as far back as the history of jaundice itself. It is however, now recognized that the processes involving bilirubin conjugation is not as simple as originally thought. [5, 6]

It is established that there are different sets of enzymes which operate at the different stages during the process of bilirubin conjugation in the liver [3]. This rather complex process initially involves the enzyme uridine diphosphoglucoronyl transferase in the conversion of the unconjugated bilirubin to bilirubin monoglucoronide and the reaction takes place at the smooth surface of the endoplasmic reticulum. The further conversion of bilirubin monoglucoronide to its final product bilirubin diglucuronide, takes place on the plasma membrane of the hepatocytes and requires a separate set of enzymes i.e. membrane esterases. This final product i.e. water soluble bilirubin diglucuronide is later excreted in the bile by a carrier-mediated protein transport system for its ultimate elimination from the body.

Similarly, the conjugating mechanism for benzoic acid with glycine and its subsequent conversion into hippuric acid (as has been carried out in the study) for final excretion in the urine is also fairly well understood and takes place as follows: [7, 8]

$C_6H_5COOH + H_2NCH_2\ COOH \rightarrow C_6H_5CONHCH_2\ COOH.$

Benzoic acid Gylcine Hippuric acid.

The reaction requires the enzyme glycine- N- acylase and co-enzyme A and the test becomes invalid if there is marked renal insufficiency. The processes which are involved in the conjugation of adrenal hormones such as glucocorticoids, mineralocorticoides and medullary catecholamines seem to be inadequately studied. It is however established [8] that these hormones are degraded in the liver by ring reduction catalyzed by NADPH- requiring

hydrogenase and by reduction of the 3-ketone group by NADH or NADPH, requiring reversible dehydrogenase. The resulting tetrahydro derivatives are in turn conjugated with mainly glucoronic acid, though not exclusively, to the corresponding carboxylic acid for elimination through the urine and bile. Hepatic inactivation and conjugation of these hormones decline in certain clinical conditions like cirrhosis, congestive cardiac failure, and prolonged malnutrition or in renal failure with the resulting retention of these hormones leading to the development of oedema and ascites [9].

This present finding suggests that the significantly lower value for benzoic acid conjugation (mean=2.96mg/4 hours urine,) in subject with high blood pressure compared with normal subjects (mean 4.24mg/4 hour urine) $t=6.7$, $df=50$, $p<0.001$) is probably due to the deficiency or diminished effectiveness of conjugating factors like glycine -N-acylase enzymes, co-enzymes A or even the glycine itself. I further believe that in addition to their usual conjugation with glucoronic acid in the liver, adrenal hormones are also partly conjugated with glycine in a similar fashion as benzoic acid.

The conjugation of retained hormones of the adrenal with glucoronic acid for elimination from the body seem to be normal in patients with idiopathic hypertension as reflected by a normal plasma bilirubin level seen in this group of patients.

This is very likely because the conjugation of bilirubin and adrenal hormones with glucoronic acid, operate on a similar mechanism. The lower value obtained in the hippuric acid synthesis test in these hypertensive subjects (see table 3), therefore, appears to indicate that the conjugation of these hormones with other conjugates such as glycine is perhaps markedly inhibited. The resulting retention of the hormone or their degraded derivative in the blood are likely to affect the blood pressure and elevate it. The renal clearance investigations carried out in these hypertensive patients were normal, a finding which suggests that the diminished values of hippuric acid seen in the urine in this group is not due to retention or renal failure. It should be mentioned also that no clinical evidence of other factors such as malabsorption or malnutrition which may interfere with the test was seen in the patients.

In conclusion therefore, it would appear from the investigations conducted in this work that there is a marked derangement of the conjugating mechanism which involves glycine or other conjugates for the removal of toxic products from the body of Nigerian patients with idiopathic hypertension. I suggest that a failure in this process is likely to affect the adrenal hormones and their break-down products as well. The disturbance of this mechanism may at least be contributory to the elevated blood pressure seen in this group.

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Alkaline
Phosphatase (K.A. units)
Aspartate transaminase
(I.U.)
Alanine transaminase
(I.U.)