HIDA Scintigraphy Diagnoses Early Hepatic Dysfunction in Diabetic Population

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Published on 30th March 2012

ABSTRACT

Introduction: Mebrofenin scintigraphy estimates hepatocellular function because of its high hepatic specificity and is correlated with histopathological severity in NAFLD.

Objective: The aim of this case control pilot study was to evaluate hepatobiliary function by 99mTc-mebrofenin scintigraphy in Type 2 diabetes.

Methods: 29 T2DM patients (with a minimum of 2 years duration of DM) and 20 BMI matched volunteers were injected with 185 MBq 99mTc- Mebrofenin intravenously. The liver function tests and viral serology of all patients and volunteers were within normal limits. Hepatic radiotracer activity accumulation rate was estimated as the T ½ of its ascending portion of the Time activity curve and maximal accumulation as percentage of total activity (FOV curve).

Results: There was a significant difference in ascending T ½ and maximal hepatic uptake between the diabetic and control population groups. The T1/2 ascend was prolonged in T2DM (70.9± 13.8 Vs. 54.2± 14.3 seconds, $p<0.0001$) suggesting slower rate of uptake and the hepatic uptake was also decreased (76.9± 5.5% Vs. 85.5± 2.6% $p<0.0001$). There is also significant difference among T2DM subgroups with BMI < 25 (n=11) and > 25 (n=18) in T ½ (64.6 vs 74.7 $p=0.04$) and uptake percentage (79.1%vs 75.6% $p=0.04$).

Conclusions: Although liver biopsy remains the gold standard for diagnosis, our pilot results suggest nuclear medicine imaging with 99mTc-mebrofenin is a useful tool in the investigation of NAFLD in T2DM.

Keywords: HIDA, Diabetes, 99mTc-mebrofenin.

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INTRODUCTION

Hepatic fatty accumulation or Non Alcoholic fatty liver disease is a Clinicopathological condition that presents with a wide spectrum of liver damage ranging from simple steatosis to steatohepatitis; a severe inflammatory form, advanced fibrosis and cirrhosis.[1] Steatosis is closely related to obesity, dyslipidemia, noninsulin-dependent diabetes and several drugs and toxins. It is currently the most common parenchymal liver disease in the Western world, affecting 20% of individuals in the general population and up to 95% among the obese, diabetics.[2,3] The prevalence is expected to increase dramatically in the near future as a consequence of the epidemic of obesity and diabetes in the Western and developing countries' population. Due to the evolving knowledge of the clinical importance of steatosis combined with its increasing prevalence, new pharmacological therapies are being developed to treat this disorder.[3] However, to assess the potential impact of the intervention, serial biopsies are required, subjecting patients to potentially harmful or even lethal complications.[4] As long as reliable non-invasive diagnostic methods are lacking, the gold standard for the diagnosis of steatosis will remain histopathological examination.[5]

The natural history is varied; at the early stages of disease, the majority remain stable at the same histological stage and grade, a proportion however will progress to cirrhosis (there is variation in the rate of progression), and finally some will have regression of disease. The variables most commonly associated with severe / progressive non alcoholic fatty liver disease (NAFLD) or fibrosis are: presence of diabetes, increasing age, increased homeostatic insulin resistance (HOMA-IR), increased aspartate aminotransferase/ alanine aminotransferase (AST/ ALT) ratio, decreased platelets, increased hyaluronic acid and high Body Mass Index (BMI). However these variables have found a consistent association only in patients with biopsy proven, severe forms of NAFLD. Hence their utility in the clinical setting of early stages of NAFLD, in

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patients prone to hepatic dysfunction, but with normal liver function tests is not as well established.

Even the latest state-of-art radiological modalities (ultrasonography, computed tomography or magnetic resonance imaging) fail to reliably identify the extent of steatosis and other pathological features related to progression of steatosis, such as parenchymal inflammation, hepatocyte ballooning, Mallory's hyaline deposition and fibrogenesis.[3,5] Therefore, a readily available, noninvasive test correlating with early histopathological features of steatosis would be of great clinical importance for the diagnosis of steatosis as well as for the follow- up of patients during pharmacological therapy.

A recent study has pointed out the potential role of biliary transport malfunction in progressive hepatocellular injury and inflammation present in steatotic livers.[6,9]

Hepatobiliary scintigraphy using 99mTc-N-(-3-bromo-2,4,6-trimethylacetanilide) iminodiacetic acid (Mebrofenin) is routinely used to assess hepatocellular function because of its high hepatic specificity and rapid transit via the main biliary transport system.[10] Furthermore, several studies in models of acute and chronic parenchymal liver disease have shown a correlation between hepatobiliary function assessed by 99mTc-mebrofenin scintigraphy and histopathological severity.[11-13] Mebrofenin scintigraphy is also clinically available as it is widely applied in the diagnosis of biliary disease as well as in the assessment of liver functional reserve prior to resection.[10,14] So far, there are no studies evaluating the correlation of hepatobiliary function with other non invasive surrogate clinical, biochemical and anthropometric markers of steatosis in humans. As NAFLD is much more frequent in the diabetic population, wherein levels as high as 40 % have been reported in newly diagnosed, untreated diabetes;[15] screening this population for early hepatic dysfunction might identify those individuals at higher risk. The aim of this study was to evaluate the utility of non-invasive assessment of hepatobiliary function by 99mTc-mebrofenin scintigraphy in a patient population with Type II diabetes; believed to have a high clinical probability of steatosis.

AIMS AND OBJECTIVES

The aim of the study is to determine if parameters generated on hepatobiliary scintigraphy [1) rate of hepatic uptake of the radiotracer; 2) maximum hepatic uptake of the injected radiotracer as a percentage of total injected activity; 3) half- time of excretion of the hepatic radiotracer and 4) time to maximal uptake in the liver] were affected in diabetic patients compared to non diabetic control population.

This was considered as a cross sectional descriptive epidemiology study.

MATERIALS AND METHODS

Patients:

The inclusion criteria for patients recruited into the study were:

- No known hepatic or gall bladder/biliary/pancreatic pathology.
- Serum markers for Hepatitis B, Hepatitis C and HIV were negative.
- Normal serum albumin levels.
- No urine microscopy evidence of albuminuria.
- Normal liver function tests.
- Normal platelet counts, INR and PT.
- Alcohol consumption below 30 gm per week for males and 18 gm per week for females. (350 mL [12 oz] of beer, 120 mL [4 oz] of wine, and 45 mL [1.5 oz] of hard liquor, each contain 10 g of alcohol).
- No exposure to potential cholestatic drugs or other medications affecting liver function.
- No historical or biochemical evidence of non insulin dependent diabetes(NIDDM) or Type I diabetes for the control population and evidence of NIDDM of at least 2 years duration for the study population.

After obtaining written, informed consent, patients recruited into the study during the study period of one year, from June 2007 to May 2008, were divided into two groups; Group I comprised those having Non Insulin Dependent Diabetes Mellitus and Group II comprised of control, non diabetic, healthy volunteer population who were undergoing work up foe other illnesses.

Nuclear medicine procedure

Camera design:

For imaging of 99mTc-mebrofenin uptake in the liver, a gamma camera (GE-SPX4, Elgems, Israel) situated in a routine clinical care facility was equipped with a

Low Energy High Resolution (LEHR) collimator. The gamma camera was interfaced to an eNTEGRA (GE medical systems, United States of America) acquisition and processing work station.

Scintigraphy and interpretation

Patients recruited were given a standard meal of two idlis and a cup of coffee followed by commencement of the scan after 3-4 hours. The meal was standardized as it was culturally acceptable and it was given under supervision to ascertain patientcompliance.3-4 hour fasting was considered adequate, as reported before, for hepatobiliary scintigraphy. Overnight fasting was not considered for this study as each acquisition was for one hour duration and the appointments for these studies were towards mid day or noon. Hence it was not considered suitable to keep the patients fasting till then. The patients were placed in the supine position after being asked to void completely. Once positioned, the patients were injected with 185 MBq99mTc-mebrofenin (TCK-39, Board of radiation and isotope technology, BARC, Mumbai, India) intravenously in the left antecubital vein. The patients were scanned immediately after injection of the radiopharmaceutical in the anterior position with the liver and the mediastinum in the field of view. Dynamic images were obtained for 60 min. Initial 10 min at 1 s per frame (liver uptake sequence) and next 50 min at 60 s per frame (bile excretion sequence) at the 140 KeV 99mTc peak with a 20% window in a 64×64 matrix. Data were processed on an eNTEGRA workstation (GE medical systems). The liver uptake was calculated based on a technique described by Ekman et al.[16] This method calculates the clearance of mebrofenin from the blood by liver uptake, based on increasing activity in the liver and the total clearance of mebrofenin from the blood. The method includes all possible routes of elimination, based on decreasing activity in the blood pool. The amount of activity that accumulates in the liver and the accumulation rate over a given period of time can be expressed as a percentage of total radiotracer activity in the field of view (FOV curve) and the T ½ of the ascending portion of the curve. The algorithm was adapted for direct clinical evaluation. Regions of interest (ROIs) were drawn around the liver, the heart and large vessels within the mediastinum (serving as blood pool) and around the total field of view (indicative of total activity). The total FOV curve generated was considered as a suitable alternative to the traditionally accepted direct injected activity calculation; as the blood pool and all possible routes of elimination (liver, kidneys, heart, spleen and the great vessels) were in the FOV. The liver ROI was drawn automatically on a threshold-based algorithm using 20% of the maximum liver value on a summed image of the first10 min of the acquisition as cut-off, ensuring uniform tracer distribution and before excretion begins. Two different time-activity curves were generated based on the liver and total field of view (FOV) (including liver, intestine and blood pool). Based on these two curve parameters and by using the curve arithmetic option of dividing the hepatic curve by total FOV curve; a derived or normalized curve was generated. This curve was analyzed for maximum % hepatic uptake, seen as the peak value of this curve. The time of this peak on the curve was analyzed as the derived T peak. Assessment of hepatic 99mTc-mebrofenin uptake rate were performed using scanned radioactivity values acquired between 30 and 120 s post injection i.e. the ascending portion of the curve before T peak, to make sure that calculations were made during a phase of homogeneous distribution of the agent in the blood pool and before the rapid phase of hepatic excretion.[16] The rate of uptake expressed as T ½ of ascending portion of the curve in seconds, for the uptake phase was calculated. Furthermore, a second liver ROI was drawn, excluding large bile ducts and superimposing bowel loops. This ROI was used to create a hepatic time-activity curve for calculation of the time at which maximal hepatic activity occurred (Raw T peak), as well as the time required for a peak activity to decrease by 50% (Raw T½excretion). All studies were processed twice by the same operator to assess the reproducibility of the hepatic uptake calculations.

STATISTICAL METHODS

Descriptive statistical analysis has been carried out in the present study. Students t test/Chi-square test has been used to find the significance of findings of correlation of scintigraphic findings with clinical parameters.

SIGNIFICANT FIGURES

+ Suggestive significance $0.05<P<0.10$

* Moderately significant $0.01<P \pounds 0.05$

** Strongly significant P£0.01

Statistical software: The Statistical software namely SPSS 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

STUDY DESIGN

A descriptive epidemiological correlation study with 49 patients is undertaken to study the correlation of Hepatic uptake and excretion parameters on scintigraphy with diabetes for detecting early hepatic dysfunction.

The mean age distribution of the patients studied is depicted in the summarization table 1. Majority of the patients in the diabetic population belonged to the age group between 51-60 years, the youngest being aged 45 yrs & the eldest was 64 yrs old. The mean age of this group is approx 54 yrs .In comparison, majority of the control, non diabetic population belonged to the age group between 25-35 years, the youngest being aged 26 yrs & the eldest was60 yrs old. The mean age of this group is approx 35 yrs.

The sex distribution of the study group is shown in summarization table 1. It shows that the studied population consisted of males predominantly (86% in diabetic and 80% in control population)

This was due to the fact that male sex is an additional risk factor for NAFLD and hence this population was considered more suitable for hepatic dysfunction analysis.

DISCUSSION

Liver biopsy is seen as the "gold standard' for diagnosis and staging of NAFLD. Its value in revealing the relationship between inflammation and fibrosis and the presence and relative contribution to other etiologies is well established. However, significant limitations to biopsy exist like post procedure pain, sampling errors, bleeding etc have been reported to occur in 0.57%.[17,18]

All staging systems of NAFLD in widespread use share common failings that have been discussed elsewhere at length.[19,20]

Non-invasive markers of liver steatosis and fibrosis have been most extensively studied in the context of hepatitis C. There has been considerable interest in extending this work into the field of NAFLD because of the increasing prevalence of disease. Currently, identification of severe disease is dependent on liver biopsy. As it is not practical to biopsy every patient with suspected NAFLD, patients are often stratified and selected for biopsy on the basis of transaminases and clinical and anthropometric parameters. This may result in underestimation of significant disease in a patient population with high clinical probability of hepatic steatosis.[21-25]

Multiple reports have stated that patients with histologically proven disease need not necessarily have any alterations in the biochemical parameters. Hence this study was considered appropriate, as it assesses hepatobiliary function non invasively in diabetic patients, when all the biochemical parameters are within the normal range.

Hepatobiliary scintigraphy using either iminodiacetic acid (IDA) analogues has been applied in several animal models of acute and chronic liver disease.[11–13] We chose the IDA analogue Mebrofenin because of its rapid and hepatocellular- specific transport. The hepatic uptake rate of Mebrofenin was chosen as it is directly applicable to the clinical situation, unlike analytical methods applied in other experimental studies using mebrofenin scintigraphy. Data in these studies are derived from time-activity curves, by invasive sampling, or without correction for blood pool activity, or a longer analysis time of up to a few hours is required.

The complexity of these data acquisition methods hinders extrapolation of results to the clinical situation .[26-28] Furthermore, multiple reports have demonstrated a good correlation of hepatic mebrofenin uptake rate with other quantitative liver function tests such as the indocyanine green clearance test.[14] More importantly, the predictive value of mebrofenin uptake rate with postoperative remnant liver function after liver resection was also reported in the clinical setting.[29]

The mebrofenin uptake rate was calculated based on the method described by Ekman et al.,[28] which is considered a true measure of uptake because it takes into account the fluxes between systemic and hepatic blood pools.[27,30] It has been shown in a previous study that calculations with this algorithm are highly reproducible and present only minor inter-subject variation under standardized conditions.[28] The dynamic acquisition was adapted to the slower metabolic rate in humans compared to rats to ensure that all calculations would be derived from the homogeneous blood distribution phase before bile excretion. This was also confirmed in previous studies in control rats, in which rapid hepatic uptake and excretion of mebrofenin occurred according to the first-pass metabolism of mebrofenin kinetics as previously reported,[9,27] In steatotic rats, hepatic mebrofenin uptake rate decreased throughout the period of steatosis progression. There was a significant inverse correlation between the uptake rate and the relevant features present in human steatosis. The decreased mebrofenin uptake rate correlated closely with hepatic fat accumulation, increased inflammation and other

histopathological changes. Also, in the presence of prominent parenchymal inflammation, the correlation between functional and structural parameters remained significant, indicating the sensitivity of mebrofenin uptake in the presence of both steatosis and inflammatory activity. Together with the mebrofenin uptake rate, the Tpeak (Time to peak hepatic activity) and T½ from peak for excretion (time to clear50% of peak hepatic activity) were calculated. Both the Tpeak and T½ peak were prolonged in steatotic rats according to the severity of steatosis but a poorer correlation with liver histopathology and biochemical parameters was observed. Calculations for Tpeak and T½ peak are derived from the time-activity curves without taking into account the clearance rate of injected mebrofenin as a quantitative parameter. Therefore Tpeak and T½ peak were considered as more descriptive parameters of the mebrofenin time-activity curve. The impairment of 99mTc- mebrofenin uptake in the steatotic rats was most likely related to the decreased hepatocyte uptake and excretion capacity, reflected also by the increased plasma bilirubin levels. The blood distribution of mebrofenin can also be influenced by hypoalbuminaemia (the main blood carrier of mebrofenin); however, this was not seen in our study.

In the present study the above mentioned parameters in a diabetic population demonstrated significant decrease in total hepatic uptake expressed as % of injected radioactivity and the reduced rate of uptake depicted by prolonged T½ of the ascending portion of the curve. This was hypothesized to be due to the presence of NIDDM and insulin resistance in these patients contributing to retarded biliary uptake and excretory parameters as mentioned above. Moreover it was found that there was further decrement in the liver function in patients who had more than one risk factor; eg diabetic patients with high BMI and dyslipidemia compared to patients with only diabetes (Refer Table 8). Similarly diabetic patients with high BMI or dyslipidemia demonstrated statistically significant decrease in hepatic uptake compared to patients with diabetes alone (Refer Tables 6 and 7). This further confirms the theory that diabetes, high BMI and dyslipidemia all play contributory roles in the development of hepatic dysfunction.

Furthermore, in the control population (nondiabetics) there was a significant difference in the T peak and T½ of excretion between the older age group versus the younger population suggesting a natural regression in hepatic function with slower uptake and excretory kinetics (Refer Table10). Hence additional studies need to be done to establish an age weighted spread of hepatobiliary uptake and excretory parameters if this modality is to be used in the clinical setting of early hepatic dysfunction.

Mebrofenin is an organic anion conjugated to an acetanilide (a lidocaine analogue) and to 99mTc compounds. The hepatic uptake occurs via the main salt and organic anion transporters (OATP) 1 and 2, and excretion into the bile occurs through multidrug resistance proteins (MRP) 2 and 3.[10,31-33] The increased pro-inflammatory response (measured by histopathology and TNF-á level) observed in other studies on the rat model of steatosis, is the most probable explanation for the impaired mebrofenin uptake and excretion. TNF-á down regulates the expression of bile excretion transporters MRP 2 and 3 and IL-6, also released by liver macrophages, down regulates the liver expression of the uptake transporters OATP 1 and2 but also the expression of MRP 2.[32-35] This negative effect of IL-6 and TNF-á is also reported in mebrofenin excretion in vivo.[36] Furthermore, in steatotic livers, the expression of bile excretion transporters is down regulated and bile secretion is impaired owing to lipid accumulation, progressive inflammation and hepatocellular injury.[3, 24] In rat models, both 99mTc-mebrofenin uptake rate and excretion were delayed, in conjunction with increased plasma bilirubin levels. Therefore, the observed changes cannot be attributable solely to uptake or excretory malfunction. It is more likely that a combination of factors, such as increased pro-inflammatory activity and ATP depletion, contribute to the affected mebrofenin uptake and excretion.

Table 1. Tabulated summary of clinical characteristics of control and diabetic population

Patients	Age (mean)	Total number	Females	Males	BMI
Controls	35.3≠10.6	20	4	16	21.48≠4.5
Diabetics	54≠5.96	29	4	25	25.2≠4.15

The mean age and BMI of the diabetic population was higher than the control population. However, there is significant overlap in these parameters suggesting partial matching of these variables.

Table 2. Tabulated summary of hepatobiliary parameters of control and diabetic population

Patients	Raw T peak (seconds)	Raw T½ excretion (seconds)	T peak (seconds)	T½ ascend (seconds)	% hepatic uptake
Controls	618≧152	2235.8≠757	551.2≠52.4	54.2≠14.3	85.5≠2.6
Diabetics	655.5≠181.9	2417.1≠794.1	557.7≠53.3	70.9≠13.8	76.9≠5.5
P Value	0.2	0.2	0.3	0.0001	$2.8x10^{-9}$

Analysis of the hepatobiliary parameters in the diabetic and control population were carried out and summarized in Table 2 above. The parameters Raw T peak and Raw T ½ refer to the values generated from the hepatic ROI curve alone, while the T peak, T ½ ascend and % hepatic uptake are generated using the hepatic ROI curve and total FOV curves. There was a significant difference in the derived parameters of T ½ of ascend and % hepatic uptake between the diabetic and control population groups. The T ½ ascend was prolonged in diabetics, suggesting slower rate of uptake and the hepatic uptake % was also decreased, suggesting decrement in overall liver function. There was no significant difference in the uncorrected parameters of T peak, T ½ of excretion and derived T peak as can be seen by the p values.

Table 3. Tabulated summary of hepatobiliary parameters in controls with BMI < 20 and >20

Controls	Raw T peak (seconds)	Raw T½ excretion (seconds)	T peak (second)	T½ ascend (second)	% hepatic uptake	Total No.
BMI <20	650≠167	2132.4≠661	553.8≠53	57.9≠14.9	85.3≠2.4	9
BMI >20	591.8≠142	2320.4≠849	549≠54.5	51.1≠13.7	85.6≠2.9	11
P Value	0.4	0.6	0.8	0.3	0.8	

To assess any change in the parameters based on BMI alone the control population was sub grouped into controls with BMI> 20 and <20. P values generated for Raw T peak, Raw T ½, T peak ,T ½ ascend and % uptake are 0.4, 0.6, 0.8, 0.3 and 0.8 respectively. To be considered significant a P value < 0.05 was deemed necessary.

There was no significant difference between the two subgroups of normal and high BMI patients in the control population on any of the parameters generated in hepatobiliary scintigraphy.

Table 4. Tabulated summary of hepatobiliary parameters of diabetics with BMI < 25 and > 28

Diabetics	Raw T peak (seconds)	Raw T½ excretion (second)	T peak (second)	T½ ascend (second)	% hepatic uptake	BMI	Age	Total No.
BMI > 28	628.5	2360.1	553.9	87.7	69.57	30.2	55.7	7
BMI < 25	610.9	2282	568.1	64.6	79.1	20.7	52.9	11
P Value	0.8	0.8	0.5	$6.4x10^{-6}$	$9.7x10^{-6}$			

The significance of BMI was analyzed in the diabetic population by sub grouping them into two groups with normal BMI i.e.. < 25 and BMI > 28 i.e. overweight category. Significant differences (p< 0.01) were noted in the derived parameters of rate of tracer uptake (T ½ asc) and total % of injected activity taken by the liver between diabetic patients with a BMI >28 and BMI < 25. The other parameters generated from the uncorrected hepatic curve were not significant (p> 0.05), though there was a trend of delayed T peak and delayed T ½ of excretion in the high BMI group.

Table 5. Tabulated summary of hepatobiliary parameters of diabetics with age < 54 and >55

Diabetics	Raw T peak (seconds)	Raw T½ excretion (second)	T peak (second)	T½ ascend (second)	% hepatic uptake	BMI	Age	Total No.
Age > 55	669	2350.9	543.8	74.8	75.1	26.5	60	12
Age < 54	645.9	2463.8	567.4	68.1	78.2	24.2	49.1	17
P value	0.7	0.7	0.2	0.2	0.1			

The significance of age was analyzed in the diabetic population by sub grouping them into < 54 and > 55 years categories. None of the parameters generated were found to be significantly different between the diabetics aged > 55 years versus diabetics aged < 55 years (p values in table 5). However there was a trend of slower uptake rate as seen by delayed T peak and T ½ of ascend in the older age group. In addition there was decreased hepatic uptake % in the older age group.

Table 6. Tabulated summary of hepatobiliary parameters of diabetics with BMI < 25 and > 25.

Diabetics	Raw T peak (seconds)	Raw T½ excretion (second)	T peak (second)	T½ ascend (second)	% hepatic uptake	BMI	Age	Total No.
BMI > 25	682.7	2450	551.2	74.7	75.6	27.8	54	18
BMI <25	610.9	2282	568.1	64.6	79.1	20.7	52.9	11
P value	0.3	0.4	0.3	0.0 4	0.0 4			

The significance of BMI was analyzed in the diabetic population by dividing them into subgroups with BMI < 25 and > 25. Statistically significant (p<0.05) difference was noted in the derived parameters of ascending T ½ and % uptake by the liver between diabetics with BMI > 25 and < 25.

Though no significant difference was noted in the directly derived hepatic ROI curve parameters of T ½ of uptake and excretion and T peak, they were both delayed in the high BMI group.

Table 7. Tabulated summary of hepatobiliary parameters of diabetics with and without dyslipidemia

Diabetics	Raw T peak (seconds)	Raw T½ excretion (second)	T peak (second)	T½ ascend (second)	% hepatic uptake	BMI	Age	Total No.
Dyslipidemia	713.3	2579.7	563	74.4	74.3	26.95	54.4	15
Nil	593.6	2242.9	551.9	67.1	79.6	23.3	52.7	14
P value	0.07	0.2	0.6	0.15	0.006			

The significance of dyslipidemia was analyzed in the diabetic population by dividing them into subgroups with and without dyslipidemia. Significant difference ($p<0.01$) in % of injected dose taken up by the liver was noted between the diabetic with dyslipidemic group compared to the diabetics with no dyslipidemia group. None of the other differences in hepatobiliary parameters were found to be statistically significant. There was a nonsignificant trend of delayed T peak and longer T ½ of ascend in the dyslipidemic group noted.

Table 8. Tabulated summary of hepatobiliary parameters of diabetics with and without dyslipidemia and BMI > 25

Diabetics	Raw T peak (seconds)	Raw T½ excretion (second)	T peak (second)	T½ ascend (second)	% hepatic uptake	BMI	Age	Total No.
Dyslipid +BMI >25	699.2	2561	560.4	76.4	73.4	28.7	53.9	12
No dyslipid + BMI< 25	551	2142	566.2	64.1	79.5	20.96	51.6	8
P value	0.03	0.2	0.7	0.04	0.004			

The significance of dyslipidemia and high BMI was analysed in the diabetic population by dividing them into subgroups with and without these added risk factors. The tabulated summary is given in above. In a diabetic population with added risk factors of dyslipidemia and high BMI there was a significant difference in some of the hepatobiliary parameters compared to the population of diabetics with no added risk factors. In addition to the % uptake which was lower ($p<0.01$), the rate of uptake was also lower ($p<0.05$) and the time to peak was significantly delayed in the diabetics ($p<0.05$) with dyslipidemia and high BMI compared to their diabetic counterparts without these added risk factors.

Table 9. Tabulated summary of hepatobiliary parameters in diabetics with dyslipidemia and BMI>25 versus control population

Diabetics	Raw T peak (seconds)	Raw T½ excretion (second)	T peak (second)	T½ ascend (second)	% hepatic uptake	BMI	Age	Total No.
Dyslipid +BMI >25	699.2	2561	560.4	76.4	73.4	28.7	53.9	12
Controls	618	2236	551.2	54.2	85.5	21.4	35.3	20
P value	0.12	0.15	0.3	0.003	$3.7x10^{-6}$			

The significance of hepatobiliary parameters in diabetics with dyslipidemia and high BMI were analyzed in comparison to the control, non diabetic population. In comparison to the control population the diabetics with added risk factors demonstrated a statistically significant (p value < 0.01) decrement in hepatic uptake % and the rate of liver uptake assessed by T ½ of ascend .In addition there was a non significant trend towards delayed T peak and delayed excretion T ½ in the diabetics with the risk factors.

Table 10. Tabulated summary of hepatobiliary parameters in controls with age < 34 years and > 35 years

Diabetics	Raw T peak (seconds)	Raw T½ excretion (second)	T peak (second)	T½ ascend (second)	% hepatic uptake	BMI	Age	Total No.
Age <34	606	1826.4	552.35	53.02	84.4	23.96	27.4	10
Age >35	630	2645.3	580	55.354	86.6	19	43.2	10
P value	0.7	0.016	0.014	0.7	0.06			

The significance of hepatobiliary parameters in controls with age group < 34 and > 35 years were analyzed. The tabulated summary is given in the Table 10 above. It is noted that there were statistically significant differences in the T ½ of excretion and derived T peak, both of which were delayed in the older population. This is in spite of the older age group having a lower average BMI, suggesting age to be an independent variable in hepatic function.

CONCLUSION

There were significant differences in the parameters generated from the normalized hepatic curve in the diabetic population compared to non diabetic control population. The T ½ ascend representing the rate of uptake of the tracer by the liver was prolonged in diabetics suggesting a slower rate of uptake in this population. In addition there was a significant decrement in total uptake % by the liver of the total injected activity, suggesting deranged global hepatic function. These findings suggest early hepatic dysfunction in the diabetic population even when the biochemical parameters of hepatic function, synthesis and excretion are with normal limits.

More importantly in diabetics with added risk factors of dyslipidemia and high BMI there was a further decrement in these two parameters compared to a diabetic population without any added risk factors or control population. These findings suggest high BMI and dyslipidemia to be additional prognostic indicators of hepatic dysfunction in diabetes and thus could play a significant contributory role in hepatic derangement. The T peak and T ½ of excretion were significantly delayed even within the control, non diabetic population when comparing them on the basis of age, suggesting a natural tendency towards slower uptake and excretory kinetics in the aged. In conclusion, the clinical utility of hepatobiliary scintigraphy in assessing

early hepatic dysfunction in a susceptible population is promising; however further large scale clinical studies are warranted to establish normal database of these parameters based on age and sex, before they can be applied in a clinical setting.

LIMITATIONS OF THE STUDY

Though there is derangement of hepatic function as analyzed on scintigraphy, these could not be directly verified histopathologically to correlate with the presence and severity of hepatic steatosis. The controls and study groups were not evenly matched with regards to age and BMI; hence the individual predictive value of these parameters could not be independently assessed. We would have liked to study a larger number of cases in each sub group category of BMI, age, sex and dyslipidemia to establish normal values and a database for further evaluative studies. We would have liked to compare the scintigraphic findings with other non invasive imaging modalities like CT and ultrasound to assess the intermodality correlation in diagnosing steatosis. The algorithm for generation of the hepatobiliary parameters described by Ekman et.al[28] in their study could not be directly incorporated in the present study due to software limitations .Hence an alternative method developed through personal communication was used for this purpose as mentioned under the Materials and Methods section.

END NOTE

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Conflict of Interest and Declaration:

There are no competing interests, financial support or prior publication to declare. Permission has been granted from all the authors and institution where work was carried out.

Cite this article as: Ajit S Shinto, A D Puranik, P Singa, G Nair. HIDA Scintigraphy Diagnoses Early Hepatic Dysfunction in Diabetic Population. Kerala Medical Journal. 2012 Mar 30;5(1):3-11

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