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Assessment of Pancreatic Function in ß Thalassemia Major Patients

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ABSTRACT

Background: In patients with ß thalassemia major, iron overload results in functional and structural changes in various organs, especially the heart, liver and endocrine glands. The pancreas is a major site of iron deposition with severe degrees of fibrosis developing in more advanced cases.

Objective: The study attempted to assess the prevalence and characteristics of pancreatic damage in a group of thalassemic patients subjected to chronic transfusional therapy.

Patients and Methods: Ninety eight patients (52 males and 46 females), previously diagnosed to have homozygous ß-thalassemia and followed-up at the Hematology Clinic of the New Cairo University Children Hospital, were randomly selected to participate in this study. Their age ranged between 5 and 32 years. None of the patients had clinical evidence of chronic pancreatitis, chronic diarrhea, malabsorption, renal failure or diabetis mellitus. The patients were further divided into 3 subgroups according to their age. Study parameters included: Serum amylase and lipase, fasting blood glucose, serum ferritin, aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT), creatinine and blood urea nitrogen (BUN), cholesterol and triglyceride.

Results: 28 patients (28.6%) showed abnormally elevated serum lipase with a range between 34 U/L to 91 U/L while 6 patients (6.1%) showed elevation in both serum lipase and amylase (range between 35 U/L to 189 U/L and between 106 U/L to 185 U/L respectively). Only 1 patient showed elevated amylase (1%) with a value of 121 U/L. Serum amylase and lipase showed a highly statistical significant increase among the group of abnormal enzymes compared to those with normal value ($p<0.001$, $p<0.001$ respectively). Blood glucose did not show any significant difference among the two groups. ALT and AST showed a statistically significant difference between the two groups ($p<0.05$). No clinical significance was observed for the patients with abnormal enzymes. In group 1 (age<12 years), 25% (5 patients) showed elevated amylase and lipase. In group 2 (age between 13-18 years), 32.1% (9 patients) showed elevated lipase only while in group 3 (age>18 years), 40% (20 patients) showed elevated amylase and lipase. Serum amylase showed a statistically significant difference between the 3 subgroups ($p<0.05$) while serum lipase showed non significant difference. On correlating serum lipase to all other parameters, lipase showed a significantly high correlation to amylase and ALT and AST ($p<0.001$, $p<0.001$, $p=0.002$ respectively). Neither serum lipase nor amylase was significantly correlated to serum ferritin or age of the patients. Serum amylase was significantly correlated to AST and ALT ($p=0.006$, $p=0.007$ respectively).

Conclusion: Our results suggest that the exocrine pancreas in ß thalassemia major patients is functionally affected in a high percentage of patients with iron overload though no clinical manifestations were observed. The possible clinical implications of this damage remain to be clarified. Further studies including more tests evaluating both the endocrine and exocrine pancreas functionally and morphologically are recommended to detect changes as early as possible.

Key Words: *ß Thalassemia major – Pancreatic function.*

INTRODUCTION

Thalassemia is considered the most common genetic disorder world wide, about 3% of the world's population carry ß-thalassemia gene **[1]** and about 60.000 children with thalassemia major are born annually **[2]**.

These ß-thalassemia patients suffer from iron overload due to increased iron absorption and regular blood transfusions. This iron results in structural and functional changes in various organs, especially the heart, liver and endocrine system resulting in progressive organ failure **[3]**.

Postmortem studies in thalassemic patients have shown that the pancreas is among the organs most severely affected by iron accumulation and fibrosis however, the frequency and the clinical relevance of these findings are little known **[4]**.

While several studies have documented an involvement of the endocrine pancreas in this disease, little is known about alterations of the exocrine pancreas **[5]**.

The aim of this study was to assess the prevalence and characteristics of exocrine pancreatic changes in *ß*-thalassemia major patients subjected to chronic transfusional therapy.

MATERIALS AND METHODS

Patients:

Ninety eight patients, previously diagnosed to have homozygous *ß*-thalassemia and followed-up at the Hematology Clinic of the New Cairo University Children Hospital, were randomly selected to participate in this study.

All the patients were on standard therapy consisting of chronic blood transfusions with desferroxamine (20-40 mg/kg/day) as iron chelator. Among the ninety eight patients recruited for this study, 52 were males and 46 females whose age ranged between 5 and 32 years with a mean age of 18.3±6.4 years and a mean hemoglobin of 7.725±1.3 g/dl.

None of the patients had clinical evidence of chronic pancreatitis, chronic diarrhea, malabsorption, renal failure or diabetis mellitus.

The medical charts of the recruited patients were reviewed. Frequency of blood transfusion and chelation therapy was recorded. A complete physical exam was performed for all patients.

The patients were further divided into 3 subgroups according to their age:

Group (1): Included patients aged 5 to 12 years, mean age of 9±2.06 years accounting for 20.4% of the total number of patients (20 patients).

Group (2): Included patients' age ranging from 13-18 years old and mean age of 15.3±1.7 years accounting for 28.6% (28 patients).

Group (3): Included patients older than 18 years with a mean age of 23.6±3.2 years and accounting for the remaining 51% (50 patients).

Study parameters included:

- Serum amylase and lipase.
- Fasting blood glucose.
- Serum ferritin.
- Aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT).
- Creatinine and BUN.
- Cholesterol and triglyceride.

Collection of blood samples and assays:

Venous blood was obtained without stasis. Blood allowed to clot & serum separated and stored at -20°C till the assay time.

All the analytes were measured on Beckman Synchron CX9 PRO autoanalyzer with Beckman Kits. Serum pancreatic lipase activity was measured based on a timed enzymatic rate method in which a diglyceride is the substrate. The diglyceride substrate is hydrolyzed by pancreatic lipase in the sample to 2-monoglyceride and fatty acid. A sequence of four coupled enzymatic steps causes the oxidative coupling of N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (TOOS) with 4-aminoantipyrine (4-AAP) to form a red quinine diimine dye. The reference value is 3-32 U/L **[6]**.

Serum pancreatic amylase activity was measured based on an immuno-inhibition method using two monoclonal antibodies with two incubation steps. In the first incubation step, the activity of human salivary α-amylase is inhibited by two monoclonal antibodies which do not affect pancreatic α-amylase. After a second incubation with the substrate, the α-amylase cleaves the substrate (4,6-Ethylidene-G7p-Nitrophenol) into fragments and these fragments are further hydrolyzed by α-glucosidase to yield p-nitrophenol and glucose with reference interval 28-100 U/L **[7]**.

Serum AST, ALT, Alkaline phosphatase and BUN activities were measured based on a kinetic rate method, Creatinine was measured based on a modified-rate Jaffé method, Cholesterol and Triglyceride were measured based on a timed-endpoint method.

Data management and statistical methods:

The data was coded and entered using the statistical package SPSS version 13. The data were summarized using mean and standard deviation for quantitative data and percent for qualitative data. The difference between studied groups were assessed using the Chi-square and Fisher's Exact tests for qualitative data and

independent samples *t*-test and ANOVA (analysis of variances) for quantitative data. The *p*-value was considered significant at a level <0.05.

RESULTS

Twenty eight patients (28.6%) showed abnormally elevated serum lipase with a range between 34 U/L to 91 U/L while 6 patients (6.1%) showed elevation in both serum lipase and amylase (range between 35 U/L to 189 U/L and between 106 U/L to 185 U/L respectively). Only 1 patient showed elevated amylase (1%) with a value of 121 U/L (Fig. 1).

Serum amylase and lipase showed a highly statistical significant increase among the group of abnormal enzymes compared to those with normal value ($p<0.001$, $p<0.001$ respectively). Blood glucose did not show any significant difference among the two groups. ALT and AST showed a statistically significant difference between the two groups ($p<0.05$) (Table 1).

No clinical significance was observed for the patients with abnormal enzymes.

In group 1, 25% (5 patients) showed elevated amylase and lipase.

In group 2, 32.1% (9 patients) showed elevated lipase only while in group 3, 40% (20 patients) showed elevated amylase and lipase.

Serum amylase showed a statistically significant difference between the 3 subgroups ($p<0.05$) while serum lipase showed non significant difference (Table 2).

On correlating serum lipase to all other parameters, lipase showed a significantly high correlation to amylase and ALT and AST ($p<0.001$, $p<0.001$, $p=0.002$ respectively) (Table 3).

Neither serum lipase nor amylase was significantly correlated to serum ferritin or age of the patients. Serum amylase was significantly correlated to AST and ALT ($p=0.006$, $p=0.007$ respectively).

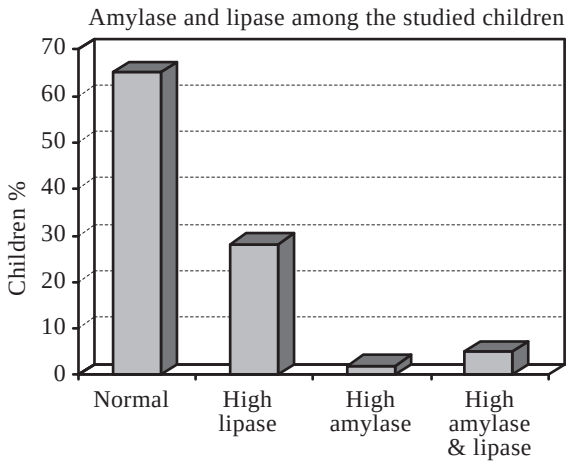

Fig. (1): Shows serum amylase and lipase among the thalassemic patients.

Table (1): Shows comparison between the mean and standard deviation of the measured parameters in patients with normal and high lipase and amylase activities.

	Patients with high lipase and amylase activity No.=34 (34.7%) 21 males & 13 females		Patients with normal lipase activity No.=64 (65.3%) 31 males & 33 females		
	Mean	SD	Mean	SD	*p* value
Age (years)	18.9	5.7	17.9	6.8	0.418
Lipase (U/L)	48.9	27.7	21.5	5.5	<0.001*
Amylase (U/L)	79.4	28.3	56.6	22.3	<0.001*
ALT (U/L)	89.7	58.1	68.1	41.4	0.036*
AST (U/L)	99.7	59.2	73.3	42.5	0.013*
BUN (mmol/L)	4.2	0.99	3.9	1.03	0.120
CRE (umol/L)	35.5	8.4	33.9	9.7	0.432
Glucose (mmol/L)	5.4	1.5	5.8	4.2	0.636
Cholesterol (mmol/L)	2.8	0.62	2.9	0.73	0.703
Triglycerides (mmol/L)	2.1	0.9	1.8	1.1	0.219
Ferritin (ng/mL)	4383	2013	3960	3290	0.495

ALT : Alanine aminotransaminase. BUN : Blood urea nitrogen. * Significant: <0.05.
AST : Aspartate aminotransaminase. CRE : Creatine.

Table (2): Shows comparison between the mean and standard deviation of the lipase and amylase in the three groups of the patients divided according to their age.

	Group 1 5-12 years No.=20 (20.4%)		Group 2 13-18 years No.=28 (28.6%)		Group 3 19-32 years No.=50 (51%)		*p* value
	Mean	SD	Mean	SD	Mean	SD	
Age	9.05	2.06	15.4	1.7	23.6	3.2	<0.001*
Lipase (U/L)	36.6	40.6	29.1	12.2	29.9	12.7	0.453
Amylase (U/L)	77.4	32.7	58.4	19.1	62.08	26.8	0.039*

Significant: <0.05.

Table (3): Shows the correlation between lipase and other parameters.

	Lipase		
	"*r*" value	*p* value	Siginificance
Age (years)	–0.073	0.476	Non
Amylase (U/L)	0.670	<0.001	High
ALT (U/L)	0.349	<0.001	High
AST (U/L)	0.304	0.002	Sig.
BUN (mmol/L)	0.026	0.798	Non
CRE (umol/L)	–0.063	0.536	Non
Glucose (mmol/L)	0.044	0.664	Non
Cholesterol (mmol/L)	0.048	0.641	Non
Triglycerides (mmol/L)	0.173	0.088	Non
Ferritin (ng/mL)	0.037	0.720	Non

DISCUSSION

The use of regular frequent blood transfusions in thalassemia major has improved the span and quality of life in these patients, but it leads to iron overload which frequently causes endocrine problems. The pancreas is a major site of iron deposition with severe degrees of fibrosis developing in more advanced cases **[8]**.

Impaired structure and function of the exocrine pancreas has been reported in patients with ß-thalassemia major. The mechanism underlying exocrine pancreatic damage in these patients is probably the infiltration of the acinar tissue with iron which causes damage to the endocrine components of the pancreas, the islets of langerhans and ß-cells in particular **[9]**.

Though measurements of both lipase and amylase are commonly used as aids in the diagnosis of pancreatic diseases, 19% of pancreatitic patients present with normal serum amylase. Thus, leaving lipase as the main hematochemical marker of pancreatic insult. **[10]**.

It was previously reported that lipase activity may be increased in the absence of clinically overt pancreatitis in thalassemic patients. This abnormality was thought to reflect a break down of the acinar-blood barrier, and their proenzymes into the blood **[11]**.

Twenty eight patients showed elevated serum lipase, six patients showed elevation in both enzymes while only one patient showed elevated serum amylase. On comparing the group of abnormal enzymes to the second group, highly significant results were observed ($p<0.001$, $p<0.001$ respectively). No subnormal results were detected among our patients.

Our results disagree with previous studies reporting a decrease in lipase enzyme in 60% and 33% respectively **[9,11]**. It was reported that on studying pancreatic enzymes (trypsin, lipase, amylase and elastase) among a group of thalassemic patients, 40% of the patients had abnormally low concentrations of one on more enzymes most commonly of trypsin and lipase being the most sensitive indicators of pancreatic insufficiency **[12]**.

During the earlier phase of pancreatic damage, pancreatic enzymes probably leak directly into the circulation causing the enzyme to increase in serum. This phase is then followed by a progressive destruction of the acinar tissue and a decline in pancreatic enzyme concentrations. These data thus confirm that the exocrine

pancreatic reserve in these patients is probably subnormal. It may be argued that the concentration of the pancreatic enzymes in serum does not reflect the extent of enzyme secretion into duodenum **[13]**.

A previous study on a group of 30 thalassemics, treated with continuous subcutaneous desferroxamine infusion for a mean period of 30 months, showed normal levels of amylase and lipase suggesting the role of iron overload on the pancreatic damage **[14]**.

The group of high lipase showed significantly higher liver enzymes (ALT and AST) than the other group (p=0.036, 0.013 respectively). This agrees with a previous study reporting that the severity of pancreatic damage is related to the progress of hepatic fibrosis and iron overload **[12]**. In a study estimating lipase activity in primary cystic fibrosis, it did not differ in any stage of the disease suggestive that pancreatic damage is not dependent on and does not parallel histological changes in the liver **[15]**. The change in cholesterol and triglycerides levels was not statistically different among our 2 groups. It was previously reported that in thalassemics with low lipase, total cholesterol decreased, thus this enzymatic activity had a major role in determining the level of lipids **[16]**.

No correlation was found between serum lipase and serum ferritin in our patients which agrees with a previous study reporting no correlation between pancreatic enzymes values and mean serum ferritin values or mean blood consumption over 3 years **[17]**. Individual tissue susceptability to iron may determine the eventual damage to pancreas as well as the degree of iron overload **[9]**.

On dividing the patients into 3 subgroups serum amylase showed statistically significant difference according to age, while no significant difference was detected between serum lipase levels. Our results disagree with a previous study denoting that the severity of pancreatic changes increased in old patient with a longer history of transfusion **[12]**.

Serum immunoreactive trypsin was supranormal in thalassemics beyond 12 years of age and subnormal in older ones **[9]**. During earlier phase of pancreatic damage, pancreatic enzymes increase to be followed by a progressive destruction of the acinar tissue and decline in the pancreatic secretion **[11]**.

Transfusion dependent thalassemic patients having major impairment of the exocrine pancreatic functions show increased echogenicity by ultrasonography **[12,18]** and higher signal intensity of the pancreas because of fatty replacement of the parenchyma by magnetic resonance imaging **[19]**.

From a clinical point of view, the possible development of a severe exocrine pancreatic insufficiency in these patients raises the question of whether this complication impairs the digestion of food and causes some degree of malnutrition especially in patients with more advanced disease **[12]**.

Though our study was not a case control study, our results suggest that the exocrine pancreas in ß thalassemia major patients is functionally affected in a high percentage of patients with iron overload inspite of absence of clinical manifestations of pancreatic insult. The possible clinical implications of this damage remain to be clarified. Further studies including more tests evaluating both the endocrine and exocrine pancreas functionally and morphologically are recommended to detect changes as early as possible.

REFERENCES

1- Bernard G, Forget. Thalassemia Syndromes. Hematology basic principle and practice. 3rd edition, Churchill Living stone, New York. 2000, 485-510.

2- Modell B, Khan M, Darlison M, et al. A national register for surveillance of inherited disorders. Bulletin of the World Health Organization. 2000, 79: 1006-1013.

3- Hoffbrand AV, Wonke B. Iron chelation therapy. Journal of Int Med. 1997, 242: 37-41.

4- Sonakul D, Pacharee P, Wasi P, Fucharoen S. Cardiac pathology in 47 patients with beta-thalassemia / hemoglobin E. Southeast Asian J Trop Med Public Health. 1984, 15: 554-563.

5- Mc Intosh N. Endocrinopathy in thalassemia major. Arch Dis Child. 1976, 51: 195-201.

6- Imamura S, Hirayama T, Arai T, Takao K, Misaki H. An enzymatic method using 1,2- diglyceride for pancreatic lipase test in serum. Clin Chem. 1989, 35: 1126.

7- Gerber M, Wulff K. Laboratoriums-medizin. 1988, 12: 110-113.

8- Suvarna J, Ingle H, Deshmukh CT. Insulin resistance and beta cell function in chronically transfused patients of thalassemia major. Indian Pediatrics. 2006, 43: 393-400.

9- Hussain M, Dandona P, Fedail SS. Serum immunoreactive trypsin in beta-thalassemia major. J Clin Pathol. 1981, 34 (9): 970-971.

10- Diani G, Poma G, Novozzi F, Zanirato S, Porta C, Moroni M, d' Eril G, Mortti R. Increased serum lipase with associated normoamylasemia in cancer patients. Clinical Chemistry. 1998, 44 (5): 1043-1045.

11- Junglee D, Katrak A, Hoffbrand AV. Pancreatic Lipase in serum of patients with ß-thalassemia major. Clin Chemistry. 1983, 29 (11): 2003-2004.

12- Gullo L, Corcioni E, Brancati C, Bria M, Pezzilli R, Sprovieri G. Morphologic and functional evaluation of the exocrine pancreas in ß thalassemia major. Pancreas. 1993, 8 (2): 176-180.

13- Taussig LM, Wolf RO, Woods RE, et al. Use of serum amylase isoenzymes in evaluation of pancreatic function. Paediatrics. 1974, 54: 229-234.

14- Kruger N, stubbe P, Tillmann W, Schroter W. Continuous subcutaneous desferroxamine infusions in thalassemia major. Improvement of glucose tolerance. Dtsch Med Wochenschr. 1986, 111 (18): 698-701.

15- Fonseca V, Epstein O, Katrak A, Junglee D, Mikhailidis DF. Serum immunoreactive trypsin and pancreatic lipase in primary biliary cirrhosis J Clin Pathol. 1986, 39: 638-640.

16- Cherchi GM, Baggi MA, Coinu R, Deiana MG, Pacifico A, Maioli M. Post-heparin lipase activity in beta thalassemia. Biol Sper. 1983, 59 (11): 1739-1943.

17- Montalto G, D'Angelo P, Locasto P, Carroccio A, Soresi M, Midiri M, Malizia R, Seafidi V. Serum and fecal pancreatic enzymes in beta thalassemia major. Int J Pancreatol. 1997, 22 (2): 131-135.

18- Theochari M, Ioannidou D, Nounopoulos H, Bouloukos A, Papadogiannis M, Katsikari M, Karpathios T, Bartsocas CS. Ultrasonography of the pancreas, as a function index, in children with beta-thalassemia. J Pediatr Endocrinol Metab. 2000, 13 (3): 303-306.

19- Midiri M, Lo Casto A, Sparacia G, D' Angelo P. Malizia R, Finazzo M, Montallo G, Solbiati L, Lagalla R, De Maria M. MR imaging of pancreatic changes in patients with transfusion dependent beta thalassemia major. AJR Am J Roentgenol. 1999, 173 (1): 187-192.