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Plasma biotinidase as a predictor for hepatic synthetic function impairment

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Abstract

Background: Biotinidase, the biotin recycling enzyme**,** synthesized by the liver and secreted into the blood. The primary function of this enzyme is to cleave biotin from biocytin, thereby conserving biotin for use as a cofactor for four biotin-dependent carboxylases .The carboxylases serve important roles in intermediary metabolism**.** Certain liver diseases can cause decreased synthesis of liver proteins or enzymes, including biotinidase, thereby reducing biotinidase activity in serum.

Aim of the work: The aim of this study was to assess plasma biotinidase level in pediatric patients with chronic liver disease and to investigate the relationship between plasma biotinidase activity and the degree of compensation of the chronic liver disease so that can identify the possible role of plasma biotinidase as a predictor for hepatic synthetic function impairment .

Patients and methods: A total of 29 pediatric patients (20 male and 9 female) with chronic liver disease were included in the study (16 compensated and 13 decompensated). Mean age were 3.3 ± 3.2 SD years that range between 0.08-13 years. The control group consisted of 40 healthy age and sex matched subjects (25 male and 15 female) with the mean age 3.8 ± 3.5 SD years, range 0.08-16 years. The total duration of the study was thirty months from July 1st 2011 up to December 31st 2013). Assay of plasma biotinidase level was done by ELIZA, using commercially available assay kit.

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Results: Plasma biotinidase activities were found 4.4±1.2, 8.2±3, 8.7±0.7 IU\L (mean±SD) (decompensated pediatric patients, compensated pediatric patients and control group respectively). Plasma biotinidase levels were statistically significant lower in pediatric patients with decompensated liver disease versus pediatric patients with compensated liver disease and control group ($p<0.001$) with no statistically significant difference in plasma biotinidase level between pediatric patients with compensated liver disease and control group. There was statistically significant inverse correlation between plasma biotinidase levels versus prothrombin time "PT" and INR ($p<0.05$) and statistically significant positive correlation between plasma biotinidase levels versus prothrombin concentration "PC" and albumin ($p<0.001$).

Conclusion: The findings of this study suggested that biotinidase level can be used as a predictor for hepatic synthetic function impairment.

Recommendation: The effect of biotin (vitamin B 7) supplementation in patients with decompensated liver disease to protect them against biotin deficiency as biotinidase is required for release of free active biotin to the body to perform its role in the metabolism need further study.

Introduction

Biotinidase, the biotin recycling enzyme is ubiquitously distributed and occurs at high levels in the liver, serum, and kidney. It is synthesized by the liver and secreted into the blood. The primary function of this enzyme is to cleave biotin from biocytin, thereby conserving biotin for use as a cofactor for four biotin-dependent carboxylases (propionyl CoA carboxylase (PCC), β-methylcrotonyl CoA carboxylase (MCC), pyruvate carboxylase (PC), and acetyl CoA carboxylase (ACC)) [(1)]. The carboxylases serve important roles in intermediary metabolism and their impairment can cause abnormalities in fatty acid synthesis, amino acid catabolism, and gluconeogenesis, resulting in the accumulation of abnormal organic acids. The absence or deficiency of biotinidase impairs the recycling of free biotin, thereby slowing the functioning of the biotin-dependent carboxylases. The liver is thought to be the source of serum biotinidase [(2)].

A wide spectrum of clinical manifestations belongs to biotin deficiency, including abnormalities of the neurological, dermatological, immunological, and ophthalmological systems has been reported[(3)]. Symptoms include ataxia, hypotonia, developmental delay, conjunctivitis, skin rash and alopecia, seizures, hearing loss, breathing problems and optic atrophy. The expression of these symptoms is variable and may be related to dietary biotin intake and the degree of residual Biotinidase enzyme activity. If untreated, patients will develop metabolic ketoacidosis and organic aciduria. Most patients with Partial biotinidase deficiency exhibit chiefly the cutaneous symptoms [(3)].

As biotinidase is synthesized in the liver [(4)], certain liver diseases can cause decreased synthesis of liver proteins or enzymes, including biotinidase, thereby reducing its activity in serum [(5)].

The aim of this study is to:

1) Assess plasma biotinidase level in pediatric patients with chronic liver disease.
2) Correlate the plasma biotinidase activity with the degree of compensation of the chronic liver disease so that can identify the possible role of plasma biotinidase as a predictor for hepatic synthetic function impairment.

Patients and methods:

This study is a case-control hospital based study was carried out on 29 pediatric patients (20 male and 9 female) with chronic liver disease (16 compensated and 13 decompensated). Mean age 3.3 ± 3.2 SD years, range 0.08-13 years, 7 patients had non-metabolic chronic liver disease (3 had secondary biliary cirrhosis, 1 autoimmune hepatitis, 3 had neonatal

hepatitis with positive TORCH) and 22 patients had metabolic liver disease (5 patients had Wilson's disease, 2 patients had galactosemia, 2 patients had Gaucher disease, 2 patients had Niemann-Pick disease, 1 patient had mucopolysaccharidosis type-I "Hurler syndrome", 1 patient have tyrosinemia type-I while the remaining 9 patients had unclassified metabolic disorder), attending the department of pediatrics of Assiut, Sohag and Qena university hospitals after approval of the university hospital ethical committee . Forty healthy age and sex matched subjects (25 male and 15 female) with the mean age of 3.8 ± 3.5 SD years, range 0.08-16 years were selected as a control group **(table. 1) .** The total duration of the study was thirty months from July 1st 2011 up to December 31st 2013). All patients and controls were subjected to the following laboratory investigations:

1. Plasma collection and storage**:** 2 mls venous blood were drawn on EDTA for assay of biotinidase ,they were then centrifuged at 3500 rpm for 15 min at 4 °C and the plasma were transferred into 1 ml cryotubes, and stored at -80 °C for later analyses.
2. Plasma albumin, alkaline phosphatase "ALP" , bilirubin (total and direct) , liver enzymes (ALT and AST) and prothrombin time and concentration were taken already from the file of the patients; which were estimated using [Cobas C311 (Roche diagnostics, Germany)]. Prothrombin time and concentration were measuredusing (BFT-II analyzer, Germany).
3. Measurement of plasma biotinidase [using enzyme-linked immune-sorbent assay (ELISA) multiskan EX microplate photomter, thermo scientific, STAT FAX-2100, USA] according to manufacturer protocol.

Statistical analyses:

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12) as follows [5]**:**

- **Description** of quantitative variables as mean, SD and range
- **Description** of qualitative variables as number and percentage
- **Chi-square** test was used to compare qualitative variables between groups.
- **Unpaired t-test** was used to compare quantitative variables, in parametric data (SD<50% mean)'
- **One way ANOVA (analysis of variance)** test was used to compare more than two groups as regard quantitative variable (LSD =least significant difference)
- **Correlation co-efficient** test was used to rank variables positively or inversely.
- P value >0.05 insignificant
- P<0.05 significant

Results:

Table (1) Comparison between the studied groups as regard age & sex:

Variables	Cases n=29	Control group n=40	p-value
Gender Male Female	 20(69%) 9(31%)	 25(62.5%) 15(37.5%)	>0.05

Age			
<1 year	15(51.7%)	16(40%)	
1-<6 years	8(27.6%)	16(40%)	
6-<12 years	3(10.3%)	4(10%)	
12+ years	3(10.3%)	4(10%)	>0.05
Age(mean±SD)	3.3±3.2	3.8±3.5	
Range of age (years)	0.08-13	0.08-16	

Table (2): Mean ± SD and p-value of plasma biotinidase and different parameters of hepatic synthetic functions in patients with compensated versus patients with decompensated liver disease:

Parameters of hepatic synthetic functions	Pediatric patients with compensated liver disease n=16	Pediatric patients with decompensated liver disease n=13	p-value
Prothrombin time "PT", seconds (Mean ± SD)	13.5±2	25±3.6	**<0.05**
Prothrombin concentration "PC", % (Mean ± SD)	89±30	49±23	**<0.001**
INR (Mean ± SD)	1.1±0.4	2.5±1.2	**<0.05**
Plasma Biotinidase Level,IU\L (Mean ± SD)	8.2±3	4.4±1.2	**<0.001**
Albumin gm\dl (Mean ± SD)	4±0.6	2.3±0.3	>0.05

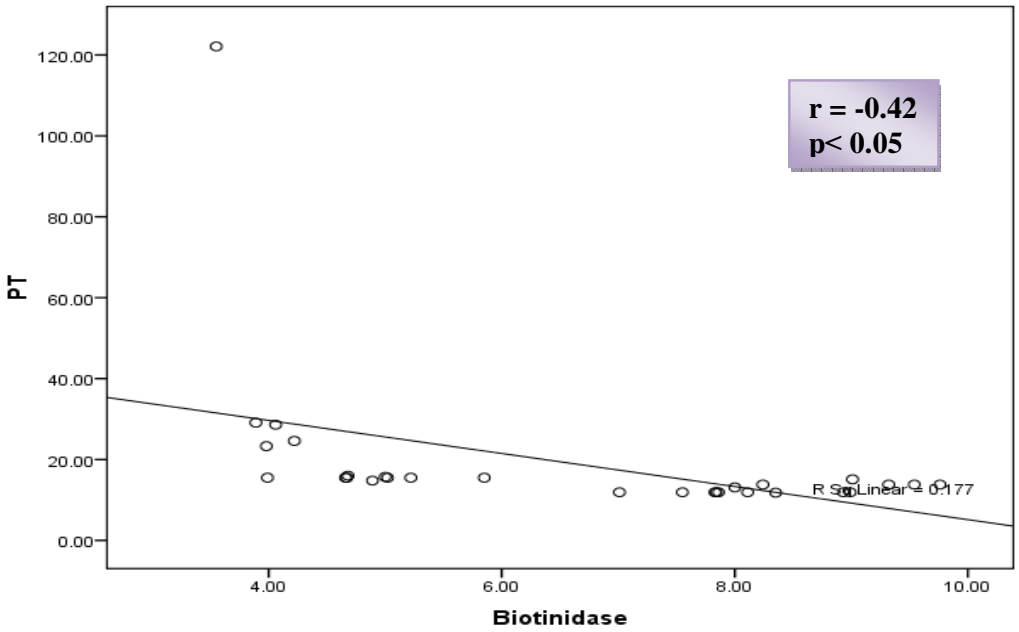

Figure (1): The inverse correlation between plasma biotinidase versus PT.

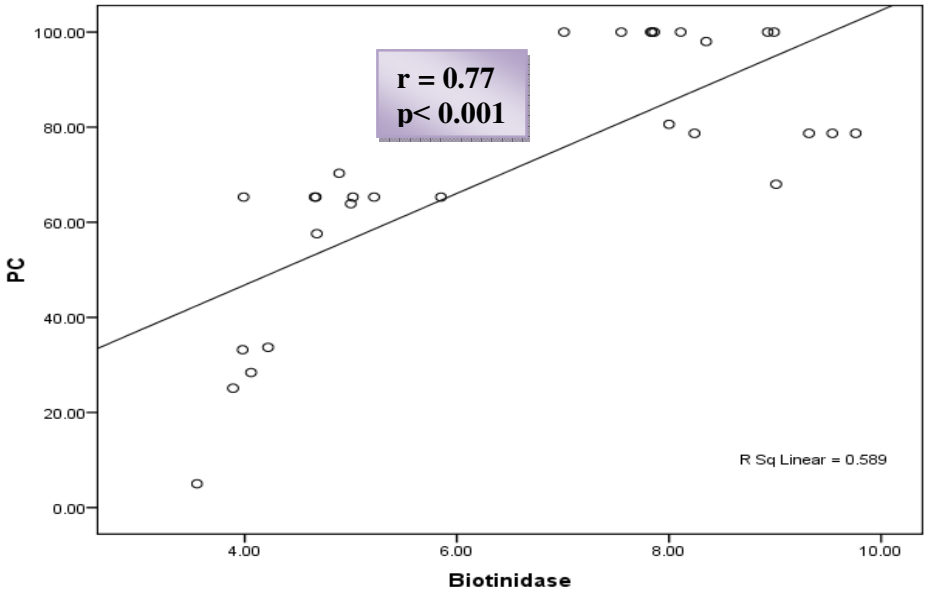

Figure (2): The positive correlation between plasma biotinidase versus PC.

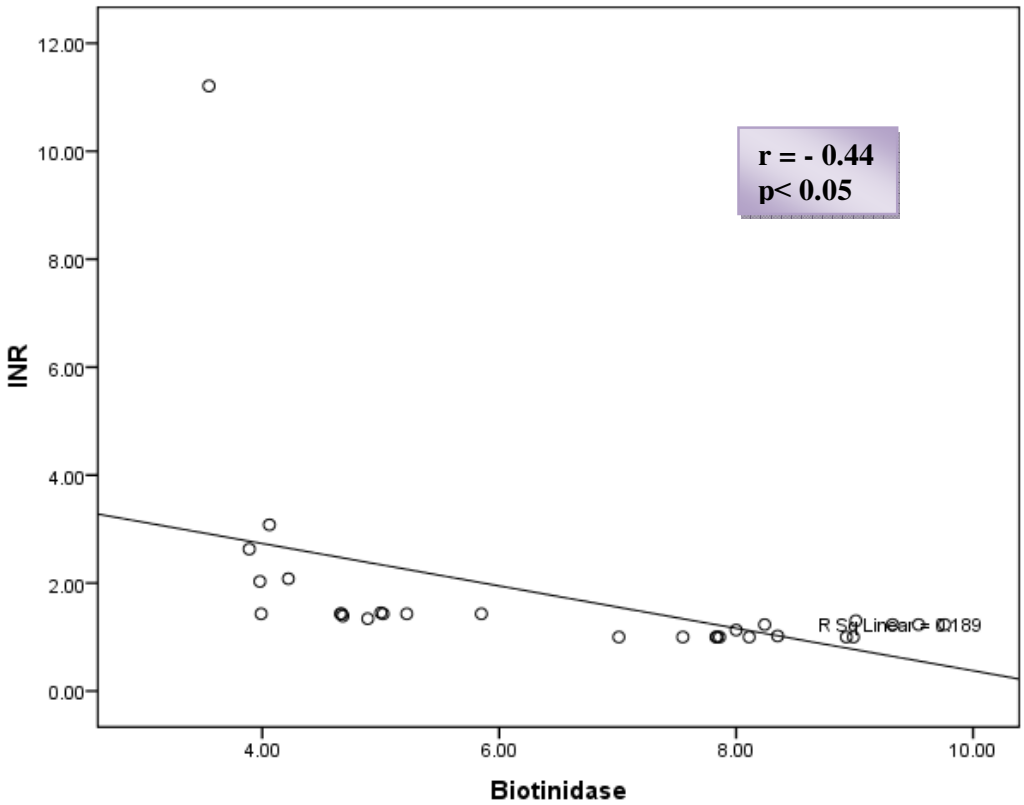

Figure (3): The inverse correlation between plasma biotinidase versus INR.

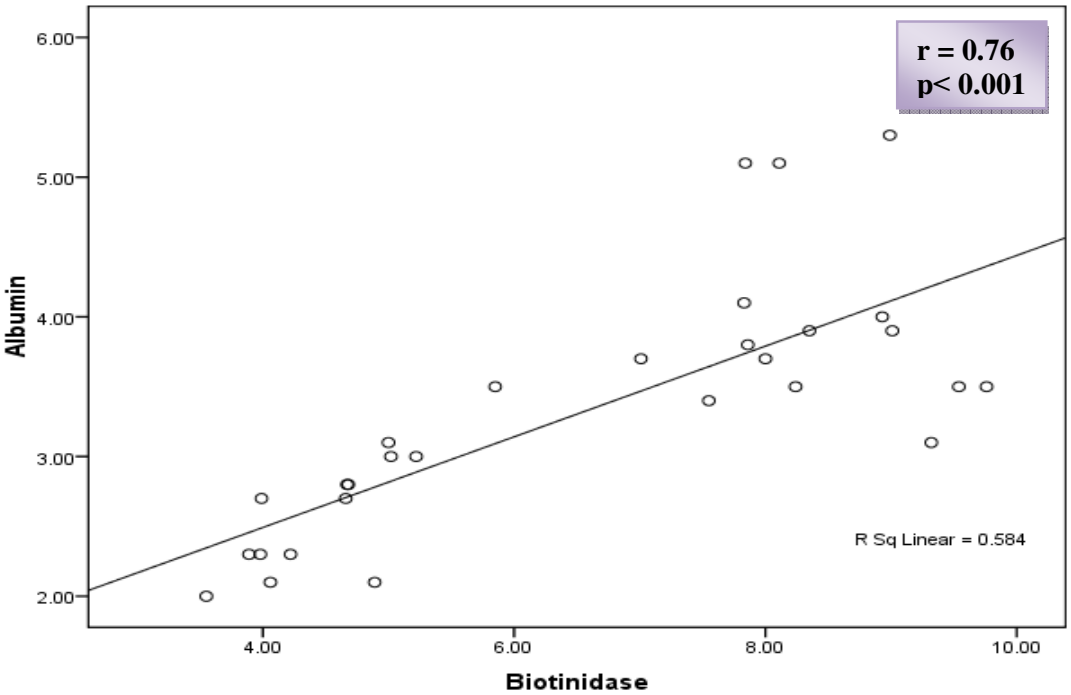

Figure (4): The positive correlation between plasma biotinidase versus albumin.

Table (3): Comparison of the mean± SD of plasma biotinidase level among pediatric patients with compensated liver disease, decompensated liver disease and control group:

	Pediatric patients with compensated liver disease n=16	Pediatric patients with decompensated liver disease n=13	Control group n=40	p-value
Plasma Biotinidase Level,IU\L (Mean ± SD)	8.2±3	4.4±1.2	8.7±0.7	**<0.001**

Discussion:

Biotinidase , the biotin recycling enzyme belongs to the nitrilase superfamily of enzymes that consists of amidases, N-acyltransferases and nitrilases[(7)]. Some members of the nitrilase superfamily (vanins-1, -2 and -3) share significant sequence similarities with biotinidase [(8)]. Biotin deficiency in humans is rare because biotin is continuously being recycled from the biotin dependent holocarboxylases by the action of biotinidase. Under normal conditions these enzymes undergo proteolytic degradation to biocytin or biotinyl peptides. Cleavage of these breakdown products by biotinidase recaptures and recycles the free biotin for continued cofactor functioning**.** Biotinidase is also important for making biotin bioavailable from bound dietary sources [(9)]. Liver injury may influence biotinidase activity because it is mainly synthesized by the liver [(10)].

In this study, the total cases were classified according to the presence or absence of impaired prothrombin time "PT", prothrombin

concentration "PC" and international normalization ratio "INR " and low plasma albumin into two subgroups, pediatric patients with decompensated liver disease (mean ±SD of PT, PC, INR & plasma albumin were 25±3.6, 49±23,2.5±1.2 &2.3±0.3 respectively) & pediatric patients with compensated liver disease (mean ±SD of PT, PC, INR & plasma albumin were 13.5±2, 89±30,1.1±0.4 &4±0.6 respectively) **(table.2).** The plasma biotinidase showed highly statistically significant lower levels in pediatric patients with decompensated liver disease (4.4± 1.2) versus both pediatric patients with compensated liver disease (8.2±3) and controls (8.7±0.7) with a p-value <0.001 **(table.3).** This is in agreement with a study that was done by **Özdemir *et al*** [10] to assess serum biotinidase activity in adult patients with liver cirrhosis and to investigate the relationship between serum biotinidase activity levels and the degree of compensation of liver cirrhosis, concluding that serum biotinidase activity was significantly lower in patients with decompensated cirrhosis.

However, the patients with compensated cirrhosis had similar biotinidase activity levels in comparison with the control group. Also, the findings of this study is in agreement with a study done by **Pabuçcuoğlu et *al*** [11] and a study done by **Nagamine *et al*** [12] who concluded that serum biotinidase activity was significantly lower in patients with cirrhosis, particularly in the patients with decompensated cirrhosis and fulminant hepatitis that was associated with severe impairment of hepatocellular function.

this study clarified that there was statistically significant positive correlation between the plasma biotinidase level and PC and plasma

albumin (p-value <0.001), the lower PC and plasma albumin the lower plasma biotinidase level. There was also statistically significant inverse correlation between the plasma biotinidase level and PT and INR with p-value <0.05, the higher PT & INR the lower plasma biotinidase level **(figure 1, 2, 3 and 4).** These results were in agreement with a study done by **Nagamine *et al*** [13] who correlate serum biotinidase activities with serum albumin and prothrombin time suggesting that biotinidase activities indicating the degree of liver damage except for both acute hepatitis and alcoholic liver disease, biotinidase activities were significantly reduced than in healthy controls.

The previous findings in this study indicate that the plasma biotinidase level is significantly decreased in patients with decompensated liver disease and so can be used as a predictor for hepatic synthetic function impairment. The effect of biotin (vitamin B 7) supplementation in patients with decompensated liver disease to protect them against biotin deficiency as biotinidase is required for release of free active biotin to the body to perform its role in the metabolism need further study.

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