Serum level of insulin like growth factor-1 in children with chronic liver disease

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Abstract

Background: Malnutrition and growth retardation are important consequences of CLD in childhood. Although IGF is a marker of protein metabolism, that can be used to assess malnutrition. However, in CLD, with impaired IGF synthesis, its use may lead to an exaggeration of the degree of malnutrition.

Objectives: To determine the level of IGF- 1 in these patients and to demonstrate the relation between its level and the degree of malnutrition and the degree of hepatic dysfunction.

Methodology: Fifty children with CLD, recruited from the outpatient clinic of pediatric hepatology and from the pediatric hepatology department of Pediatric Hospital, Cairo University, were enrolled in the study. Their mean age was 2.05 years ranged from (0.5 to 5.75) years. They were compared with an age and sex- matched normal healthy children (control group). Anthropometric measurements, liver function tests and serum level of IGF- 1 were performed. Assessment of severity of liver disease was done using the modified Child- Pugh score.

Results: Results revealed that serum IGF- 1 level was significantly lower in patients compared to controls, and it was significantly lower in Child Pugh C compared to Child Pugh B and A, and it was significantly lower in Child Pugh B compared to Child Pugh A. Moreover, there was no significant correlation between any of the anthropometric parameters and serum IGF- 1.

Conclusion: In CLD, IGF- I level is inversely correlated to the degree of liver dysfunction rather than the degree of malnutrition.

Key words: IGF- 1, Children, and Chronic Liver Disease

مستوى عامل النمو شبيه الإنسولين- ١ فى مصل الأطفال المصابين بأمراض الكبد المزمنة

خلفية: إن تأخر النمو وسوء التغذية من أكبر المشاكل التى يعانى منها الأطفال المصابون بأمراض الكبد المزمنة .بالرغم من أن عامل النمو شبيه الإنسولين- ١ هو أحد دلائل عملية أيض البروتينات وبالتالى إمكانية إستخدامه فى تقييم الحالة الغذائية إلا أن إنخفاض تكوينه عن طريق الكبد فى حالات الإصابة بأمراض الكبد المزمنة يجعل إستخدامه فى تقييم الحالة الغذائية غير دقيق حيث يؤدى إستخدامه إلى تقييم زائد لدرجة سوء التغذية.

الهدف: قياس مستوى عامل النمو شبيه الإنسولين- ١ عند الأطفال المصابين بأمراض الكبد المزمنة وتحديد مدى علاقة مستواه بدرجة تدهور الحالة الوظيفية للكبد ودرجة سوء التغذية.

المنهجية: تم إجراء هذه الدراسة علي٥٠ طفل (٢٥ ذكر- ٢٥ أنثى) مصابين بأمراض الكبد المزمنة بأسبابها المختلفة والذين يترددون على عيادة الكبد والمحتجزين بقسم الكبد بمستشفى الأطفال جامعة القاهرة وقد تراوحت أعمارهم من ٦ شهور- ٧٥, ٥ سنة (متوسط العمر ٢,٠١ سنة) وتمت مقارنتهم بأطفال أصحاء من نفس الفئة العمرية للمرضى ومن كلا الجنسين (المجموعة الضابطة). وتم عمل قياسات أنثروبومترية، وظائف كبد وقياس مستوى عامل النمو شبيه الإنسولين- ١. كما تم قياس درجة الخلل الوظيفى للكبد باستخدام مقياس تشايلد باج المعدل.

النتائج: أظهرت النتائج إنخفاض مستوى عامل النمو شبيه الإنسولين- ١ فى المرضى عنه فى المجموعة الضابطة. وكان مستواه يتناسب عكسيا مع درجة الخلل الوظيفى للكبد. كما لم تثبت الدراسة وجود علاقة ذات قيمة إحصائية بين مستوى عامل النمو شبيه الإنسولين- ١ وأى من المعايير الأنثروبومترية.

الخلاصة: مستوى عامل النمو شبيه الإنسولين- ١ يتناسب مع الحالة الوظيفية للكبد وليس الحالة الغذائية.

الكلمات المفتاحية: عامل النمو شبيه الإنسولين- ١، الأطفال، وأمراض الكبد المزمنة.

Introduction:

Chronic liver disease (CLD) is a disease process of the liver that involves a process of progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis (Shepherd, 2008).

Insulin- like growth factor- I (IGF-1) is a polypeptide hormone that functions as the major mediator of growth hormone (GH) - stimulated somatic growth, as well as a mediator of GH- independent anabolic responses in many cells and tissues. (Bonefeld and Møller, 2011; Clemmons, 2012; Puche and Castilla- Cortázar, 2012).

Malnutrition and growth retardation are important consequences of CLD in childhood. They are associated with frequent complications, hospitalization, poor outcome after liver transplantation, and ultimately death (Roongpisuthipong, 2001; Hurtado- López et al., 2007).

The pathogenesis of malnutrition in CLD is multifactorial and includes a reduction in nutrient and caloric intake, anorexia and dietary restrictions, impaired intestinal absorption, abnormalities in nutrient metabolism, and increased proinflammatory cytokine levels, resulting in a hypermetabolic state (Sanchez and Aranda- Michel, 2006; Hurtado- López et al., 2007; Nightingale and Ng, 2009). A disturbed growth hormone (GH) - insulin- like growth factor (IGF-1) axis may also contribute to wasting and growth failure in children with liver disease, by virtue of IGF-1 deficiency and GH resistance (Shepherd, 2008).

The nutritional status has a great influence on IGF-1. Both the energy and protein content of the diet are important in the maintenance of IGF-1 (Livingstone, 2013). Although IGF is a marker of protein metabolism, that can be used to assess malnutrition. However, in CLD, with impaired IGF synthesis, its use may lead to an exaggeration of the degree of malnutrition (Stephenson et al., 2001; Taylor and Dhawan, 2005; Socha, 2008). Moreover, Colakoğlu et al., 2007; Dehghani et al., 2012; Khoshnood et al., 2013 and Ronsoni et al., 2013 reported a decrease of IGF level in patients with CLD, and they found that its level was correlating to the extent of hepatic dysfunction rather than the degree of malnutrition.

The IGF-1 deficiency in CLD is thought to result primarily from the reduced synthetic capacity of the hepatocellular mass, combined with a decrease in GH receptors in the cirrhotic liver (Donaghy et al., 2002).

Aims:

To measure the level of IGF-1 in children with CLD and to identify the relation between its level and the degree of malnutrition and the degree of hepatic dysfunction.

Subjects And Methods

Subjects:

This is a cross- sectional case control study that included 50 children with CLD (25 males and 25 females) recruited from the outpatient clinic of pediatric hepatology and from the pediatric hepatology department of Pediatric Hospital, Cairo University in the period from April 2012 to April 2013. Their mean age was 2.05 years ranged from (0.5 to 5.75) years. They were compared with an age and sex- matched normal healthy children (26 males and 24 females) attending the pediatric general clinics and pediatric emergency department, with a mean age of 2.01 years (ranged from0.5 to 5.83 years).

All the subjects met the inclusion and exclusion criteria mentioned below:

- Inclusion Criteria:
  1. Children with chronic liver disease.
  2. Age range: 6 months to 6 years.
  3. Both Sexes Were Included.
- Exclusion Criteria:
  1. Associated chronic disease such as neurological, heart, or renal diseases.
  2. Children With Diabetes Mellitus.
  3. Age less than 6 months or more than 6 years.
- Ethical Considerations: The parents were informed about the purpose of the study and cases were included in the study only after written consent was given by parents. The study protocol was approved by the Ethical Committee of the National Research Centre and the Institute of Postgraduate Childhood Medical Studies, Ain Shams University.

Methods:

All participating children were subjected to:

1. History taking: This include: age, sex, age at onset of the liver disease, symptoms of liver cell failure.
2. Physical Examination: Involved
   a. General examination: Head, neck, limbs, skin, back, spine, and genitalia.
   b. Systemic examination: Neurological, cardiovascular, chest, abdominal examination to identify level of consciousness, signs of liver cell failure, organomegaly, ascites, and to exclude associated chronic diseases such as neurological, heart, or renal diseases.
3. Anthropometric Assessment: Anthropometric assessment was performed using standardized equipments, and following the recommendations of the International Biological Program (Tanner et al., 1969). All bilateral measurements were taken on the left side. Three consecutive measurements were taken and when the differences between the readings were acceptable the mean was recorded.
   a. Body weight (Kg): Children < 2 years old were weighed on Seca scale. While children ≥ 2 years of age were weighed while standing on a digital platform scale. Subjects were measured without shoes and minimal clothing. The measure was recorded to the nearest 0.1 Kg.
   b. Body length in cm (for children <3 years of age): Length was measured and recorded to the nearest0.1 cm in a recumbent position using an infantometer. The assistant held the child's head in firm contact with the headboard, so that the Frankfurt plane is vertical. At the same time the legs are straightened, holding the feet with toes pointed up and moving the footboard against the feet.
   c. Height in cm (for children >3 years): Height was measured and recorded to the nearest0.1 cm using a standiometer with a movable block. The subjects were measured while standing, without shoes, with their heels together and back as straight as possible and arms hanging freely; the head was positioned in the Frankfort horizontal plane and the movable block was brought down until it touched the subject's head.
   d. Mid upper arm circumference (MUAC) in cm: It was measured using a flexible, non- stretchable measuring tape with the arm completely relaxed and the measurement was taken horizontally, midway between the inferior border of the acromion process and the tip of the olecranon process. The tape was just touching the skin but not compressing the

tissue. The measure was taken to the nearest 0.1 cm.

e. Skin- fold thickness in mm: This was measured by using Holtain skin-fold caliper. The thumb and four fingers of the left hand picked up a fold of skin and subcutaneous tissue and pinched it away from the underlying muscle. Readings were taken to the nearest 0.2 mm as soon as the caliper came in contact with the skin and the dial reading stabilized.

- Triceps skin- fold thickness in mm: The tips of the acromion process and olecranon were palpated, and a mark was made on the skin (a point midway between them and parallel to the long axis of the arm). Then the skin- fold was picked up between the index finger and the thumb of the left hand, over the posterior surface of the triceps muscle, one centimeter above the mark then the caliper jaws were applied.
- Subscapular skinfold thickness in mm: The subject's shoulders were erect and the arm beside the body. The skinfold was picked up at the inferior angle of the scapula then the caliper jaws were applied.

Total upper arm area (TUAA), mid upper arm muscle area (MUAMA), and mid upper arm fat area (MUAFA) were calculated with MUAC and TSFT measurements according to the formulas described by Jeliffe (1963); Gurney and Jelliffe (1973); Sann et al. (1988) and Frisancho (1990) and the results were expressed in square millimeters.

$$\text{MUAMA } (cm^2) = (\text{MUAC [cm]} - [\text{TSF} \times \pi])^2 / (4 \times \pi)$$

$$\text{TUAA} = (\text{MUAC})^2 \text{ [cm]} / (4 \times \pi)$$

$$\text{MUAFA } (cm^2) = \text{TUAA} - \text{MUAMA}$$

$$\text{AFI} = 100 \times (\text{AFA} / \text{TUAA})$$

Where $\pi = 3.14$

The results of anthropometric data of these patients were compared with that obtained from the measurements on normal healthy Egyptian children (Egyptian Growth Charts, 2002). All anthropometric data were expressed in standard deviation score (Z score) to allow comparison of data irrespective of age and sex. The calculation was made according to the following formula:

$$\text{Z Score} = \frac{\text{Individual's value - Mean of the reference population}}{\text{SD of the reference polpulation}}$$

4. Laboratory Investigations:

a. The following investigations were done: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gama glutamyltransferase (GGT), alkaline phosphatase (ALP), serum albumin, serum bilirubin (total and direct), prothrombin time, and international normalized ratio (INR). Procedures: Venous blood samples (5mL) were withdrawn, 1mL was collected into heparinized tube for determination of prothrombin time. The rest of the sample was collected into plain tube and allowed to clot, and then serum was separated and stored at- 20 °C until assayed by Hitachi automated chemical analyzer using commercially available kits according to the manufacturer's instructions.

b. Serum IGF-1 was measured using quantitative Enzyme- Linked Immuno- Sorbent Assay (ELISA) using commercial kit provided by DIAsource, Belgium according to the manufacturer's instructions.

5. Assessment of the severity of liver disease: It was done using Modified Child- Pugh score which classifies severity of liver disease according to the degree of ascites, the plasma concentrations of bilirubin and albumin, the prothrombin time, and the degree of encephalopathy.

Table (1): Modified Child- Pugh score

Parameter		Points Assigned		
		1	2	3
Ascites		Absent	Slight	Moderate
Bilirubin (mg/dL)		< 2	2 To 3	> 3
Albumin (g/dL)		> 3.5	2.8 to 3.5	< 2.8
Prothrombin Time	Seconds Over Control	1 To 3	4 To 6	>6
	INR	< 1.7	1.8 to 2.3	> 2.3
Encephalopathy		None	Grade 1 To 2	Grade 3 To 4

Patients were grouped into three categories:

- Class A: Well compensated disease, scores (5- 6).
- Class B: Significant functional compromise, scores (7- 9).
- Class C: Decompensated disease, scores (10- 15). (Pugh et al., 1973; Lucey et al., 1997).

Statistical Analysis:

Data analysis was assisted by Statistical Package for Social Science (SPSS V.16). Nominal and categorical data were expressed as frequency and percentage. Numerical data were expressed as mean, SD, median, minimum, and maximum. The difference between two groups was calculated using unpaired T- test, while the difference between more than two groups was calculated using one- way analysis of variance (ANOVA). Pearson's correlation was used to evaluate correlations between numerical variables. P value less than 0.05 was considered significant (Machin et al., 2007).

Results:

According to the degree of liver dysfunction (assessed by Child Pugh score) patients were divided into 3 groups (classes). It was found that 22 patients (44%) were in grade A (well- compensated disease), 17 patients (34%) were in grade B (Significant functional compromise) and 11 patients (22%) were in grade C (Decompensated disease).

Table (2): Comparison of means of IGF-1 level between patient and control groups

	Controls (n= 50)				Patients (n= 50)				P- Value
	Mean± SD	Median	Min	Max	Mean± SD	Median	Min	Max	
IGF-1 (Ng/Ml)	40.76± 4.82	40	33	53	26.93± 6.33	26.58	13.7	39	<0.001

Comparison of means of IGF-1 level between patient and control groups was shown in table (2). It was found that IGF-1 level was significantly lower in the patients compared to controls.

Table (3): Correlation between IGF-1 level and (serum albumin, total bilirubin, direct bilirubin, PT, INR, AST, ALT, ALP and GGT)

Liver Function Tests	IGF-1	
	r	P- Value
Albumin	0.456	0.001
Total Bilirubin	- 0.400	0.004
Direct Bilirubin	- 0.401	0.004
PT	- 0.326	0.021
INR	- 0.335	0.018
AST	- 0.366	0.009
ALT	0.098	0.499
ALP	- 0.013	0.927
GGT	- 0.134	0.355

ALP: Alkaline phosphatase. ALT: Alanine aminotransferase
AST: Aspartate aminotransferase GGT: Gama glutamyltransferase
PT: Prothrombin time INR: International normalized ratio.

The correlation between serum IGF-1 level and (serum albumin, total bilirubin, direct bilirubin, PT, INR, AST, ALT, ALP and GGT) was illustrated

in table (3). It was found that serum IGF-1 level had positive significant correlation with serum albumin and had negative significant correlation with total bilirubin, direct bilirubin, PT, INR, and AST.

Table (4): Comparison of means of IGF-1 level between Child Pugh classes

	Mean ± SD of IGF-1 in different Child Pugh class			P-Value
	Child Pugh A (N= 22)	Child Pugh B (N= 17)	Child Pugh C (N= 11)	
IGF-1 Level	30.68 ± 5.67[a]	25.60 ± 4.82 [a]	21.47 ± 5.00 [a]	<0.001

*Child Pugh classes sharing the same letter (a) are significantly different from each others ($p<0.05$).

Comparison of means of IGF-1 level between Child Pugh classes was demonstrated in table (4). Results showed that IGF-1 level was significantly lower in Child Pugh C compared to Child Pugh B and A. Moreover, IGF-1 level was significantly lower in child Pugh B compared to Child Pugh A.

Table (5): Correlation between anthropometric parameters z scores and serum IGF-1 level

Anthropometric Parameters	IGF1	
	r	P- Value
Wt Z Score	0.119	0.411
Ht Z Score	- 0.005	0.972
Wt/Ht Z Score	0.067	0.645
MUAC Z Score	0.044	0.763
TSFT Z Score	0.188	0.191
SSFT Z Score	0.165	0.252
TUAA Z Score	0.038	0.794
MUAMA Z Score	- 0.098	0.497
MUAFA Z Score	0.185	0.199
AFI Z Score	0.265	0.063

AFI: Arm fat index- Ht: Height- MUAC: Mid upper arm circumference- MUAFA: Mid upper arm fat area- MUAMA: Mid upper arm muscle area- SSFT: Subscapular skinfold thickness- TSFT: Triceps skin- fold thickness- TUAA: Total upper arm area- Wt: Weight- Wt/ht: Weight for height

Correlation between anthropometric parameters and serum IGF-1 level was presented in table (5). Results showed that there was no significant correlation between any of anthropometric parameters and serum IGF-1 level

Table (6) Comparison of means of IGF-1 level between patients with anthropometric parameters z score above and below- 2SDS

Anthropometric Parameters	Mean ± SD of IGF-1		P- Value
	Patients with anthropometric parameters z score above- 2 SDS	Patients with anthropometric parameters z score below- 2 SDS	
Weight Z Score	26.96 ± 6.61	26.89 ± 6.09	0.970
Height Z Score	27.65 ± 6.39	26.30 ± 6.33	0.459
Wt/Ht Z Score	27.38 ± 6.91	25.87 ± 4.76	0.447
MUAC Z Score	27.77 ± 6.98	26.41 ± 5.96	0.466
TSFT Z Score	28.09 ± 6.24	26.01 ± 6.36	0.253
SSFT Z Score	28.02 ± 6.32	26.07 ± 6.32	0.283
TUAA Z Score	28.48 ± 6.97	26.26 ± 6.02	0.260
MUAMA Z Score	26.36 ± 7.06	27.58 ± 5.43	0.503
MUAFA Z Score	27.77 ± 6.33	26.15 ± 6.35	0.370
AFI Z Score	27.23 ± 6.27	25.84 ± 6.72	0.527

AFI: Arm fat index Ht: Height MUAC: Mid upper arm circumference
MUAFA: Mid upper arm fat area MUAMA: Mid upper arm muscle area
SSFT: Subscapular skinfold thickness TSFT: Triceps skin- fold thickness
TUAA: Total upper arm area Wt: Weight Wt/ht: Weight for height

Comparison of means of IGF-1 level between patients with anthropometric parameters z score above and below- 2 SDS was illustrated in table (6). It was found that there was no significant difference between the 2 groups.

Discussion:

In the current study, IGF-1 level was significantly lower in patients compared to controls. Moreover, IGF-1 level positively correlated with serum albumin, and negatively correlated with total bilirubin, direct bilirubin, PT, INR and AST. In addition, IGF-1 level was significantly lower in Child Pugh C compared to Child Pugh B and A. Similarly, the IGF-1 level was significantly lower in child Pugh B compared to Child Pugh A. These results come in accordance with Sedlaczek et al., (2003); Vyzantiadis et al., (2003); Wu et al., (2004); Colakoğlu et al., (2007); Dehghani et al., (2012); Khoshnood et al., (2013); and Ronsoni et al., (2013) who reported similar findings and they mentioned that IGF-1 level negatively correlates to the degree of liver dysfunction and they also concluded that the combined detection of serum IGF-1 with Child- Pugh score is more effective in predicting prognosis than Child- Pugh score alone.

The IGF-1 deficiency in CLD is thought to result primarily from the reduced synthetic capacity of the hepatocellular mass, combined with a decrease in GH receptors in the cirrhotic liver (Donaghy et al., 2002). The level of bioactive IGF-1 is further reduced because of elevated levels of IGFBP-1 and IGFBP-2, which act primarily as blockers of IGF actions..Another contributing factor is the often reoccurring periods of spontaneous bacterial peritonitis, during which the level of IL- 6 is increased. A negative correlation between IL- 6 and IGF-1 has been reported, possibly owing to IL- 6- mediated blockade of the IGF-1 production in the liver (Bonefeld and Møller, 2011).

Our results revealed insignificant correlation between any of the nutritional anthropometric parameters and serum level of IGF-1. Moreover, the mean serum level of IGF-1 of patients with anthropometric parameters z score more than- 2 SD was not significantly different from patients with theses parameters less than- 2 SD. These results are in agreement with Caregaro et al., (1997) and Colakoğlu et al., (2007) who reported that the decrease in IGF-1 concentration correlates better with the degree of liver dysfunction rather than the degree of malnutrition.

Conclusion:

It could be concluded that IGF-1 level was inversely correlated to the degree of liver dysfunction rather than the degree of malnutrition.

Recommendations:

In CLD, serum IGF-1 can be used as an index of severity of liver disease along with Child Pugh score.

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