

## Dental Abnormalities in Children Affected with Hyperbilirubinaemia

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### Key words

Dental abnormalitie, Hyperbilirubinaemia.

### Abstract

This study was carried out to evaluate the relationship between hyperbilirubinaemia and dental abnormalities (D.A). Group of patients composed of 52 patient, and control group composed of 35 healthy child. This study was conducted on children that ages between (6-12 years). The markers measured in this study were total serum bilirubin (TSB) (conjugated and unconjugated), alanine amino-transferase (ALT), gamma-glutamyltransferase (GGT), calcium (Ca), and vitamin D.

This study show a significant decrease ( $P < 0.05$ ) in levels of serum calcium and vitamin D, a significant increase ( $P < 0.05$ ) in levels of serum ALT and GGT in patients as compared with healthy persons, and also these changes proportional with hyperbilirubinaemia due to conjugated type increase (normal unconjugated type), also dental abnormalities proportional with these changes.

### Introduction

Bilirubin is a byproduct of heme catabolism from aged red blood cells. Bilirubin is primarily produced in the liver, spleen, and bone marrow. Total bilirubin is the sum of unconjugated bilirubin, monoglucuronide and diglucuronide, conjugated bilirubin, and albumin-bound delta bilirubin<sup>(1)</sup>.

Alanine transaminase (ALT) is a cytosolic enzyme which is specific for liver function examination. ALT is found in large amounts in the liver, and small amounts of this enzyme are also found in the heart, muscle, and kidney. Gamma-glutamyltransferase (GGT) is a cell-surface protein contributing to the extracellular catabolism of glutathione (GSH).

The enzyme is produced in many tissues, but most GGT in serum is derived from the liver<sup>(2,3)</sup>.

Calcium is an important part of the daily diet, especially for children. It is essential for the growth of strong bones and teeth. Vitamin D refers to a group of compounds that are formed in different parts of the body. Vitamin D play a role in various physiological processes including bone and calcium metabolism, cellular growth and differentiation, immunity and cardiovascular function. In the absence of vitamin D, intestinal calcium and phosphorus absorption diminish<sup>(4,5)</sup>.

Dental abnormalities (especially dental caries) is still one of the most common diseases in the world today<sup>(6)</sup>. Many constituent of serum and saliva, both organic and inorganic have potentially protective role. These include calcium, phosphate, fluoride ions and bicarbonate

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buffer systems<sup>(7)</sup>. The associations between dental diseases such as periodontitis or dental caries and the general health condition have been reported, therefore; dental caries might be a risk marker or factor for the general health condition<sup>(8,9)</sup>.

Aim of the study:

This study was done to detect the alterations in vitamin D synthesis and calcium metabolism and percent of dental abnormalities in patients with hyperbilirubinaemia.

### Subjects and Methods:

This study included 87 children (52 patients with hyperbilirubinaemia, and 35 healthy children), and the age was ranged between (6-12 years). This study was conducted from November, 2013 till May 2014 in Tikrit city (Tikrit general hospital and college of dentistry).

Instruments used were spectrophotometer for estimation of TSB, ALT, GGT, and Ca. Mini Vidas was used for estimation of vitamin D.

Biochemical Analysis:

1- Total (conjugated and unconjugated) serum bilirubin Assay

Serum bilirubin was estimated by the use of biolabo kit (Maizy, France). Reaction between bilirubin and diazotized sulfanilic acid which leads to a compound, the azobilirubin, coloured in very acid or basic medium. This principle modified in an aqueous solution, only for direct (conjugated) bilirubin reacts. To enable the assay of total bilirubin, it is necessary to break the link between unconjugated bilirubin and albumin. This step is done by adding dimethylsulfoxide (DMSO).

The absorbance of azobilirubin is proportional to the concentration of bilirubin and is measured at 550 nm.

2- Determination of serum ALT

Serum ALT was estimated by the use of biolabo kit (Maizy, France). Colorimetric method used which started by the reaction and conversion of alanine to pyruvate. Then, Pyruvate reacts with 2, 4 DNP to form 2, 4 Dinitrophenylhydrazones, which absorbance at 505 nm in alkaline solution is proportional to ALT activity in the reactional mixture.

3- Determination of serum GGT

GGT was estimated by the use of CYPRESS DIAGNOSTICS kit, Belgium (Kinetic test. Carboxy substrate). Kinetic determination of  $\gamma$ -glutamyltransferase activity according to the following reaction:

L-  $\gamma$ -glutamyl-3-carboxy-4-nitroanilide + glycylglycine  $\rightarrow$  L-  $\gamma$ -glutamyl-glycylglycine + 5-amino-2-nitrobenzoate.

4- Determination of serum calcium

Serum calcium was estimated by use of CPC (O-Cresol Phtalein Complexone) method. In alkaline solution, CPC reacts with calcium to form a dark-red coloured complex which absorbance measured at 570 nm is proportional to the amount of calcium in specimen.

5- Determination of serum vitamin D

Vitamin D was determined by VIDAS 25 OH Vitamin D TOTAL (VITD). VIDAS 25 OH Vitamin D TOTAL (VITD) is an automated quantitative test for use on the instruments of the VIDAS family for the determination of 25-hydroxyvitamin D Total in human serum or plasma using the ELFA technique (Enzyme Linked Fluorescent Assay).

Biostatistical analysis:

The results were expressed as mean  $\pm$  SD. Student's t-test and bivariate correlation [Pearson correlation coefficient (r)] was used for assessment of the results of patients and control groups. Significant variation was considered when P value less than 0.05.

### Results:

1- Dental abnormalities percent and measurements of biochemical markers in patients with high conjugated bilirubin and healthy persons.

Serum ALT and GGT levels were increased significantly ( $P < 0.05$ ) and serum calcium and vitamin D<sub>3</sub> were significantly ( $P < 0.05$ ) decreased in patients with high conjugated bilirubin as compared with healthy persons. The percent of dental abnormalities was higher in patients (80.65%) than in healthy persons (25.5%).

2- Dental abnormalities percent and measurements of biochemical markers in patients with low conjugated bilirubin and healthy persons.

Serum ALT and GGT levels were increased significantly ( $P < 0.05$ ) and serum calcium and vitamin D3 were significantly ( $P < 0.05$ ) decreased in patients with low conjugated bilirubin as compared with healthy persons. The percent of dental abnormalities was higher in patients (52.38%) than in healthy persons (25.5%).

3- Dental abnormalities percent and measurements of biochemical markers in patients with high conjugated bilirubin and low conjugated bilirubin.

Serum ALT and GGT levels were increased significantly ( $P < 0.05$ ) and serum calcium and vitamin D3 were significantly ( $P < 0.05$ ) decreased in patients with high conjugated bilirubin as compared with patients with low conjugated bilirubin. The percent of dental abnormalities was higher in patients with high conjugated bilirubin (80.65%) than in patients with low conjugated bilirubin (52.38%).

4- Correlation of liver function markers (ALT and GGT) with calcium and vitamin D.

There was a significant negative correlation between liver markers (ALT and GGT) with calcium and vitamin D, and this negative correlation was higher in patients with high conjugated bilirubin than in patients with low conjugated bilirubin.

## Discussion:

The results show a significant increase in concentrations of serum ALT and GGT in patients with high conjugated hyperbilirubinaemia as compared with low conjugated hyperbilirubinaemia and with healthy children, and also in low conjugated hyperbilirubinemic patients in relation to healthy children. However; Burtis<sup>(10)</sup>, Cabrera-Abreu<sup>(11)</sup>, and Fevery<sup>(12)</sup> mentioned that the increase of ALT and GGT happen in case increase of conjugated bilirubin due to hepatitis and liver diseases affecting the biliary system, which mean a disturbance in liver function.

Also results show a significant decrease of vitamin D and calcium as the same pattern of patients above, this is due to disturbance of vitamin D synthesis due to liver disease and this lead to alteration of calcium metabolism. This is in agreement with Amano<sup>(5)</sup>, and Garcia-Bailo<sup>(13)</sup> which are mentioned that liver diseases lead to decrease vitamin D synthesis and this lead to disturbances of calcium and phosphorous metabolism. Therefore; this study shows a significant negative correlation of both ALT and GGT with vitamin D and calcium.

All these results explain why the percent of dental abnormalities was higher in patients with conjugated type hyperbilirubinaemia.

**Table (1): Dental abnormalities percent and biochemical markers in patients with high conjugated bilirubin and healthy persons.**

	TSB (mg/dl) ±S.D	ALT (U/l) ±S.D	GGT (U/l) ±S.D	Ca (mg/l) ±S.D	Vit.D (ng/ml) ±S.D	D.A. (yes/%)
Patients with high conjugated bilirubin	4.58 ±1.54	35.26 ±9.46	49.14 ±13.15	7.17 ±1.10	27.78 ±7.85	80.65
Normal subjects	0.93 ±0.17	17.32 ±2.53	22.19 ±3.77	9.25 ±0.55	54.48 ±6.20	25.5
P <sub>value</sub>	0.0001	0.0001	0.0001	0.0001	0.0001	

**Table (2): Dental abnormalities percent and biochemical markers in patients with low conjugated bilirubin and healthy persons.**

	TSB (mg/dl) ±S.D	ALT (U/l) ±S.D	GGT (U/l) ±S.D	Ca (mg/l) ±S.D	Vit.D (ng/ml) ±S.D	D.A. (yes/%)
Patients with low conjugated bilirubin	4.23 ±1.26	27.41 ±5.63	36.23 ±11.48	8.25 ±0.75	44.08 ±8.57	52.38
Normal subjects	0.93 ±0.17	17.32 ±2.53	22.19 ±3.77	9.25 ±0.55	54.48 ±6.20	25.5
P <sub>value</sub>	0.0001	0.0001	0.0001	0.007	0.005	

**Table (3): Dental abnormalities percent and biochemical markers in patients with high conjugated bilirubin and low conjugated bilirubin.**

	TSB (mg/dl) ±S.D	ALT (U/l) ±S.D	GGT (U/l) ±S.D	Ca (mg/l) ±S.D	Vit.D (ng/ml) ±S.D	D.A. (yes/%)
Patients with high conjugated bilirubin	4.58 ±1.54	35.26 ±9.46	49.14 ±13.15	7.17 ±1.10	27.78 ±7.85	80.65
Patients with low conjugated bilirubin	4.23 ±1.26	27.41 ±5.63	36.23 ±11.48	8.25 ±0.75	44.08 ±8.57	52.38
P <sub>value</sub>	>0.05	0.007	0.023	0.006	0.0001	

**Table (4): Correlation of liver function markers (ALT and GGT) with calcium and vitamin D.**

Patient type	Markers	Calcium		Vitamin D	
		r	P <sub>value</sub>	r	P <sub>value</sub>
Patients with high conjugated bilirubin	ALT	-0.825	0.0001	-0.915	0.0001
	GGT	-0.637	0.0001	-0.835	0.0001
Patients with low conjugated bilirubin	ALT	-0.527	0.005	-0.635	0.004
	GGT	-0.487	0.006	-0.565	0.005

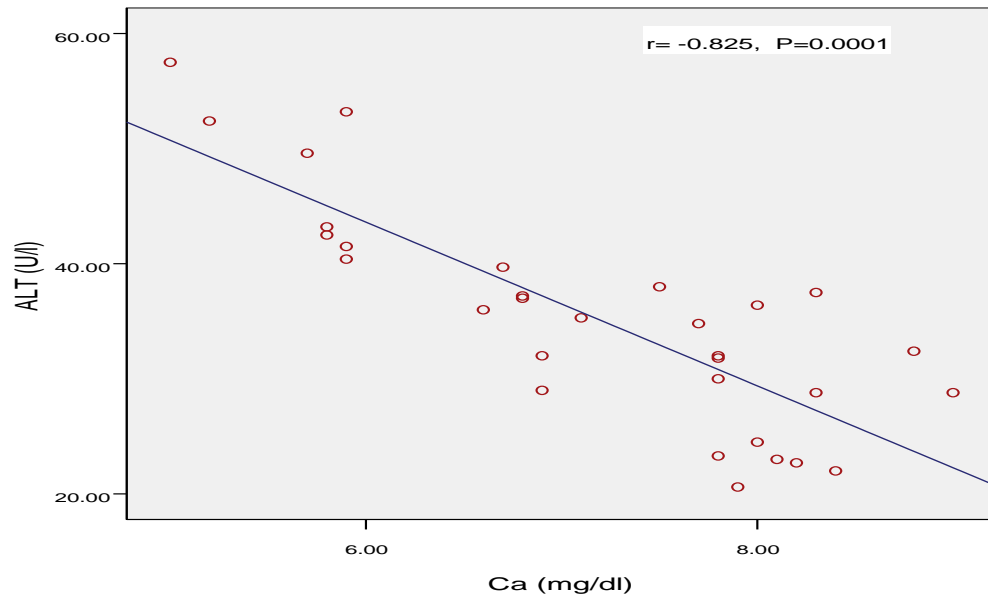


Figure (1): The association between serum ALT and serum calcium in patients with high conjugated bilirubin.

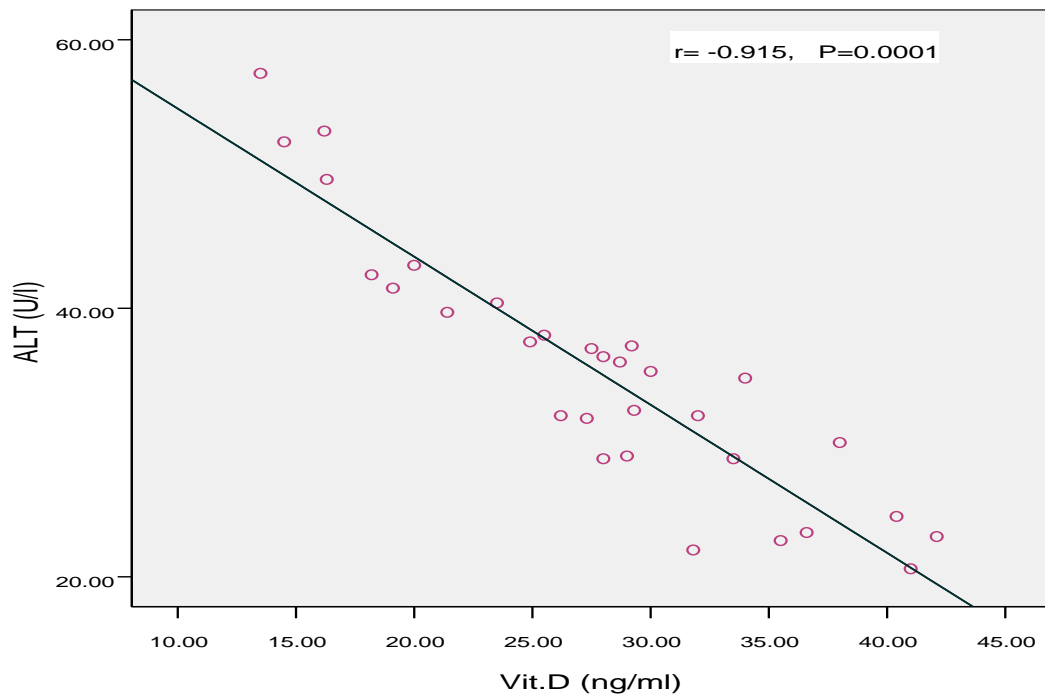


Figure (2): The association between serum ALT and vitamin D in patients with high conjugated bilirubin.

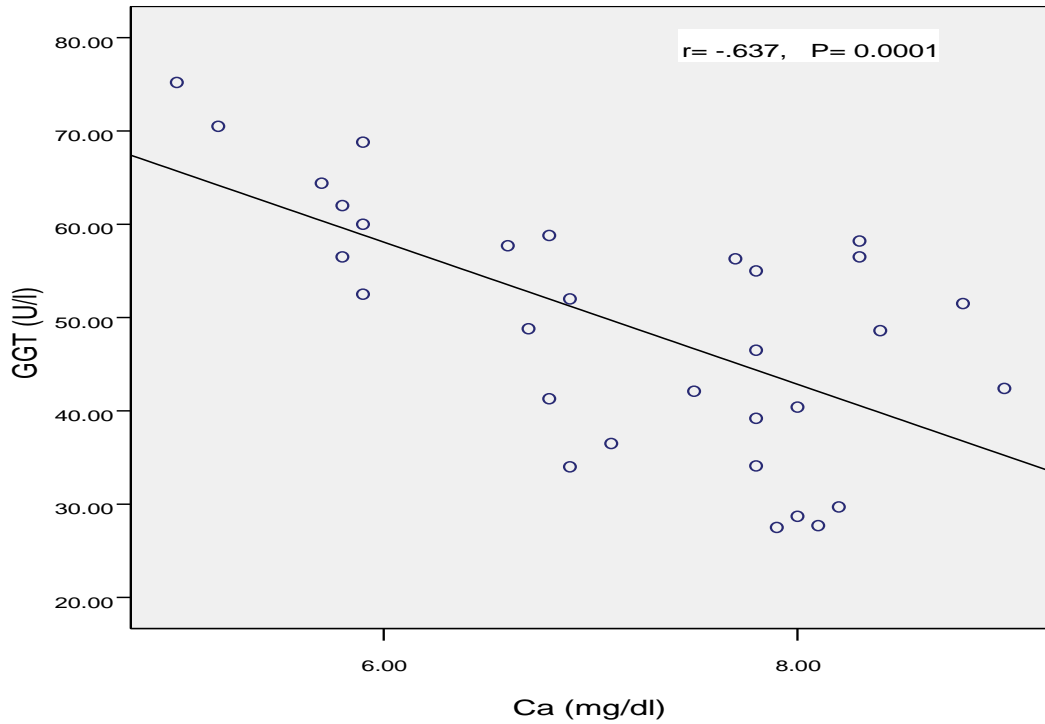


Figure (3): The association between serum GGT and serum calcium in patients with high conjugated bilirubin.

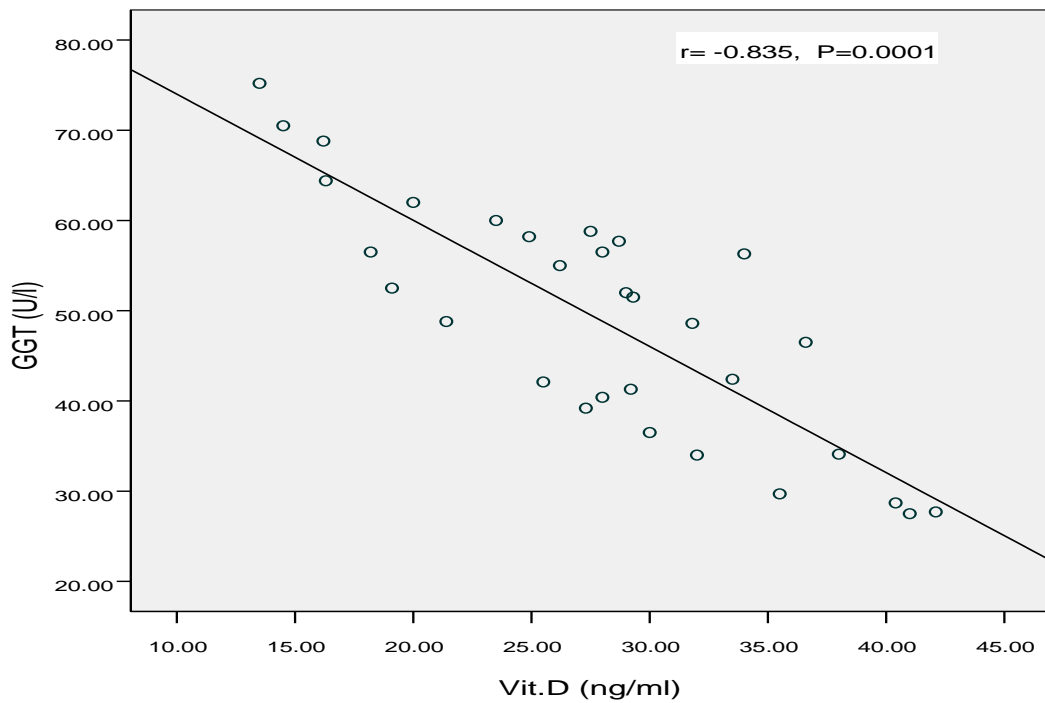


Figure (4): The association between serum GGT and vitamin D in patients with high conjugated bilirubin.

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