

Assessments of Serum 25-Hydroxy Cholecalciferol Levels in Neonates with Physiological Jaundice Candidate for Phototherapy

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Abstract

Background

In newborns, jaundice is the most common ailment that necessitates medical treatment and hospital readmission. The aim of this study was to evaluate vitamin D3 status and investigate the role of phototherapy in the treatment of jaundice and the improvement of vitamin D3 status in neonates with physiological jaundice.

Materials and Methods: This prospective research included 50 full-term neonates with physiological hyperbilirubinemia who were phototherapy candidates. They came from Egypt's Qena University Hospitals' Neonatal Intensive Care Units. Colorimetric assays of albumin and ionized calcium, as well as daily serum bilirubin and an ELISA assay of vitamin D3 were performed on the included cases before and 5 days after phototherapy, in addition to clinical assessments.

Results: The findings revealed a high prevalence of vitamin D3 deficiency (96.7%), and a lower frequency of optimal and insufficient vitamin D3 status (3.3%) with lack of optimal vitamin D3 status among cases. Post-phototherapy total, direct, and indirect bilirubin levels were significantly lower than pre-therapy levels, with substantial improvement in vitamin D3 status ($p < 0.05$ for all). Furthermore, both serum total bilirubin ($r = -0.703$, $p < 0.001$) and serum indirect bilirubin ($r = -0.710$, $p < 0.001$) had significantly negative associations with vitamin D3 serum levels.

Conclusion

Without vitamin D3 supplementation, neonates with low vitamin D and physiological jaundice who were received phototherapy had considerably improved vitamin D status 5 days later.

Key Words: Egypt, Neonatal hyperbilirubinemia, Physiological jaundice, Vitamin D3.

*Please cite this article as: Rashwan NI, El-Abd Ahmed A, Hassan MH, Bakheet Taqi F, Mohamed Ahmed ME, Helmi Bakri A. Assessments of Serum 25-Hydroxy Cholecalciferol Levels in Neonates with Physiological Jaundice Candidate for Phototherapy. *Int J Pediatr* 2021; 9(5): 13445-454. DOI: 10.22038/ijp.2021.56258.4427

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Received date: Jan.15, 2021; Accepted date: Apr.12, 2021

1- INTRODUCTION

In newborns, jaundice is the most common ailment that necessitates medical treatment and hospital readmission (1). Unconjugated hyperbilirubinemia is a common transitional syndrome in most babies. However, serum bilirubin levels in certain infants may rise too fast, which is worrying since unconjugated bilirubin is neurotoxic and may cause death in newborns as well as lifelong neurologic sequelae in those who live (kernicterus) (1, 2). Since newborns' capacity to conjugate bilirubin is minimal, unconjugated bilirubin accumulates and is difficult to excrete (3). Increased bilirubin production due to accelerated erythrocyte destruction, decreased excretory potential due to low levels of ligand in hepatocytes, and low activity of the bilirubin-conjugating enzyme uridine diphosphor glucuronyl transferase (UDPGT) cause physiologic jaundice (1). Bilirubin encephalopathy can be caused by conditions that cause low albumin or bilirubin displacement from albumin sites (4).

The American Academy of Pediatrics developed recommendations for the treatment of hyperbilirubinemia in newborns with a gestational age of 35 weeks or more. This guideline provides an equation for treating jaundice in the newborn nursery, as well as recommendations for phototherapy initiation based on total serum bilirubin levels, gestational age, and infant age in hours (5). Albumin infusion dramatically improves total serum bilirubin (TSB) levels, suggesting that albumin has a strong attractive effect on tissue bilirubin. Albumin, when given one to two hours before an exchange transfusion, increases bilirubin removal dramatically (6). "K" is referred to as a bilirubin-albumin equilibrium binding constant and represents the capacity of albumin to bind bilirubin according to a biochemical theory, namely the mass action equation.

The binding of bilirubin to albumin varies considerably in the first few days after birth (7). There is no particular range for neonates available at this time. Vitamin D deficiency and inadequacy are global issues with high prevalence even in developing and sunny countries (8). The metabolisms of bilirubin and vitamin D are two distinct pathways that are very different; however, at least one part of the synthesis takes place in the same organ, the liver. As a consequence, one's metabolism or synthesis can have an effect on the other (9). Infants that have been exposed to sunlight have less jaundice (10). This suggests that there may be a correlation between vitamin D and jaundice. As a result, the current study aimed to evaluate and compare serum vitamin D3 levels with serum bilirubin, albumin, and the bilirubin/albumin ratio in neonates with physiological jaundice requiring phototherapy, as well as to assess the impact of phototherapy in neonates with abnormally low serum vitamin D3 levels in enhancing optimal vitamin D levels without the need for vitamin D3 supplementation.

2- MATERIALS AND METHODS

2-1. Study design and participants

This prospective study included 50 full-term neonates with physiological hyperbilirubinemia who were candidates for phototherapy (290–315 nm was used) and were admitted to the neonatal intensive care unit, Pediatric Department, Qena University Hospitals, Qena, Egypt, between January 2019 and January 2020.

2-2. Patients' selection criteria

Any neonate who met one or more of the following conditions was eligible: postnatal ages of 3-10 days, gestational ages of 37-40 weeks, and neonates with jaundice and their mothers without vitamin D3 supplementation. The level of bilirubin in the blood is higher than the

pre-determined threshold for phototherapy. Any participant who met the previous eligibility requirements but whose parents refused to participate in the study, or who had isoimmunization, red cell sequestration, polycythaemia, or infections were excluded from the study.

2-3. Data collection

Mode of delivery, gestational age, gender, admission diagnosis, prenatal history, natal history, postnatal history, family history, and maternal history were all taken from the mothers of the included neonates. Nutritional history was recorded including breast feeding (exclusive or predominant,

number of feeds per day, duration of feeding, supplementation), formula feeding (complementary, supplementary or substitutive, used milk, number of feeds per day and amount of milk per feed). Weaning (onset, given food, amount and how taken). History of seizures or tetany. Medical history with special concern on previous vitamin D3 therapy was considered. All neonates who appear jaundiced were evaluated with a risk score or TSB (total serum bilirubin)/TcB (transcutaneous bilirubin) measurement (11). The bilirubin level was interpreted according to the infants' age in hours using bilirubin charts [Figures1 and 2 (12, 13)].

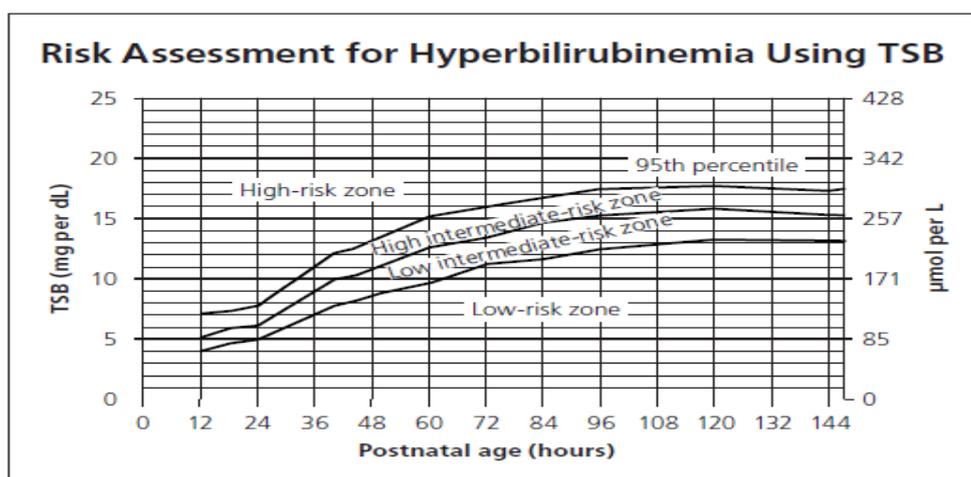


Fig.1: Total serum bilirubin (TSB) nomogram for designation of risk (12).

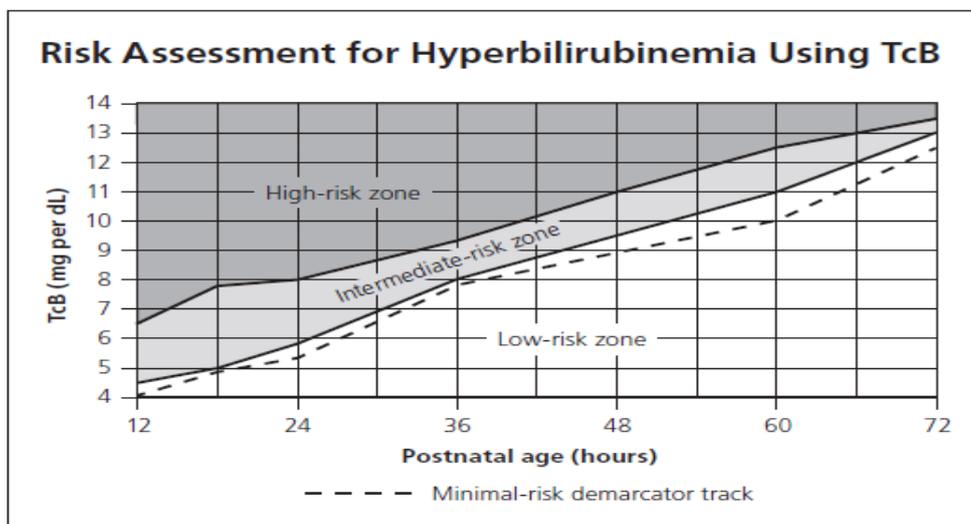


Fig.2: Transcutaneous bilirubin (TcB) nomogram for assessing the risk of subsequent significant hyperbilirubinemia in healthy term and near-term newborns (13).

2-4. Hematological and Biochemical work up

Five ml venous blood was withdrawn from each participant in the study divided into 2ml on EDTA tube for CBC analysis and the remaining 3 ml on serum gel separator tubes where were allowed to clot for 30 minutes at 37°C before centrifugation for 10 minutes at 3,500 rpm. The separated sera were evacuated into 1 ml cryo tubes and preserved at -80°C till time of biochemical assays in the form of 25-hydroxy cholecalciferol, ionized calcium, albumin, bilirubin (total & direct) and bilirubin / albumin ratio.

2-4-1. Analysis of CBCs: Performed using (Cell Dyn 1800-Abbott diagnostics, Germany).

2-4-2. Serum vitamin D3 assays: Measurement of Vitamin D3 was performed using commercially available ELISA assay kit supplied by Chongqing Biopsies Co., Ltd (Chongqing, People's Republic of China) with the catalog number: BYEK1472 using microplate ELISA reader (EMR-500, Labomed, Inc., LA, USA). The serum 25-OHD3 level of: ≥ 30 ng/ml was considered optimal, ≤ 20 ng/ml was considered to have vitamin D deficiency, vitamin D insufficiency was diagnosed at serum levels from 21 to 29 ng/ml (14-17). It was measured twice, before starting phototherapy and 5 days after therapy (12, 18).

2-4-3. Ionized Calcium assay: Commercially available colorimetric assay kits were used for assays of the serum levels of ionized calcium supplied by Spectrum Diagnostics, Egypt, using a spectrophotometer (Chem-7, Erba Diagnostics Mannheim GmbH, Germany).

2-4-4. Serum bilirubin (total and direct) assay: Measurement of total and direct bilirubin were performed using colorimetric kit supplied by Spectrum Diagnostic, Egyptian Company for

Biotechnology (S.A.E) using (Chem-7, Erba Diagnostics Mannheim GmbH, Germany). It was measured daily and the value 5 days after therapy was used for evaluation of the effect of vitamin D co-therapy.

2-4-5. Serum albumin assay: Measurement of albumin was performed using colorimetric kit supplied by Spectrum Diagnostic, Egyptian Company for Biotechnology (S.A.E) using (Chem-7, Erba Diagnostics Mannheim GmbH, Germany).

2-4-6. Serum bilirubin / albumin ratio:

Serum bilirubin / albumin ratio was calculated by dividing the serum total bilirubin by the serum albumin level.

2-5. Ethics approval and consent to participate

The study has been conducted in accordance to the Declaration of Helsinki and after approval of the Ethics Committees of Faculty of Medicine, South Valley University, Qena, Egypt. Qena 83523, Egypt.

2-6. Statistical data analysis

The data were tested for normality using the Kolmogorov-Smirnov test and for homogeneity variances prior to further statistical analysis. Categorical variables were described by number and percent (Number, %), where continuous variables described by mean and standard deviation (Mean, SD). Chi-square test and fisher exact test used to compare between categorical variables where compare between continuous variables by t-test (Independent-samples t-test, Paired-samples t-test). A two-tailed $p < 0.05$ was considered statistically significant. All analyses were performed with the IBM SPSS 20.0 software.

3- RESULTS

3-1. Demographic and clinical data of the study groups

The current study included 50 neonates with physiological jaundice with their demographic and clinical data were presented in (**Table.1**). The mean \pm SD of age (days) in cases was 5.17 ± 1.21 days.

The mean \pm SD of gestational age (weeks) in cases was 37.93 ± 0.87 weeks. Among cases, 29(58%) were males and 21(42%) were females. The mean weight of cases was 2.6 ± 0.56 kg. Twenty two cases (42%) were breast fed, 20(40.0%) were mixed, and 8(16%) were artificial feeding.

Table -1: Demographic and clinical data of the study group

Variables	Cases, (n=50)	
	No.	%
Age (days) Mean \pm SD	5.17 \pm 1.21	
Gestational age (weeks) Mean \pm SD	37.93 \pm 0.87	
Gender (Number, %)		
• Male	29	58
• Female	21	42
Weight (kg) Mean \pm SD	2.6 \pm 0.56	
Mode of delivery (Number, %)		
• NVD	24	48
• CS	26	52
Feeding history (Number, %)		
• Artificial feeding	8	16
• Breast feeding	22	44
• Mixed	20	40
Respiratory rate (cycle/min) Mean \pm SD	52.63 \pm 4.87	
Heart rate (beat/min) Mean \pm SD	129.03 \pm 12.53	
Temperature ($^{\circ}$ C) Mean \pm SD	36.17 \pm 0.77	

SD: Standard deviation, CS: Caesarian section; NVD: Normal vaginal delivery.

3-2. Hematological and biochemical data of the study groups

Regarding to the serum bilirubin levels (mg/dl), the mean \pm SD of total, direct and indirect bilirubin levels among cases were (16.3 ± 2.01 , 0.98 ± 0.92 and 15.47 ± 1.82

respectively), (**Table.2**). The mean \pm SD of serum vitamin D3 levels (ng/ml) among cases was (8.2 ± 0.8), with frequently occurring deficient vitamin D3 status and less frequency of optimal and insufficient vitamin D3 status among cases, (**Table.2**).

Table-2: Hematological and biochemical data of the study group.

Variables (Mean \pm SD)	Cases, (n=50)
Complete blood count parameters	
• Hemoglobin (g/dl)	15.1 \pm 2.37
• White blood cell count (10^6 /microlitre)	8.85 \pm 2.59
• Platelet count (10^3 /microlitre)	324.9 \pm 107.39
• Reticulocyte count (%)	2.63 \pm 1.42
Serum ionized calcium (mg/dl)	1.065 \pm 0.119
Serum bilirubin level (mg/dl)	
• Serum total bilirubin	16.3 \pm 2.01

• Serum direct bilirubin	0.98±0.92
• Serum indirect bilirubin	15.47±1.82
Serum albumin (g/dl)	3.75±0.72
Serum bilirubin / albumin ratio	2.62±0.95
Serum 25OH(D3) (ng/ml)	8.2±0.8
Vitamin. D 3 status (Number, %)	
• Deficient	29(96.7%)
• Insufficient	1(3.3%)
• Optimal	0(0.0%)

3-3. Effect of vitamin D co-therapy with phototherapy on serum bilirubin and vitamin D3

Five days following combined phototherapy and vitamin D3 supplementation for neonates with physiological jaundice and abnormally low serum vitamin D3 levels, there were significant lower mean ± SD of total, direct and indirect bilirubin levels among cases post-therapy (8.56±2.31, 0.61±0.52, and 8.02±2.35, respectively) compared with the pre-therapy levels, p< 0.05 for all

(Table.3). Additionally, there were significantly higher mean serum vitamin D3 levels (ng/ml) among cases post-therapy (19.29±8.18) compare to the pre-therapy levels with significant improvement in vitamin D3 status where there were significantly higher frequency of optimal and insufficient vitamin D3 status and significantly lower frequency of deficient vitamin D3 status in cases post-therapy compared to their status pre-therapy, p< 0.05 for all (Table.3).

Table-3: Comparison of the effects of phototherapy on serum bilirubin and vitamin D3 in neonates with physiological jaundice.

Measured Biochemical parameters, (Mean ± SD)	Pre -phototherapy level, (n=50)	Post-phototherapy level, (n=50)	P- value
Serum bilirubin level (mg/dl)			
• Serum total bilirubin	16.3±2.01	8.56±2.31	<0.001**
• Serum direct bilirubin	0.98±0.92	0.61±0.52	0.072
• Serum indirect bilirubin	15.47±1.82	8.02±2.35	<0.001**
Serum 25OH (D3) (ng/ml)	8.2±0.8	19.29±8.18	<0.001**
Vit. D3 status (Number, %)			
• Deficient	29(96.7%)	2(6.7%)	<0.001**
• Insufficient	1(3.3%)	24(80.0%)	
• Optimal	0(0.0%)	4(13.3%)	

Used Chi-square test for categorical variables & Paired-sample T test for continuous variables.

* Statistically significant difference (P<0.05), ** highly statistically significant difference (P<0.01).

3-4. Correlations between serum vitamin D 3, serum bilirubin, serum albumin and serum bilirubin / albumin ratio in neonates with physiological jaundice

Correlation analysis among the included cases revealed significantly negative correlations between vitamin D3 serum

levels with both serum total bilirubin (r=-0.703, p<0.001 and serum indirect bilirubin (r= -0.710, p<0.001), (Table.4 and Figure.3) with no significant correlations between vitamin D3 with serum direct bilirubin, or albumin or bilirubin/ albumin ratio.

Table-4: Correlations between serum vitamin D3 with serum bilirubin, serum albumin and serum bilirubin / albumin ratio in neonates with physiological jaundice.

Variables	Serum vitamin D 3 (ng/ml)
Serum total bilirubin (mg/dl)	r= -0.703
	P= <0.001**
Serum direct bilirubin (mg/dl)	r= -0.200
	P= 0.126
Serum indirect bilirubin (mg/dl)	r= -0.710
	P= <0.001**
Serum albumin (g/dl)	r= 0.117
	P= 0.380
Serum bilirubin \albumin ratio	r= -0.119
	P= 0.366

Used Pearson Correlation coefficients.

* Statistically significant difference (P<0.05), ** highly statistically significant difference (P<0.01).

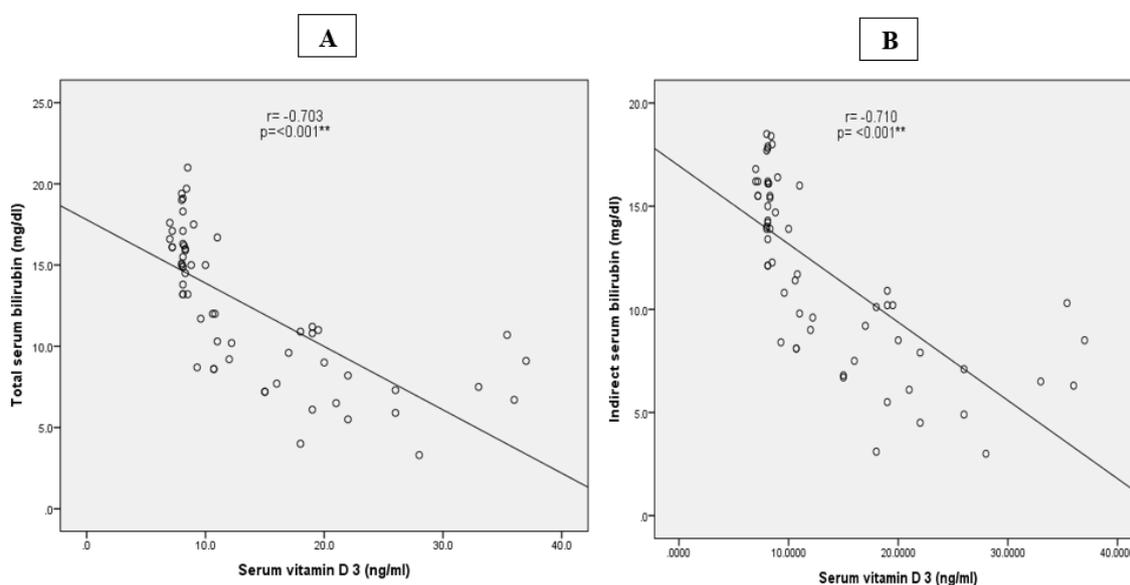


Fig.3: Negative correlations between serum vitamin D3 with both total serum bilirubin (A) and indirect serum bilirubin (B).

4- DISCUSSION

The aim of this study was to determine vitamin D3 status and whether there is a relationship between indirect bilirubin and serum 25(OH)D levels in term newborns with indirect hyperbilirubinemia at a level that necessitates phototherapy, as described by the American Academy of Pediatrics (12). Neonatal hyperbilirubinemia has a well-understood etiology. Despite years of research, the factors that contribute to hyperbilirubinemia in neonates remain poorly understood. Hyperbilirubinemia is a

multifactorial mechanism correlated with both biochemical and physiological immaturity (19). Few studies examined the relationship between neonatal serum vitamin D and hyperbilirubinemia (20). The current study found significantly low vitamin D3 levels in full-term neonates with physiological hyperbilirubinemia; however, whether this is an association or a pathogenic mechanism would require further investigation. Vitamin D3 deficiency in neonates has been linked to hyperbilirubinemia in many recent studies (19, 21-25). The mechanism underlying

this connection is unclear, but since bilirubin and vitamin D3 are both metabolized in the liver, there may be an interaction that worsens hyperbilirubinemia (26).

Sunlight, specifically UVB between the wavelengths of 290 and 315 nm, is the primary source of vitamin D3 for the body and is the key source of vitamin D3 production in the skin. Sunlight exposure is thought to provide 90 % of body requirements (27). Phototherapy with UV light has been used to treat vitamin D deficiency in places where sunlight is scarce or in conditions where patients cannot absorb vitamin D3 from their diet (28). The present research showed significant improvement in vitamin D3 status among the included cases 5 days following phototherapy without need for vitamin D3 supplementation, indicating that such treatment stimulates photobiosynthesis of vitamin D3.

In fact, further research is required to determine the optimal duration of narrow band ultraviolet (UVB) exposure and the long-term benefits of correcting vitamin D3 status through UVB rather than oral supplementation, especially in pediatric patients with fat malabsorption. A study by Dan-Ierodiaconou et al. (1980) on the effect of phototherapy on vitamin D metabolism examined ten infants with jaundice under phototherapy treatment.

The infants' 25-hydroxyvitamin D3, 24,25dihydroxyvitamin D3, calcium and phosphorous levels were measured before phototherapy and 24 and 48 h after the procedure was over (29). Correlation analysis in the current study revealed significantly negative correlations between vitamin D3 serum levels with both serum total bilirubin and serum indirect bilirubin. In line with our findings, two Egyptian studies (19, 26) reported a strong negative correlation between neonatal total serum bilirubin level and neonatal serum vitamin D3.

4-1. Study Limitations

Lack of comparison of the possible vitamin D3 effect as adjuvant therapy on neonates with physiological hyperbilirubinemia with normal vitamin D3 status without using phototherapy which will be approached in future studies. The findings of the current study required to be confirmed using larger scale studies.

5- CONCLUSION

The current study confirms association of abnormally low vitamin D3 status with the occurrence of physiological neonatal hyperbilirubinemia and explores the dual role of phototherapy in neonates with physiological jaundice candidate for it and had abnormally low vitamin D3 via improving both the hyperbilirubinemia and vitamin D3 status without need to add vitamin D3 supplementation.

6- AVAILABILITY OF DATA

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

7- AUTHORS' CONTRIBUTIONS

Study concept and design: AEA, MHH, NIR and AHB; Clinical evaluation of the cases: AEA, NIR and AHB; Literature research: FBT, MHH, NIR and AHB; Sample collections: MHH, MEMA and FBT; Biochemical and laboratory assays: MHH and MEMA; Data analysis: MHH, FBT, AHB, NIR and MEMA; drafting the manuscript: MHH; All authors revised and approved the final version of the manuscript.

8- CONFLICT OF INTEREST: None.

9- ACKNOWLEDGMENTS

This study was funded partially by Faculty of Medicine, South Valley University, Egypt. Qena 83523, Egypt.

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