

Adrenomedullin Level in Neonatal Hyperbilirubinemia

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Abstract

Introduction: Cellular and molecular mechanisms responsible for neurotoxicity have not yet been determined despite several studies conducted. Recent studies show that bilirubin may create oxidative stress. Adrenomedullin (AM) decreases endothelial permeability by increasing transendothelial electrical resistance (TEER), and with cAMP increase, it plays an important role in the regulation of this barrier.

Aim of the study: To investigate the relation between serum bilirubin and AM.

Patients and methods: This case control study included seventy neonates divided into two groups jaundiced and normal control groups. Complete blood count (CBC), liver function tests, kidney function tests and serum adrenomedullin: measured by Human Adrenomedullin Enzyme Linked Immunosorbent Assay for both groups.

Results: Serum adrenomedullin level was 48.64 ± 0.39 ng/L in jaundiced group (Group 1) while in the control group (Group 2), the level was 1.37 ± 0.01 ng/($p < 0.01$).

Conclusion: We can conclude that the role, effects and physiopathological basis of AM in neonatal hyperbilirubinemia should be established especially with further animal studies.

Keywords: Adrenomedullin (AM); neonatal hyperbilirubinemia; bilirubin

Introduction

Jaundice refers to the yellow colour of the skin and sclera of the eyes caused by excess bilirubin in the blood. Jaundice occurs when bilirubin builds up faster than a newborn's liver can break it down and pass it from the body. Clinically apparent jaundice occurs when the serum bilirubin level is 5-7 mg/dl in neonates. In adults sclera appears jaundiced when serum bilirubin exceed 2 mg/dl [1].

Cellular and molecular mechanisms responsible for bilirubin neurotoxicity have not yet been determined despite too many studies conducted. Recent studies show that bilirubin may create oxidative stress [2]. Free bilirubin is the bilirubin with lipophilic properties that can pass into the brain tissue and is primarily responsible for neurotoxicity. Immature blood-brain barrier (BBB) is more permissive to bilirubin. Cytokine release and inflammation also play a role in bilirubin neurotoxicity [3].

Adrenomedullin (AM) is a peptide with 52 amino acids and has tyrosine amino acid at carboxyl terminal. It was classified in the calcitonin/calcitonin gene-related peptide (CGRP)/amylin peptide family as it shows homology with CGRP that contains disulfide bond between 16th and 21st amino acids [4].

Cyclic adenosine monophosphate (cAMP) has long been known to contribute to the regulation of BBB functions; for example, cAMP elevates transendothelial electrical resistance and decreases paracellular permeability [5] reduces the rate of fluid phase endocytosis [6], and increases P-glycoprotein function [7].

Activation of adenylate cyclase is a common consequence of AM receptor activation in a wide variety of cells. So AM has an important neuroprotective role. The fact that AM is much more secreted in cerebral endothelial cells than the other endothelial cells also suggests that this peptide may have important functions in central nervous system (CNS). AM decreases endothelial permeability by increasing transendothelial electrical resistance (TEER), and with cAMP increase, it plays an important role in the regulation of this barrier. AM is a peptide, which may demonstrate neuron protective effects by inhibiting apoptosis, inducing angiogenesis and stimulating neuronal differentiation [8].

Aim of the Study

To investigate the relation between serum bilirubin and AM.

Patients and Methods

In this case control study - seventy neonates were recruited from the inpatient and outpatient clinic of Pediatric Hepatology department, National Liver Institute, Menoufiya University and Pediatric department in Benha teaching hospital from April 2012 to April 2013 to study the levels of AM in cases of neonatal hyperbilirubinemia.

Newborns was divided into two groups according to the serum bilirubin levels: those with hyperbilirubinemia constituted Group I which included thirty seven neonates, and those with normal bilirubin level on the basis of age specific guidelines of American Academy of Pediatrics served as the control group (Group II) which included thirty three neonates. Neonatal hyperbilirubinemia is considered when the serum bilirubin level is above 5-7 mg/dl in neonates.

Exclusion criteria included infants aged above 28 days, perinatal asphyxia and infants with proven sepsis, those whose mothers had chronic diseases, such as hypertension, pre-eclampsia and diabetes mellitus, those with jaundice due to haemolytic causes excluded from the study considering the oxidative stress possibly resulting from haemolysis.

All neonates were subjected to the following: Pregnancy data: previous and current pregnancies, mode of delivery, neonatal feeding, anthropometric measurements, the presence of jaundice and mode of treatment including phototherapy (age at which phototherapy was initiated, duration of phototherapy, and exchange transfusion), thorough clinical examination and laboratory investigation included: Complete blood count (CBC), liver function tests, kidney function tests, serum adrenomedullin: measured by Human Adrenomedullin Enzyme Linked Immunosorbent Assay ELISA Kit.

Assay Procedure

All reagents and samples were brought to room temperature before use and protected from strong light. A face mask and gloves were used and the tips and test tubes in hand used once, to protect kit reagents from contamination.

In Blank well: We added only Chromogenic solution A and B, then stop solution.

In Standard wells: We added standard 50µl, Streptavidin-HRP 50µl (since the standard already has combined biotin antibody, it is not necessary to add the antibody)(then sample 40µl was added then added both AM-antibody 10µl and Streptavidin-HRP 50 µl. gently shaken and, incubated 60 minutes at 37°C.

- Then was washed and shaken away the remaining water.
- We added chromogen solution A 50 µl, then chromogen solution B 50 µl to each well. Was added and gently mixed, incubated for 10 min at 37°C away from light.
- We added stop Solution 50 µl was added into each well to stop the reaction the blue changes into yellow immediately.
- Final measurement: blank well was taken as zero the optical density (OD) was measured under 450 nm wavelength which should be carried out within 10 min after adding the stop solution.
- According to standards' concentration and the corresponding OD values, we calculated out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation.

Calculation of results

The O.D values of all the wells were calculated, standard curve diagram with concentration of the standard from the low to the high and the left to the right as abscissa (X) axis and O.D values of the wells at 450 nm as ordinate (Y) axis was done. In the (Log it-Log), linear regression model to map out a standard curve. The corresponding concentration range of each sample was assayed on the standard curve diagram according to their OD values.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows 15.0 (St. Louis, MO). All the newborns in the study were analyzed regarding demographic, anthropometric and biochemical parameters including serum AM levels. Number and percent were used for discrete variables, and mean & standard deviation (SD) was used for continuous variables.

Chi-square test was used for the comparison of the discrete variables, and Student's t-test and ANOVA was used for the comparison of continuous variables in the study and control groups.

Pearson correlation analysis was used in assessing the relationship between serum adrenomedullin and serum bilirubin in all newborns in the study.

Non-parametric tests were used when the data showed non-parametric distribution. A p-value of < 0.05 was considered statistically significant. All tests were two-sided.

Results

Seventy neonates were included in this study, 33 males (47.2%), 37 female (52.8%). All were full term, 37 were jaundiced (52.8%) while 33 were not apparently jaundiced (42.2%).

No complication was found among the studied cases due to neonatal jaundice, jaundice improved with treatment of 37 cases (100%) by phototherapy while no cases (0%) needed exchange transfusion.

Serum adrenomedullin level was 48.64 ± 0.39 ng/L in jaundiced group (Group 1) while in the control group (Group 2), the level was 1.37 ± 0.01 ng/($p < 0.001$). Serum indirect bilirubin levels were 12.74 ± 5.36 mg/dl and 1.8 ± 0.95 mg/dl in Groups I and II, respectively ($p < 0.001$).

Discussion

Jaundice is the most common condition requiring medical attention in newborns. The yellow coloration of the skin and sclera in newborns with jaundice is the result of accumulation of unconjugated bilirubin. Jaundice is the most common physiological problem in newborn and is of concern because of the current early hospital discharge patterns. Most cases of jaundice will not occur until the infant is at home. For this reason, in addition to monitoring the infant's weight, it is imperative, the infants to be seen by their health care provider within a day or two days of hospital discharge [9].

There are a number of epidemiologic risk factors related to neonatal jaundice that have been reviewed elsewhere. Some of the factors associated with increased neonatal bilirubin levels are male sex, low birth weight, prematurity, certain races (Asian, American Indian, Greek) maternal medication (e.g. oxytocin, promethazine hydrochloride), premature rupture of membrane, increased weight loss after birth, breast-feeding and neonatal infection. Delivery with vacuum extractor increases the risk of cephalhematoma and neonatal jaundice. Data suggest that pancuronium is associated with an increased risk of hyperbilirubinemia [10].

This study, to our knowledge, is the second one investigating the relationship between serum AM levels and hyperbilirubinemia, which previously studied by Kemal E., *et al.* [11]. In this study, there was a significant positive correlation between serum bilirubin levels and simultaneously measured serum AM levels in total of the study population $p < 001$, which is in agreement with Kemal E., *et al.* [11].

In addition, we found a significant positive correlation between serum direct bilirubin and AM while we could not find any correlation between plasma AM levels and liver tests (AST, ALT and serum albumin). This correlation suggests that oxidative stress created by increasing bilirubin levels somehow activates endothelial synthesis of AM, being a strong antioxidant, as a protective measure [12].

Reactive oxygen species (ROS) contribute to increased blood brain barrier (BBB) permeability. In fact, experiments in frogs had shown a correlation between increased ROS levels and decreased electrical resistance across the brain endothelium indicated an increase in BBB permeability [13]. AM regulates the peripheral localization of F-actin bands, and decreases cellular permeability occurring due to H_2O_2 and thus has a protective effect on BBB, and ultimately demonstrates an antioxidant brain protecting efficacy against injury resulting from oxidant stress [8].

Although increasing bilirubin levels create oxidative stress and destroy BBB, increasing AM levels in response to increasing bilirubin levels regulate and protect BBB by increasing TEER and reorganizing actin filaments. One study has shown decreasing effect on electrical resistance in intestinal barrier and supported the inverse relationship between bilirubin levels and TEER [14].

The positive correlation between indirect bilirubin levels and AM, which increases in inflammation and of which both peripheral and neuronal anti-inflammatory efficacy have been proven, suggests that AM increases as a preventive response in the steps of inflammation and apoptosis at the level of CNS [15].

In most infants, unconjugated hyperbilirubinemia reflects a normal transitional phenomenon. However, in some infants serum bilirubin levels may rise excessively, which can be a cause for concern because unconjugated bilirubin is neurotoxic and cause death in newborns and lifelong neurologic sequel in infants who survive [16].

In this study, there was a significant positive correlation between serum total bilirubin level and the simultaneously measured serum AM levels ($P < 0.001$) in jaundiced patients, also there was a statistically significant difference between the study and control groups regarding both serum indirect bilirubin level and AM level ($p < 0.001$).

Adrenomedullin has vasodilator and blood pressure lowering properties and plays important role in maintaining electrolyte and fluid homeostasis [17]. Endogenous AM may protect from organ damage by inhibiting oxidative stress production [18] and raised AM levels correlated with increased oxidative stress.

Chemical, multifunctional properties of AM opened new scopes for researchers to utilize these features in the field of diagnosis and therapy. Till now researches are done either laboratory or on animal models this may be due to incomplete picture about its fate, its short half-life (22 mins), high cost, limitation of routes of administration, etc. to the best of our knowledge if these obstacles are removed AM will be promising therapeutic approach that will surely leave a thumb print in medicine and put the answer to questions remained unanswered for long time [19].

Conclusion

According to the results of the present study, we can conclude that endogenous AM may protect from brain damage by inhibiting the oxidative stress of significant hyperbilirubinemia. Further studies will clarify the role, effects and physiopathological basis of AM in neonatal hyperbilirubinemia.

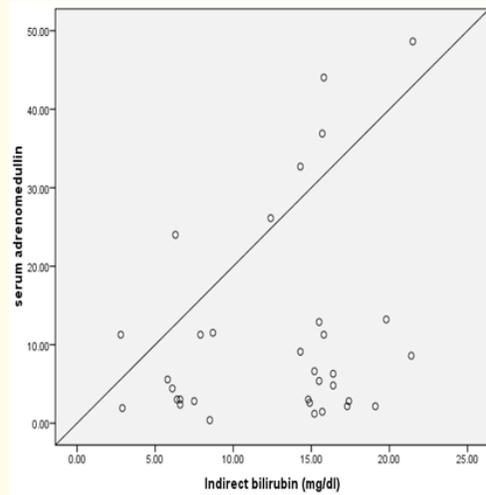


Figure 1: Correlation between serum adrenomedullin and serum indirect bilirubin levels in the newborns with jaundice (group 1), correlation coefficient (r) 0.26.

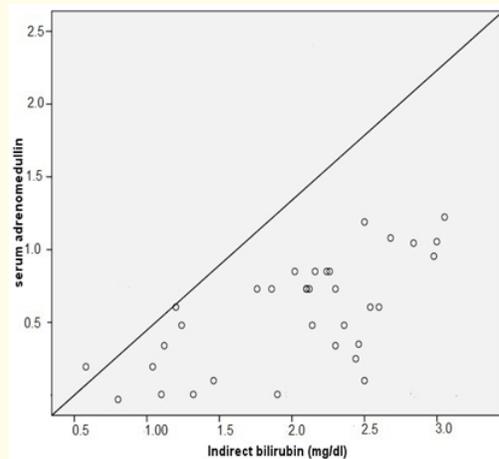


Figure 2: Correlation between serum adrenomedullin and serum indirect bilirubin levels in the control group (group 2). Correlation coefficient (r) 0.07.

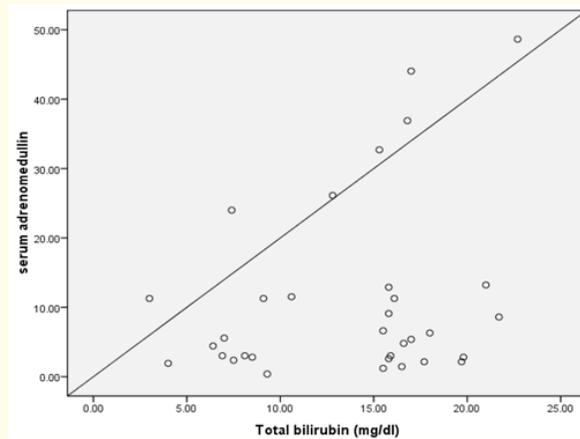


Figure 3: Correlation between serum adrenomedullin and serum total bilirubin levels in the newborns with jaundice (group 1), cases correlation coefficient (r) 0.27.

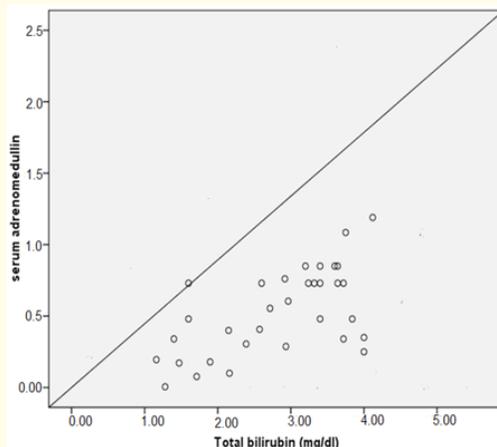


Figure 4: Correlation between serum adrenomedullin and serum total bilirubin in the control group (group 2). Correlation coefficient (r) 0.09.

	All subjects No. 70 No (%)	Patients No. 37 No (%)	Controls No. 33 No (%)	P Value
Sex				> 0.05
Males	33 (47.2)	20 (54.1)	13 (39.4)	
Females	37 (52.8)	17 (45.9)	20 (60.6)	
Mode of Delivery				> 0.05
NVD	37 (52.8)	18 (48.6)	19 (57.6)	
CS	33 (47.2)	19 (51.4)	14 (42.4)	
Anaesthesia				> 0.05
Not Given	37 (52.8)	18 (48.6)	19 (57.6)	
Given	33 (47.2)	19 (51.4)	14 (42.4)	
Total	70	37 (100%)	33 (100%)	

Table 1: Distribution of the studied cases (patients and controls) according to gender, mode of delivery and anaesthesia given.

NVD = normal vaginal delivery.

CS = cesarean section.

Studied Variables	All Subjects No. 70	Patients No. 37	Controls No. 33	P Value
	Mean ± SD	Mean ± SD	Mean ± SD	
Mother Age (Years)	25.8 ± 3.48	25.30 ± 3.86	26.24 ± 3.08	> 0.05
Parity	2.38 ± 1.23	2.30 ± 1.28	2.46 ± 1.19	> 0.05
Age at which blood was withdrawn (hours)	172.86 ± 176.97	88.85 ± 85.32	207.08 ± 189.20	< 0.005*
Birth Weight (kg)	3.18 ± 0.41	3.01 ± 0.32	3.34 ± 0.43	< 0.005*
Height (cm)	49.31 ± 2.12	48.87 ± 1.89	49.70 ± 2.26	> 0.005
Chest Circumference (cm)	31.92 ± 1.41	31.91 ± 1.26	31.95 ± 1.54	> 0.005
Head Circumference(cm)	35.25 ± 1.36	35.42 ± 1.37	35.09 ± 1.34	> 0.005

Table 2: Relationship between number of pregnancy (parity), mother age, anthropometric and neonatal jaundice.

Studied Variables	Patients No. 37	Controls No. 33	P Value
Serum Adrenomedullin Mean ± SD	11.02 ± 12.82	0.38 ± 0.41	< 0.001*
Serum Adrenomedullin level (ng/L)	48.64 ± 0.39	1.37 ± 0.01	

Table 3: Serum Adrenomedullin in the studied groups.

Studied Variables	Patients No. 37	Controls No. 33	P Value
	Mean ± SD	Mean ± SD	
Total Bilirubin mg/dl	13.66 ± 5.38	2.9 ± 0.96	< 0.001*
Direct Bilirubin mg/dl	0.88 ± 0.55	0.21 ± 1.09	< 0.05*
Indirect Bilirubin mg/dl	12.74 ± 5.36	1.8 ± 0.95	< 0.001*

Table 4: Serum bilirubin in the studied groups.

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