



RESEARCH ARTICLE

Can we use umbilical cord hydrogen peroxide as an early predictor for neonatal hyperbilirubinemia?



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Abstract

Introduction: Most neonatal hyperbilirubinemia is benign, but because of the potential toxicity of bilirubin, neonates should be monitored to identify those who might develop severe hyperbilirubinemia.

Objective: To determine if the cord blood hydrogen peroxide level can be used as an early predictor of neonatal hyperbilirubinemia or not and if there is a correlation between its levels and the severity of hyperbilirubinemia.

Methods: This is a prospective comparative study included 59 full term neonates. Forty-one neonates developed jaundice (group I) and 18 neonates did not develop jaundice (group II) as controls. For all studied groups, we measured the cord blood bilirubin using modified diazo method and hydrogen peroxide levels using ELISA Kits and we measured their levels at 5th and 7th days of life for patients.

Results: The cord blood H₂O₂ levels as well as the bilirubin levels were higher in patients than controls. Significant positive correlations between cord blood H₂O₂ and cord blood bilirubin levels were present (r=0.81& p<0.001), bilirubin levels at the 5th day of life (r=0.46& p<0.001) and bilirubin levels at the 7th day (r=0.60 & p<0.001). Moreover, there was a significant positive correlation between the 5th day H₂O₂ and the 5th day bilirubin levels (r=0.75& p<0.001) and a strong significant correlation between 7th day H₂O₂ and 7th day bilirubin (r=0.94 & p= 0.001). The sensitivity of the cord blood H₂O₂ in cases as a predictor to neonatal hyperbilirubinemia was 92.6% and the specificity was 100% with a cutoff point >35 (p-value 0.001).

Conclusion: Cord blood hydrogen peroxide (H₂O₂) levels can be used as an early predictor of neonatal indirect hyperbilirubinemia and can determine which neonates should be followed after discharge from the hospital.

Key words: cord blood, hydrogen peroxide, neonatal hyperbilirubinemia, prediction.

Introduction

Hyperbilirubinemia is the most common clinical condition requiring evaluation and treatment in the newborn and is a frequent reason for hospital admission during the first week of life [1]. Neonatal hyperbilirubinemia is generally considered a benign self-limiting condition affecting more than 60% of healthy term and late preterm infants [2]. However, severe neonatal hyperbilirubinemia can lead to irreversible brain damage and kernicterus [3, 4]. Therefore, researchers have been enthusiastic about identifying early predictors of neonatal hyperbilirubinemia to assist in the early detection of neonates at high risk of severe hyperbilirubinemia and therefore neurological sequelae. A number of previous studies have highlighted the relationship between bilirubin and nitric oxide and reactive oxygen/nitrogen species [5].

Hydrogen peroxide (H_2O_2) is a freely miscible with water and apparently able to cross the cell membranes readily [6].

In low concentrations, it is not toxic for the body and it plays a certain role in the fulfillment of very important physiological functions, however if it is produced in excess, this free radical is harmful due to its cytotoxic properties [7].

The fetal tissues which are in a permanent state of low oxygenation during intrauterine life are subjected to oxidative damage by the increase of free radicals such as nitrous oxide (NO) and (H_2O_2). Soon after birth part of these free radicals is directed against the circulating erythrocytes and thrombocytes and may cause hemolysis and thrombolysis [8].

Hydrogen peroxide is a chemical compound with the formula (H_2O_2), it is one of the reactive chemical species containing oxygen, its normal blood level is between 2.53 and 5.75 $\mu\text{mol/liter}$ [8].

Oxygen free radicals have been incriminated as the causative mechanism for many neonatal diseases including broncho-pulmonary dysplasia (BPD), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), intracranial hemorrhage (ICH) and hypoxic-ischemic encephalopathy (HIE) [9].

Given the relationship between bilirubin and hydrogen peroxide, which is one of reactive oxygen species, it could be suggested that if hydrogen peroxide levels in cord blood could be used to predict neonatal hyperbilirubinemia or not.

Aim of the work

The study aimed to answer if the cord blood hydrogen peroxide level can be used as an early predictor for neonatal hyperbilirubinemia or not

and if there is a correlation between its levels and the severity of hyperbilirubinemia?

Subjects and Methods

This is a prospective comparative study included 59 full-term neonates delivered at Minia University hospital for gynecology & obstetrics and children during the period from June 2016 to December 2016. The patients were followed up for development of neonatal jaundice. Forty-one neonates developed neonatal jaundice, 14 of them were missed during the study follow-up, while 27 patients continued the follow-up and were assigned as patients (group I). Eighteen neonates did not develop neonatal jaundice and were assigned as a control group (II).

Inclusion Criteria: full-term (37-42 weeks) neonates according to [New Ballard Score] for assessment of gestational age, birth weight 2500 – 4000 grams and Apgar score of ≥ 7 at 1 and 5 minutes of life.

Exclusion Criteria: Preterm neonates delivered before 37 weeks gestation, suspected patients of hemolytic diseases of the newborn, patients with cephalohematoma, cholestasis, major congenital or chromosomal abnormality and suspected sepsis,

Both groups of patients and controls were subjected to:

(1) Full history taking, including maternal medical problems, mode of delivery and Apgar score.

(2) Clinical examination including: assessment of gestational age using New Ballard Score, anthropometric measurements and systemic examination.

3. For all neonates, the following investigations were done

1) Complete blood count using electronic counter Sysmex kx-21N

2) Reticulocyte count by Billiant Cresyl blue stain (supravital stain).

3) Maternal and neonatal blood group and Rhesus factor (Rh)

4) Serum bilirubin (total and direct) using modified diazo method (Bilirubin diamond diagnostic kits)

Principle:

Quantitative determination of bilirubin:

Two mls of cord blood and 2 mls of venous blood from all studied groups (patients and controls) at fifth and seventh days of life were collected for separation of enough serum required for the test. Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photo-metrically. The intensity of the color formed is proportional to the bilirubin concentration in the sample [10].

5) Serum hydrogen peroxide level.

Measurement of serum hydrogen peroxide levels (H_2O_2) from cord blood sample (5ml) for both groups (patients and controls) and from venous blood (3 ml) sample postnatal at fifth and seventh days of life for patients only (group I) using human hydrogen peroxide (H_2O_2) ELISA Kit, Glory Science co., Ltd

Principle of the test:

The kit is for the quantitative level of H_2O_2 in the sample, purified human H_2O_2 was adopted to coat micro titer plate, solid –phase antibody was made then samples or standards were added to wells with a labeled antibody specific to H_2O_2 then labeled HRP to well. The concentration of H_2O_2 in the samples is then determined by comparing the O.D. of the samples to the standard curve [11].

Statistical analysis:

SPSS (statistical package version 22) was used for statistical analysis of data. The p-value < 0.05 was considered the cut-off value of significance. Receivers Operating Characteristic (ROC) curve: was used to illustrate the diagnostic properties of a test on a numerical scale. Quantitative results were presented as mean \pm SD while qualitative data were presented by frequency distribution as percentage (%). Correlation was performed by using Pearson correlation coefficient.

Results

There were no statistically significant differences between patients and controls regarding the age, sex, weight, mode of delivery, RBCs, reticulocyte count or platelets count, while there were significant lower levels of hemoglobin, total leucocytic count (TLC) and hematocrit levels in patients group than controls (p-values were $p<0.001$, $p<0.001$, $p<0.008$ respectively). The cord blood H_2O_2 levels as well as the bilirubin levels were higher in patients than controls (Table 1and figure 1). The bilirubin levels of cord blood, 5th and 7th days were higher in patients than controls with a peak level at the 5th day of life (mean \pm SD 13.8 ± 2.9 in patients)(Figure 2).

Significant positive correlations between cord blood H_2O_2 and cord blood bilirubin levels were present ($r=0.81$ & $p<0.001$) (Figure 3) as well as between the cord blood H_2O_2 and bilirubin levels at the 5th day of life ($r=0.46$ & $p<0.001$) (Figure 4) and significant correlations between cord H_2O_2 and bilirubin levels at the 7th day ($r=0.60$ & $p<0.001$) (Figure 5).

Moreover, there was a significant positive correlation between the 5th day H_2O_2 and the 5th day bilirubin levels ($r=0.75$ & $p<0.001$) and a strong significant correlation between 7th day H_2O_2 and 7th day bilirubin ($r=0.94$ & $p= 0.001$) (Table 3).There were non-significant correlations between H_2O_2 levels and each of

weight and gestational age among patients (Table 4).

The sensitivity of the cord blood H_2O_2 in cases as a predictor to neonatal hyperbilirubinemia was 92.6% and the specificity was 100% with a cutoff point >35 (p-value 0.001)(Figure 6)

Discussion

Hyperbilirubinemia is the most common condition in neonates that requires medical attention. About 50% to 70% of term babies and 80% of preterm babies develop hyperbilirubinemia in the first week of life [12]. Although most neonates with hyperbilirubinemia are otherwise healthy, they need to be monitored. Hydrogen peroxide (H_2O_2) is the simplest peroxide present normally in the body [13].

Erythrocyte of newly born is sensitive to hydrogen peroxide and this sensitivity may be associated with deficiency of vitamin E or erythrocyte glutathione peroxidase or catalase [14].

If an excess of free radicals such as H_2O_2 is produced, as occurs in the case in the newborn, part of it is directed against the circulating erythrocytes and thrombocytes, and may cause hemolysis.

Early discharge of healthy term neonates is a common practice because of medical, social and economic constraints and in a significant

number (6.5%) of neonates; the neonatal hyperbilirubinemia is a cause for readmission [15].

The American Academy of Pediatrics (AAP), 2004 [16] recommended that neonates discharged within 48 hours should have a follow-up visit after 2-3 days to detect significant hyperbilirubinemia and other problems.

Our results revealed that there were no significant differences between neonates with hyperbilirubinemia and controls regarding the age, sex, weight, and mode of delivery. These results agreed with the results of a study published in 2009 [17]. However, these results disagreed with another study found that hyperbilirubinemia was more frequent in boys than in girls and more in neonates delivered by normal vaginal delivery than by Caesarean section with no explanation [18].

In this study, we found that the cord blood hemoglobin, TLC, and hematocrit were significantly lower in patients than controls and this may be attributed to hemolysis of RBCs in patients which cause bilirubin levels rise. These results were in concordance with the previous study [18] that found lower levels of hemoglobin, TLC in patients than controls.

The results of this study showed that levels of bilirubin in patients were more than in controls

with a peak level at 5th day (mean value of 13.8 mg /dl \pm 2.9) and this was expected. Those results agree with other studies reported that the peak of hyperbilirubinemia also was at the 5th day with mean \pm SD of 10.81 \pm 2.81mg/dl [19].

This study revealed significant higher levels of serum hydrogen peroxide in patients at 5th and 7th days with a peak level at 5th day and mean \pm SD 128 \pm 37.7 pg/ml(P value<0.001) and there was a significant higher cord hydrogen peroxide levels in comparison to controls.

The results agreed partly with a study reported significant higher levels of hydrogen peroxide in hyperbilirubinemia in neonates at 3rd and 5th days more than in controls with a peak level at the 3rd day [20].

In the present study, there were significant positive correlations between cord H₂O₂ and cord bilirubin levels (r=0.81& p<0.001) , between cord H₂O₂ and bilirubin at 5th day (r=0.46& p<0.014) and between cord H₂O₂ and bilirubin in 7th day (r=0.60 & p<0.001) respectively among patients.

In the present study there was no-significant correlation between H₂O₂ levels and each of weight or gestational age among patients.

Chou et al, 2014 [20] recorded a sensitivity of 74.3% and a specificity 54.9% for cord blood hydrogen peroxide as a predictor for hyperbilirubinemia while in this study using

cord blood hydrogen peroxide level of \geq 35 pg/ml as a cut-off value, neonatal indirect hyperbilirubinemia can be predicted with a sensitivity of 92.6% and a specificity of 100% for the same markers. These results may be explained in the light of association between bilirubin concentrations and reductive and oxidative reactions.

It was found that the level of hydrogen peroxide increases rapidly during the first few days of life, parallel with the increase in bilirubin concentrations [17]. There is a strong correlation between hydrogen peroxide levels and bilirubin concentration. The increase in hydrogen peroxide levels is caused by increasing oxygen levels in the transition from fetal to the extra-fetal environment [17]. Hydrogen peroxide may damage red blood cell membranes, leading to the production of bilirubin [21]

The greater the reactive oxidative product, such as hydrogen peroxide level after birth, the more bilirubin is produced. Hence, we can measure hydrogen peroxide levels as an indicator of severe hyperbilirubinemia.

The significant positive correlations between cord blood hydrogen peroxide (H₂O₂) levels and bilirubin concentrations in the immediate neonatal period suggest that cord blood hydrogen peroxide levels can be used to predict

severe neonatal indirect hyperbilirubinemia.

Limitation of this study was in the form of small sized sample of the study and difficulty in measurement of hydrogen peroxide (H₂O₂) at the fifth and seventh days of life for controls due to financial aspects.

Conclusion

Cord blood hydrogen peroxide (H₂O₂) levels can be used as an early predictor of neonatal indirect hyperbilirubinemia and can determine which neonates should be followed after discharge from the hospital.

Conflict of interest No Conflict of interest

Author's contributions (NS) carried out the study design, (LM) coordinated the implementation, (RH) helped to perform the statistical analysis, (NO) carried out the laboratory investigations, (All authors) drafted the manuscript. (All authors) collected the data revision of the manuscript and approved the final manuscript.

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Table (1) Demographic and laboratory data of studied groups

Item		Patients N = 27	Controls N = 18	P-Value
Gestational age (Weeks)	Range	37 – 39	37 – 40	0.91
	Mean ± SD	38.6 ± 0.8	38.6 ± 0.1	
Weight (Kg)	Range	2.5 – 3.7	2.5 – 3.2	0.71
	Mean ± SD	3 ± 0.4	3 ± 0.32	
Sex	Male	13 (48.1 %)	4(22.2 %)	0.17
	Female	14 (51.9 %)	14 (77.8 %)	
Mode of delivery	NVD	11 (40.7 %)	10 (55.6 %)	0.43
	CS	16 (59.3 %)	8 (44.4 %)	
Cord Hemoglobin (g/dl)	Range	10-16.8	13-18	0.001**
	Mean ± SD	12.7±2.1	15.8±1.5	
WBCs (x10³/ul)	Range	2.1-15.1	9-14	0.001**
	Mean ± SD	8.2±3.01	11.2±1.8	
Hematocrit (%)	Range	31.5-65	45-60	0.008*
	Mean ± SD	44.4±8.6	53.1±5.4	
Reticulocytes (%)	Range	0.4-2.5	0.2-2	0.55
	Mean ± SD	0.94±0.5	0.81±0.56	
RBCs (x10⁶/ul)	Range	4-5.5	4-5.3	0.49
	Mean ± SD	4.5±0.7	4.7±0.4	
Platelets count (x10³/ul)	Range	116-450	150-450	0.64
	Mean ± SD	259.5±65.3	273.1±99.9	
Bilirubin (mg/dl)	Range	2.7-9	1-2	0.001**
	Mean ± SD	5.2±2.2	1.5±0.52	
Cord H₂O₂ (pg/ml)	Range	10-81	14-35	0.001**
	Mean ± SD	42.7±12.4	24.7±6.1	

*Significant; ** highly significant

SD: standard deviation; NVD: normal vaginal delivery; CS: Caesarean section

Table (2): Comparison between cord blood, 5th day and 7th day H₂O₂ levels in patients

	Cord H ₂ O ₂ Patients	5 th day H ₂ O ₂	7 th day H ₂ O ₂	P1-value	P2-value	P3-value
Range	10-81	51-212	42-164	0.001**	0.01*	0.01*
Mean ±SD (pg/ml)	42.7±12.4	128.4±37.7	84.5±33.4			

*Significant; ** highly significant

P1: between cord & 5th day levels

P2: between cord & 7th day levels

P3: between 5th & 7th day levels

Table (3): Correlations between H₂O₂ and bilirubin levels in patients

Item	Cord blood H ₂ O ₂ (pg/ml)		5 th day H ₂ O ₂ (pg/ml)		7 th day H ₂ O ₂ (pg/ml)	
	R	p-value	r	p-value	r	p-value
Cord bilirubin (mg/dl)	0.81	0.001**	0.12	0.52	0.02	0.89
5th day Bilirubin (mg/dl)	0.46	0.014*	0.75	0.001**	0.39	0.04*
7th day Bilirubin (mg/dl)	0.60	0.001**	0.21	0.28	0.94	0.001**

*Significant; ** highly significant

Table (4): Correlations between H₂O₂ (pg/ml) levels and gestational age and birth weight in patients

Item	Cord H ₂ O ₂		5 th day H ₂ O ₂		7 th day H ₂ O ₂	
	r	p-value	r	p-value	r	p-value
Gestational Age	0.20	0.30	-0.18	0.36	- 0.06	0.97
Birth Weight	-.11	0.57	0.09	0.64	0.24	0.21

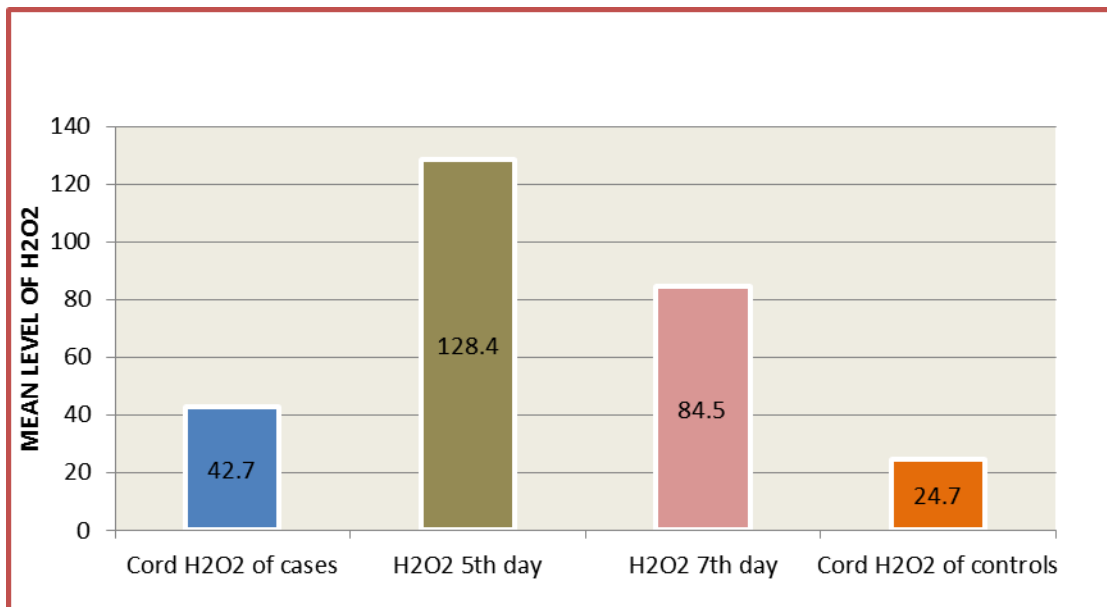


Figure (1): Comparison between patients cord & serum (5th -7th days) H₂O₂ levels and that of control cord blood (pg/ml).

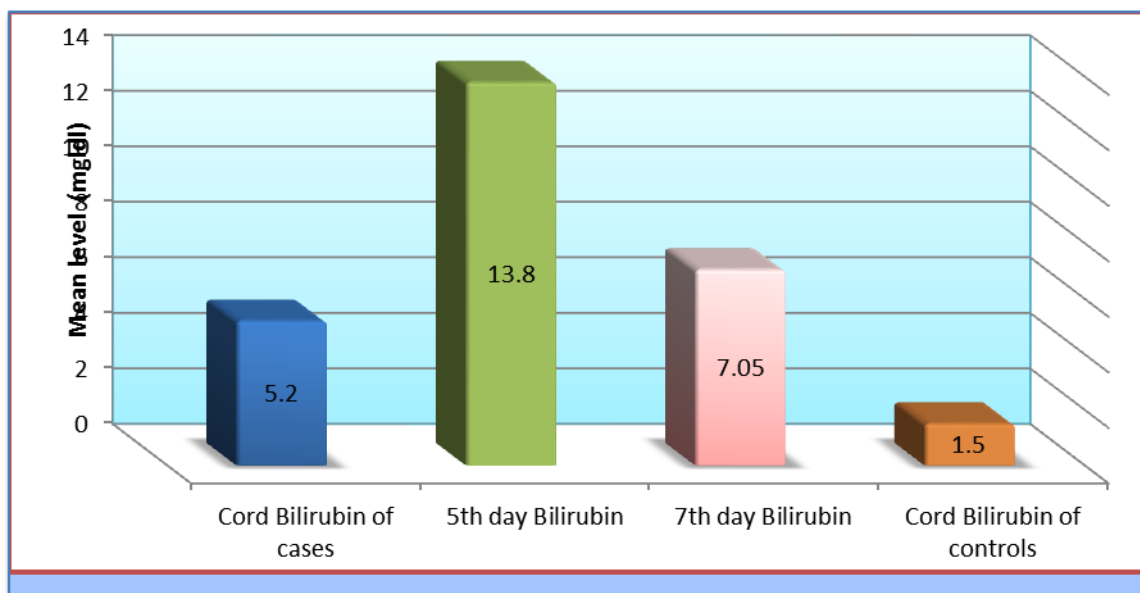


Figure (2): Comparison between patients cord & serum (5th -7th days) bilirubin levels and that of control cord blood (mg/dl).

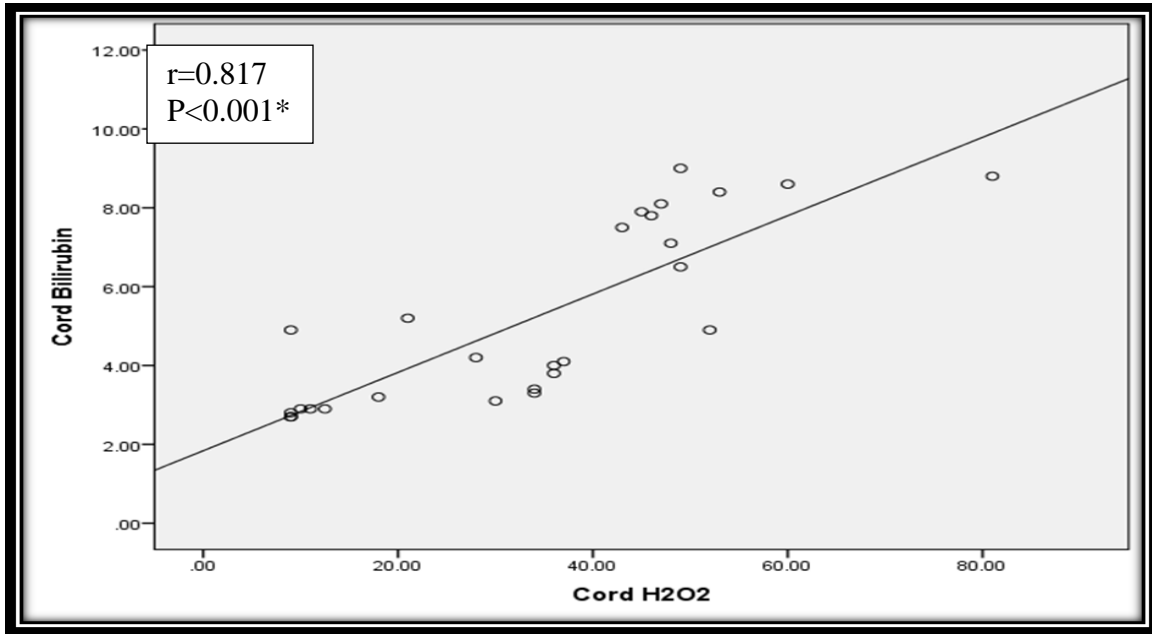


Figure (3): Correlation between cord H₂O₂ (pg/ml) and cord bilirubin levels among all neonates (mg/dl)

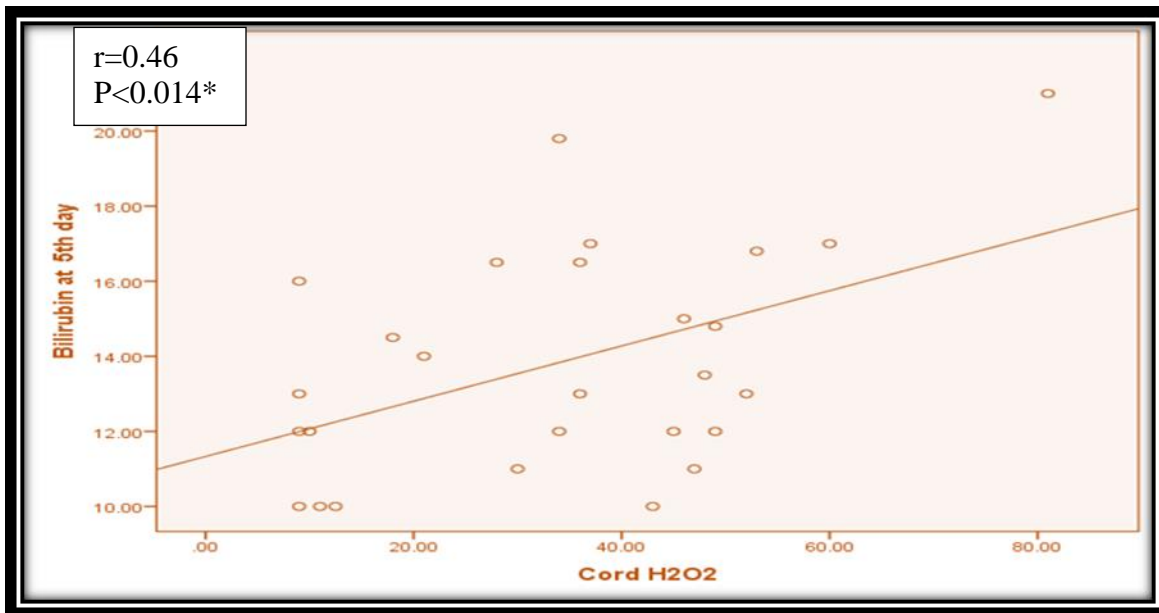


Figure (4): Correlations between cord H₂O₂ and 5th day bilirubin levels

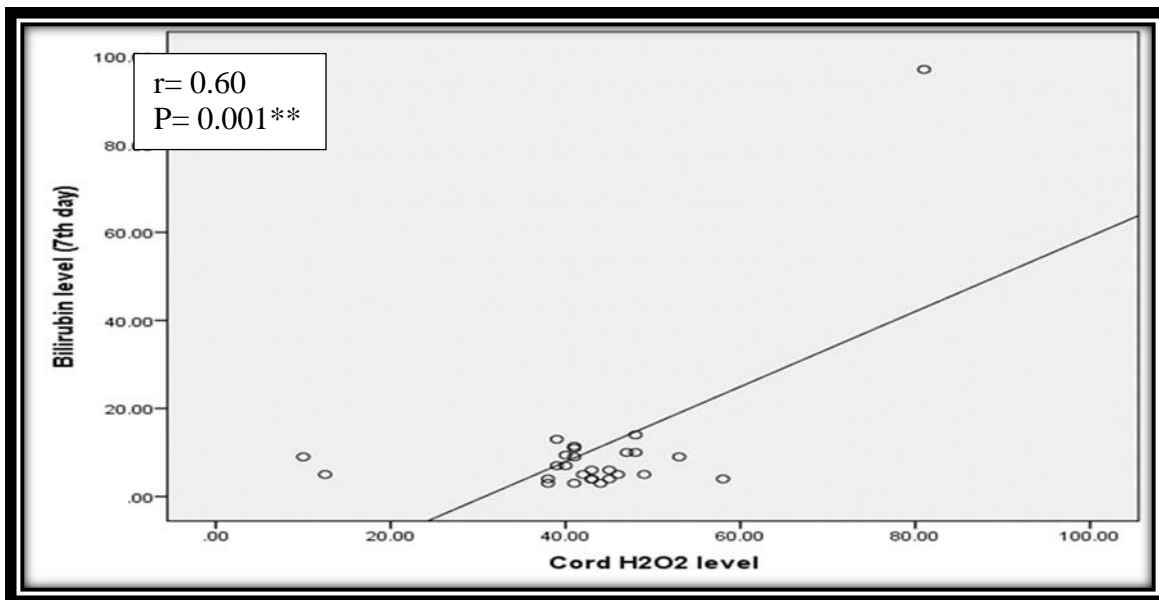


Figure (5): Correlation between cord H₂O₂ and bilirubin levels at the 7th day

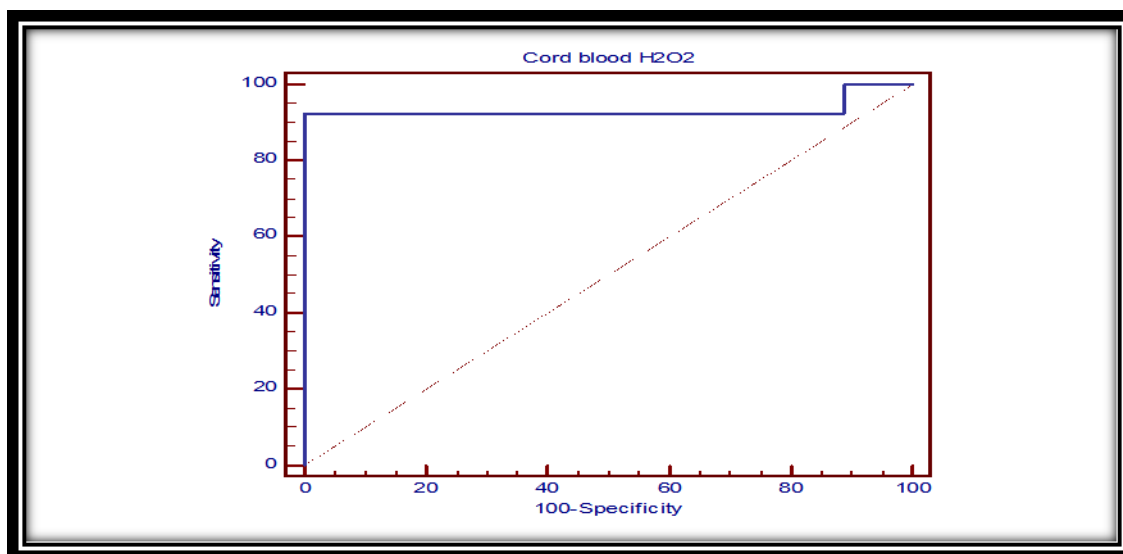


Figure (6): Sensitivity and specificity of cord H₂O₂ as a predictor for neonatal indirect hyperbilirubinemia.

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