

## Comparative Study of Liver Function and Rh Blood Group between both Physiological and Pathological Neonatal Jaundice

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### Abstract

Jaundice occurs in most newborn due to increase bilirubin concentration. Jaundice is detected at first week after birth in approximately 60% of full-term neonates. A high level of bilirubin is neurotoxic and may cause neonatal kernicterus, auditory neuropathy or death. The article aims to compare the Rh group compatibility, serum bilirubin (total and direct), serum albumin and several liver enzymes (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase) between physiological and pathological neonatal jaundice. The study was conducted for one hundred neonates with jaundice divided into two groups, group one includes fifty newborns with physiological jaundice, Group two includes fifty newborns with pathological jaundice. Blood samples were taken from each patient, used to determine the Rh group, serum bilirubin (total and direct, liver enzymes and albumin levels. In the present study, it was found that Rh compatibility show a significant relationship between newborns with physiological and pathological jaundice ( $p < 0.05$ ). Serum bilirubin (total and direct), serum albumin concentration and the liver enzymes were significantly higher when compare between newborns and pathological jaundice with newborns of physiological jaundice ( $p < 0.05$ ).

These findings demonstrate that the newborns with pathological jaundice have significantly higher levels of the studied parameters (Serum bilirubin, albumin and several liver enzymes) in comparison with those with physiological jaundice.

**Keywords:** Neonatal jaundice, Liver enzymes, Bilirubin, Albumin.

### دراسة مقارنة بين مصل البيليروبين ومصل الألبومين و البعض من إنزيمات الكبد بين اليرقان الفسيولوجي واليرقان المرضي للأطفال حديثي الولادة أية ناظم كمال<sup>\*</sup>، و علي فارس حسن<sup>\*\*</sup>

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#### الخلاصة

ينتج اليرقان في معظم حديثي الولادة بسبب زيادة مستوى البيليروبين في الدم عن الحد الطبيعي. تتم ملاحظة اليرقان خلال الأسبوع الأول بعد الولادة في حوالي 60٪ من حديثي الولادة. زيادة تركيز البيليروبين في الدم تكون سامة حيث تسبب تسمم في الاعصاب وقد يؤدي الى التلف الدماغي و اعتلال الأعصاب السمعية أو الوفاة. لأجراء مقارنة في توافق مجموعة Rh ، تركيز البيليروبين (الكلي والمباشر) ، تركيز الألبومين وتراكيز بعض انزيمات الكبد في مصل الدم بين اليرقان الفسيولوجي واليرقان المرضي في الاطفال حديثي الولادة حيث تم جمع مئة عينة دم من حديثي الولادة المصابين باليرقان و تقسيمهم الى مجموعتين المجموعة الاولى 50 طفل يعانون من اليرقان الفسيولوجي، المجموعة الثانية 50 طفل يعانون من اليرقان المرضي واجريت الفحوصات التالية لتحديد توافق Rh ، مستوى البيليروبين الكلي والمباشر ، تركيز انزيمات الكبد (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase) وكذلك تركيز الألبومين. وقد أظهرت الدراسة علاقة معنوية عند المقارنة بين توافق Rh بين الأطفال حديثي الولادة المصابين باليرقان الفسيولوجي والذين يعانون من اليرقان المرضي ( $p < 0.05$ ) كذلك مستوى البيليروبين الكلي والمباشر في مصل الدم ، تركيز الألبومين وتركيز انزيمات الكبد كانت مرتفعة معنويا للأطفال المصابين باليرقان المرضي عند مقارنتهم مع الاطفال ذوي اليرقان الفسيولوجي ( $p < 0.05$ ).

وبذلك تم الاستنتاج أن الأطفال الذين يعانون من اليرقان المرضي لديهم تراكيز أعلى من هذه المؤشرات (الالبومين والبيليروبين في مصل الدم وانزيمات الكبد المتعددة) مقارنة باليرقان الفسيولوجي.  
الكلمات المفتاحية: اليرقان الوليدي ، انزيمات الكبد ، البيليروبين ، الألبومين

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## Introduction

Jaundice (hyperbilirubinemia) is an elevated level of the pigment bilirubin in the blood, causing yellow discoloration of the body tissue resulting from the accumulation of an excess of bilirubin in skin <sup>(1)</sup>. The Neonates jaundice is clinically diagnosed when total serum bilirubin level (TSB) above 5 mg per dL <sup>(2)</sup>. Bilirubin is produced from the catabolism of heme by heme oxygenase with liberation of iron, carbon monoxide and biliverdin. The biliverdin is reduced by biliverdin reductase to form bilirubin <sup>(3)</sup>. The bilirubin produced is then transported to the liver in the conjugated form with plasma albumin. bilirubin conjugation occur in the liver by UDP-glucosyltransferase enzyme and this conjugation increase water solubility which ease the removal of bilirubin. Conjugated bilirubin is discharged into the bile and intestine <sup>(4)</sup>.

In the newborns, greatly of the conjugated bilirubin in the gut is decomposed back to unconjugated bilirubin by the action of the enzyme beta-glucuronidase that is present in the intestinal mucosa. The reabsorptions of unbound bilirubin into the blood stream take place by the action of enterohepatic circulation, this leads more of bilirubin load to the already overloaded liver <sup>(5)</sup>. Severe hyperbilirubinemia (more than 20 mg/dL) that could possibly cause kernicterus and neurodevelopmental problems less than of 2% of neonatal infants may be affected <sup>(6)</sup>.

Various risk factors increase the incidence of jaundice this includes preterm neonatal, low birth weight, hemolysis, sepsis, cephalohematoma or easy bruising, and only breastfeeding <sup>(7)</sup>.

Physiological Jaundice is the one of more common type of neonatal jaundice, do not cause serious problems <sup>(8)</sup>. Physiological jaundice usually appears after at least 24 hours of birth, and peak after four or five days. It later disappears after about 2 weeks of life.

The bilirubin associated with physiologic jaundice is the mostly unbounded, and its levels in serum do not excess 15 mg/dl<sup>(9)</sup>. Many clinical conditions can lead to the occurrence of physiologic jaundice in the neonatal like high bilirubin load of relative polycythemia, immature hepatic uptake, a shortened life span of red blood cell, and higher enterohepatic circulation<sup>(10)</sup>.

Other type of neonatal jaundice is pathologic jaundice which is characterized by rapid onset of jaundice ( first 24 hours after delivery), the rapid elevation of total serum bilirubin level (elevate of more than 5 mg/dL/day), and a total serum bilirubin concentration more than (17 mg/dL) in a full-term infant <sup>(7)</sup> frequently happens as a result of iso-immunisation (mostly ABO or Rhesus incompatibility) or another reasons of large hemolysis <sup>(11)</sup>.

The regimens is used to treat jaundice: phototherapy <sup>(12)</sup>, exchange transfusion this is used to remove antibodies that are causing hemolysis and is used in RH iso-immunization and ABO Incompatibility, drugs therapy of neonatal hyperbilirubinemia may be classified into phenobarbitone<sup>(13)</sup>, intravenous immunoglobulin (IVIG)<sup>(14)</sup> and metalloporphyrins <sup>(15)</sup>.

## Patients and Methods

**Table 1.** List of reagents, their companies, and countries

Material	Company	Country
Alanine transaminase (ALT) kit	Biosystems	Spain
Albumin kit	BIOLABO	France
Alkaline Phosphatase (ALP) kit	Biosystems	Spain
Aspartate transaminase (AST) kit	Biosystems	Spain
Direct Serum Bilirubin	Linear	Spain

### Patients' selection

A cross-sectional study of 100 newborns with jaundice, diagnosed by pediatrician. They were selected from the Salah Al-Din General Hospital in Tikrit city and Al-Alwiya Children's Hospital in Baghdad. According to the pediatrician diagnosis and biochemical tests, the newborns allocated into two groups

Group 1: 50 newborns with physiological jaundice

Group 2: 50 newborns with pathological jaundice

The 100 neonate undergo the followings information were recorded, that data categorized into:

1-Pateints demographic data which include (sex of newborns, weight, age, gestational period, residency, source of pollution such as presence of gas station, factory, high way road and the job of mother)

2-Clinical Examination which includes (onset of jaundice, state of newborn, feeding status, bowel motion, level of yellowish discoloration, moro reflex, sucking reflex and gasping reflex)

3-Laboratory Data which include (Rh group, total serum bilirubin, Direct Serum Bilirubin, serum albumin, ALT, AST and ALP).

### Blood sampling

Blood sample (1 ml) taken from each patient and put in to serum separator tube then centrifuged to isolate the serum which used for measurements of direct serum bilirubin, liver function test (ALT, AST and ALP) and albumin concentration. liver function test (ALT, AST and ALP) and albumin concentration done in private

laboratory meanwhile other tests achieved in hospital laboratory.

### Biochemical assay

#### Determination of Rh group by slide method

Rh antigens, termed for the rhesus monkey in which they were first determined, are as well as surface antigens noted on erythrocytes. There are certain Rh antigens (prevalent one is named D). Red cells showing the Rh antigens are termed Rh positive. Red blood cells which do not exhibit this surface antigen are Rh negative (approximately 15% of the people are Rh negative)<sup>(16)</sup>.

#### Procedure

Take pre-warm a clean glass slide to 40-50 C on a lighted view box and then place one drop of Immucor Anti-D Series 4 (Monoclonal Blend) on the slide and add one drop of blood sample to be tested to drop of Anti D. Using a clean applicator stick, mix the blood -reagent mixture over an oval area of approximately 20 mm x 40 mm. Rock the view box back and forth and observe for macroscopic agglutination for a period not to exceed 2 minutes.

**Positive Test:** agglutination of red blood cells at the immediate spin, or 37 C or antiglobulin phases.

**Negative Test:** no agglutination of red blood cells at any test phase<sup>(17)</sup>.

#### Measurement of total bilirubin.

The measurement of serum total bilirubin is achieved by using Dual Wavelength Total Bilirubin Meter (APEL BR-501) device which is use a dual wavelength (461 nm, 551 nm), this device minimizes the interference of hemolysis and turbidity<sup>(18)</sup>.

#### Procedure

50-60 µL of whole blood sample collected by heparinized capillary tube and measure directly by Dual Wavelength Total Bilirubin Meter device and record the result.

#### Measurement of direct serum bilirubin

Bilirubin is transformed to colored azobilirubin by diazotized sulfanilic acid and is calculated photometrically. The two bilirubin parts in serum-bilirubin glucuronide and free bilirubin which is conjugated to albumin-only the former reacts directly, free albumin reacts after being removed from protein in an accelerator<sup>(19)</sup>.

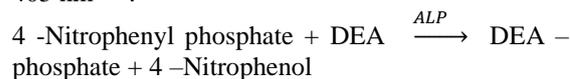
#### Procedure

Mix thoroughly 100µL of serum sample with 1 ml of working reagent and let tubes stand for exactly 5minute at 37c. Read the absorbance (A) of the samples blanks at 540 nm against distilled water and read the absorbance (A) of the samples at 540 nm against the reagent blank by BioSystem bts 350 device.

#### Measurement of Alkaline phosphatase (ALP)

The action of Alkaline phosphatase (ALP) enzyme in alkaline medium is the removal of the phosphate group from 4-nitrophenylphosphate to

diethanolamine (DEA), releasing 4-nitrophenol. The amount of 4-nitrophenol production which determine the catalytic concentration, calculated at 405 nm<sup>(20)</sup>.



#### Procedure

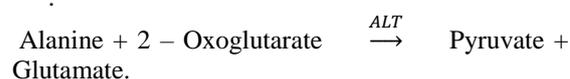
Bring the Working Reagent and the instrument to reaction temperature and then mix 1ml of reagent A, 200 µl of reagent B with 20 µl of serum sample into a cuvette after that insert the cuvette into the spectrophotometer, start the stop wash and record initial absorbance and at 1 minute intervals thereafter for 3 minutes by BioSystem bts 350 device. Calculate the difference between consecutive absorbance, and the average.

#### Calculations

- $\Delta A = \text{Final Absorbance} - \text{Initial Absorbance}$
- $(\Delta A/\text{min}) \times 2764 = \text{IU/L}$  absorbance difference per minute ( $\Delta A/\text{min}$ ).

#### Measurement of Alanine aminotransferase (ALT).

Alanine aminotransferase (ALT) stimulate the transport of the amino group from alanine to 2-oxoglutarate, producing pyruvate and glutamate. The catalytic concentration is evaluated from the value of decrease of NADH, by the mode of the lactate dehydrogenase (LDH) conjugated reaction<sup>(21)</sup>.



#### Procedure

Bring the Working Reagent and the instrument to reaction temperature and then mix 1ml of reagent A, 200 µl of reagent B with 100 µl of serum sample into a cuvette, insert the cuvette into the spectrophotometer, measured at 340 nm, Start the stop wash. After 1 minute, record initial absorbance and at 1 minute intervals thereafter for 3minutes by BioSystem bts 350 device. Calculate the difference between consecutive absorbance, and the average absorbance difference per minute ( $\Delta A/\text{min}$ ).

#### Calculations

- $\Delta A = \text{Final Absorbance} - \text{Initial Absorbance}$
- $(\Delta A/\text{min}) \times 3333 = \text{IU/L}$

#### Measurement of Aspartate Aminotransferase (AST/GOT)

The formation of oxalacetate and glutamate by the Aspartate aminotransferase (AST or GOT) enzyme by activated the transport of the amino group from aspartate to 2-oxoglutarate. The catalytic concentration is estimated from the value of diminish of NADH by means of the malate dehydrogenase (MDH) coupled reaction, measured at 340 nm<sup>(21)</sup>.

Aspartate + 2 - Oxoglutarate  $\xrightarrow{AST}$  Oxalacetate + Glutamate.

Oxalacetate + NADH + H<sup>+</sup>  $\xrightarrow{MDH}$  Malate + NAD.

#### Procedure

Bring the Working Reagent and the instrument to reaction temperature and then mix 1ml of reagent A, 200  $\mu$ l of reagent B with 100  $\mu$ l of serum sample into a cuvette, insert the cuvette into the spectrophotometer, measured at 340 nm, Start the stop wash. After 1 minute, record initial absorbance and at 1 minute intervals thereafter for 3 minutes by BioSystem bts 350 device. Calculate the difference between consecutive absorbance, and the average absorbance difference per minute ( $\Delta A/\text{min}$ ).

#### Calculations

- $\Delta A = \text{Final Absorbance} - \text{Initial Absorbance}$
- $(\Delta A/\text{min}) \times 3333 = \text{IU/L}$

#### Measurement of serum albumin

The bromocresol green in buffered solution at PH 4.2 binds albumin to produce a colored compound which absorbance, calculated at 630 nm (620-640) is relative to albumin concentration in the sample<sup>(22)</sup>.

#### Procedure

Mix well 5 UI of serum sample with 2.5ml of reagent. Record absorbance at 630 nm (620-640) within 3 minutes against reagent blank or better after exactly 1 minute by spectrophotometer PD-303(Apel JAPAN).

#### Calculations

- $\Delta A = \text{Final Absorbance} - \text{Initial Absorbance}$ .

- Concentration of albumin =  $(\Delta A_{\text{sample}} / \Delta A_{\text{standard}}) \times \text{concentration of standard (5g/dl)}$

#### Statistical analysis

The entire results were demonstrated as mean  $\pm$  standard deviation (SD). The statistics were evaluated by computerized statistical package for the social sciences SPSS program. Unpaired student t-test was achieved for each group pair includes a comparison between two groups-values ( $P < 0.05$ ) is express to be statistically significant. A chi-square test is used to determine the statistical significance in the distribution between different various variables.

#### Ethical consideration

All the administrative agreements were taken from the parents, the administrative staff of the hospital including the managing director, and the responsibility of the departments.

#### Results

In table (2), according to the (age of newborns, weight of newborns, the duration of gestation of newborns) there are no significant differences when compared between the newborns with pathological jaundice and physiological jaundice ( $P\text{-value} > 0.05$ ).

As regard to the gender of newborns, residency and source of air pollution, no significant relationship when comparing newborns with physiological jaundice and newborns with pathological jaundice ( $P\text{-value} > 0.05$ ), meanwhile according to the job of mothers, there is a significant relationship between the job of the mothers and both types of newborn jaundice ( $P\text{-value} < 0.05$ ).

**Table 2. Demographical information of newborns with physiological jaundice and pathological jaundice.**

Characters		Newborns with Physiological Jaundice	Newborns with Pathological Jaundice	P-value
Age of Newborn (hours)		131.2 $\pm$ 48.8	115.1 $\pm$ 38.8	0.073 <sup>A</sup>
Weight of Newborn (kg)		2.76 $\pm$ 0.79	2.92 $\pm$ 0.37	0.214 <sup>A</sup>
Gestational Durations (weeks)		36.1 $\pm$ 3.3	36.6 $\pm$ 1.5	0.246 <sup>A</sup>
Gender	Male	27 (54%)	22 (44%)	0.3172 <sup>B</sup>
	Female	23 (46%)	28 (56%)	
Residency	Rural	29(58%)	26(52%)	0.5464 <sup>B</sup>
	Urban	21(42%)	24(48%)	
Sources of air pollution	Positive	22(44%)	27(54%)	0.328 <sup>B</sup>
	Negative	28(56%)	23(46%)	
Job of Mother	Employee	7(14%)	1(2%)	0.0269 <sup>B</sup>
	Housewife	43(86%)	49(98%)	

- N=50 newborn for each group
- Superscript A refer to t-test biostatic analysis, in which  $P\text{-value} < 0.05$  mean significant differences between the means of test groups
- Superscript B refer to chi-square biostatic analysis, in which  $P\text{-value} < 0.05$  mean significant relationship between the test groups

Table 3 represents the onset of jaundice and physical examination findings, there is a significant difference between the onset of Physiological jaundice and pathological jaundice ( $77.28 \pm 27.9$  vs  $23.56 \pm 41.5$ ) ( $P$ -value  $< 0.05$ ). According to the general status, feeding status, the stage of yellowish discoloration and opisthotonus status of newborns there is a significant relationship when comparing

recently born babies with physiological jaundice and newborns with pathological jaundice ( $P$ -value  $< 0.05$ ), meanwhile the bowel motions, moro reflex, sucking reflex and grasping reflex of newborns show no significant relationship between infants with physiological jaundice and infants with pathological jaundice ( $P$ -value  $> 0.05$ ).

**Table 3. Clinical examinations of newborns with physiological and pathological jaundice**

Characters		newborns with physiological jaundice	newborns with pathological jaundice	$P$ -value
Onset of jaundice(hours)		77.28±27.9	23.56±41.5	0.615 <sup>A</sup>
Status of newborn	Active	36(72%)	7(14%)	4.69×10 <sup>-9B</sup>
	Lethargic	14(28%)	43(86%)	
Feeding	Good	32(64%)	19(38%)	0.009308 <sup>B</sup>
	Poor	18(36%)	31(62%)	
Bowel motion	Positive	44(88%)	45(90%)	0.749271 <sup>B</sup>
	Negative	6(12%)	5(10%)	
Yellowish discoloration*	Stage I	21(42%)	3(6%)	1.123×10 <sup>-15B</sup>
	Stage II	28(56%)	31(62%)	
	Stage III	1(2%)	16(32%)	
Moro reflex	Positive	48(96%)	44(88%)	0.140369 <sup>B</sup>
	Negative	2(4%)	6(12%)	
Sucking reflex	Positive	48(96%)	45(90%)	0.239678 <sup>B</sup>
	Negative	2(4%)	5(10%)	
Grasping reflex	Positive	48(96%)	46(92%)	0.39912 <sup>B</sup>
	Negative	2(4%)	4(8%)	
Opisthotonus status	Positive	0(0%)	6(12%)	0.01152 <sup>B</sup>
	Negative	100(100%)	44(88%)	

- N=50 newborn for each group
- Superscript A refer to t-test biostatic analysis, in which  $P$ -value  $< 0.05$  mean significant differences between the means of test groups
- Superscript B refer to chi-square biostatic analysis, in which  $P$ -value  $< 0.05$  mean significant relationship between the test groups
- \* Yellowish discoloration stages, stage I only the face and serum bilirubin range was (4-6mg/dl), stage II reach to the abdomen and serum bilirubin range was (8-14mg/dl), stage III reach to the extremities and serum bilirubin range was (15-20mg/dl)<sup>(23)</sup>.

Table 4 represents the measured biochemical parameters (Bilirubin, ALT, AST, ALP, Albumin and Rh factor). There was significant differences ( $P < 0.05$ ) in the levels of total serum bilirubin, direct bilirubin, ALP, ALT, AST and serum albumin

between the 2 groups of jaundiced infants. there is a significant difference if compare between the Rh Compatibility of newborns with physiological jaundice and newborns with pathological jaundice ( $P$ -value  $< 0.05$ ).

**Table 4. Biochemical assay of newborns with physiological jaundice and newborns with pathological jaundice.**

Parameters *	Newborns with Physiological Jaundice	Newborns with Pathological Jaundice	P-value
Total Serum Bilirubin (mg/dL)	10.11± 3.6	14.34± 4.6	2.443×10 <sup>-6</sup>
Direct Serum Bilirubin (mg/dL)	6.36± 2.54	9.82± 2.4	7.24×10 <sup>-6</sup>
Alkaline Phosphatase (ALP)(IU/L)	284.57± 50.8	388.71± 52.7	0.002728
Alanine Aminotransferase (ALT/GPT)(IU/L)	13.2± 3.49	27.37± 2.64	4.28×10 <sup>-6</sup>
Aspartate Aminotransferase (AST/GOT)(IU/L)	34.12± 6.1	62.62± 9.47	0.00516
Serum Albumin Concentration (g/dL)	3.37± 0.25	3.83± 0.41	0.0201
Rh Compatibility	Compatible	50(100%)	44(88%)
	Incompatible	0(0%)	6(12%)

- \*Normal values for TSB in newborn less than 2 mg / dl, normal value for direct serum bilirubine in newborn 0.1-0.3mg/dl, normal value for ALP in newborn is 150-420 IU/L, normal value for ALT in newborn is 13-45 IU/L , normal value for AST in newborn is 30-150 IU/L and normal value for Serum albumin in newborn is 3-4.3g/dL

## Discussion

All newborns are at risk of jaundice; most cases of jaundice will have a good prognosis with prompt intervention. Bilirubin has certain anti-oxidative effects; an appropriate concentration of bilirubin might have a protective effect within normal value<sup>(24)</sup>. However, if the concentration of unconjugated bilirubin is too high, and the binding capacities of plasma proteins are limited, free bilirubin might raise and pass through the newborn blood-brain barrier and have toxic complications, producing in permanent neurological injury (bilirubin encephalopathy)<sup>(25)</sup>.

Table (2) has showed that, there is no significant differences when comparing the age, weight, gender, gestational age, sources of air pollution and residency of newborn with both types of newborn jaundice ( $p>0.05$ ), this indicates that these factors did not have a significant effect on incidence of different types of jaundice (pathological – physiological). A previous study had been studied the influence of these factors on the jaundice in newborns, they found that gestational age range between 35-36 weeks considered as a major risk factor<sup>(26)</sup> This finding is a match with the present study results in which both groups have gestational age about 36 weeks beside it seems that gestational age had no effect on the incidence of either type of jaundice. Other study found that gender has an influence on the incidence of jaundice in newborns, they found that male babies have minor risk compared to female<sup>(27)</sup> this finding was counteracted with the present study in which there is no significant influence of gender on the incidence of both type of jaundice.

Air pollution and residency had no significant effect on the incidence on both types of jaundice in newborns meanwhile a previous study show that air pollution causes an increase in incidence of jaundice in newborns without mention which type of it<sup>(28)</sup>.

Table (3) has showed that there is a significant difference when compared between the onset of jaundice in newborns with physiological jaundice and newborns with pathological jaundice; the appearance of pathological type is within 24 hrs. while the appearance of physiological jaundice after 24 hrs. of birth this finding is compatible with previous studies which found that physiologic jaundice in newborn mostly occurs on days 2 to 4, peaks between 4 to 5 days, and disappears in two weeks. Physiologic jaundice does not appear in the first 24 hours<sup>(29)</sup>. Another study shows that physiological jaundice usually appears after at least 24 hours of birth, and peak after four or five days. It later disappears after about 2 weeks of life<sup>(30)</sup>. Appearance of pathological jaundice in newborns during 24 hours, the levels of peak total serum bilirubin more than the expected normal value<sup>(31)(32)</sup>. According to the general status of newborn, the patients with pathological jaundice more lethargic and less active in compare to patients with physiological jaundice, regarding to the feeding status of newborn, the patients with pathological jaundice more poor feeding if compared to patients with physiological jaundice and also according to opisthotonus status the patients with pathological jaundice more susceptible to opisthotonus state in comparatively with patients with physiological jaundice. This finding completely agrees with another a previous study conducted by Shapiro SM

in 2003, which found that newborns with severe hyperbilirubinemia which characterized by poor feed, high-pitched cry, abnormal tone (hypertonia and/or hypotonia), lethargy, retrocollis and opisthotonus, the sunset eye signs, seizures, and possibly death<sup>(33)</sup>.

In table (4), the total serum bilirubin levels of newborns with pathological jaundice were significantly higher than newborn of physiological jaundice. Bryon J, *et al* in 2011 have been found that, in physiologic jaundice the average total serum bilirubin level mostly peaks at 5 to 6 mg/dL on the third to fourth day after birth and then decrease over the 1st week of life. Bilirubin level may reach of up to (12 mg/dL), can some time occur. Newborns with many risk factors may suffer from an exaggerated form of physiologic jaundice in which the total serum bilirubin concentration may reach to 17 mg/dl. So, bilirubinemia more than this value is no longer consider as a physiologic jaundice<sup>(34)</sup>.

According to the direct serum bilirubin of newborn, there are significant difference between the direct serum bilirubin of newborns with physiological jaundice and newborns with pathological jaundice ( $P$ -value  $<0.05$ ). Several clinical studies have emphasized on the importance of measuring direct serum bilirubin for newborns with jaundice. They found that direct serum bilirubin has been increased more than 2 mg/dl is a which is a diagnostic feature for pathological jaundice<sup>(35)</sup>. According to the Rh compatibility of newborns, there is significant relationship between the Rh compatibility of newborns with physiological jaundice and newborns with pathological jaundice this finding is compatible with previous study done by Johnson LH, *et al* in 2002 which found the elevated production of bilirubin (like newborn with blood group and Rh incompatibilities), insufficiency of hepatic uptake, impaired conjugation of bilirubin and raised enterohepatic circulation of bilirubin report for most cases of pathological jaundice in neonatal infant<sup>(36)</sup>.

Maisels MJ in 2005 found that more production of bilirubin occurs in newborns with red cell-enzyme deficiencies, incompatibility of blood group, or structural deformity in red cells<sup>(37)</sup>. Other study conducted by Blanchette VS in 1984 also show that the causes of raised bilirubin production in pathologic jaundice are immune-mediated hemolysis like incompatibility of ABO, Rh and non-immune mediated causes like cephalhematoma, erythrocyte membrane defects such as hereditary spherocytosis and elliptocytosis, defect in enzymes such as pyruvate kinase deficiency and glucose-6-phosphate dehydrogenase (G6PD) deficiency<sup>(38)</sup>.

The results demonstrated that the concentration of Alkaline phosphatase in newborns with pathological jaundice is significantly higher than that in newborns with physiological jaundice. ALP is present in all cells including erythrocytes and is

released to plasma after the cells destruction. Accordingly, it hypothesized that it could be used as a primary marker of hemolysis of red blood cells and estimation of significant hyperbilirubinemia. Alike to the current findings, other study utilized the alkaline phosphatase concentration in blood six hours after delivery. They establish that ALP concentrations were significantly greater in newborns with jaundice need treatment, either by phototherapy or exchange transfusion<sup>(39)</sup> besides other study carried by Mousa AK, *et al* in 2015 found that there is a the concentrations of cord blood alkaline phosphatase between the non-jaundiced and clinically jaundiced infants, and it was significantly larger in patients with hyperbilirubinemia need treatment. Furthermore, the ALP concentrations were significantly higher in infants whose serum bilirubin level arrived a level  $\geq 10$  mg/dL<sup>(40)</sup>. Other study also found that huge levels of ALP in a patient with incompatibility of Rh and hemophagositic syndrome<sup>(41)</sup>.

The serum concentration of (ALT/ GPT) and (AST/GOT) in newborns with pathological jaundice were significantly higher than that of newborns with physiological jaundice. There is no pervious study linked between these two enzymes and jaundice in newborn but many previous study link the concentration of these enzymes with jaundice in adults in which they found that the elevation in the concentration of these enzymes occur in patients with different types of hyperbilirubinaemia if compare with control group, which may be as a result of defects in the liver function due to damage of hepatic cells, liver dysfunction biliary obstruction, viral infections, or any another deformities which cause releasing of these enzymes into the circulation, this study demonstrate that there were a significant rise of activities of AST and ALT enzymes in patients with hepatic jaundice. Generally GOT or GPT is evaluated as a marker of damage in liver<sup>(42)</sup>.

Serum Albumin concentration of newborns with pathological jaundice was significantly higher when compared with that of newborns with physiological jaundice this finding is compatible with previous study conducted by Taksande A, *et al* in 2005 which found that Physiological hyperbilirubinemia is a result of immature liver cell which have low uridine diphosphoglucuronosyl transferase activity when compared to mature hepatocyte, low concentration of albumin which is a bilirubin binding ligand and increased number of erythrocytes which have a shorter life span<sup>(43)</sup>. Another previous study deduced by Gaurav AKC in 2017 which they found that cord albumin concentrations in a healthy term newborn used to predict the probability of the neonate having jaundice. It aids to determine the newborn that are at a more risk of developing hyperbilirubinemia. A value lower than 2.8 mg/dl has found to be more associated with clinical

jaundice. So routine determination of cord Albumin together with TSH can be achieved to keep path of at risk newborn<sup>(44)</sup>. Another previous study which found that jaundice and the following neurotoxic side effect of bilirubin on the brain is determined by numerous factors among these are inadequate oxygen supply, acidosis, the level of bilirubin in the brain, gestational age, the period of exposure and the level of albumin bound<sup>(45)</sup> and also bilirubin can pass through the brain if it is free or it is unconjugated, consequently if the serum albumin levels is low, the binding of bilirubin is compromised and the unconjugated bilirubin increase, may exposure the newborn for the danger of encephalopathy<sup>(46)</sup>

## Conclusion

These findings demonstrate that newborns with pathological jaundice have higher Level of studied parameters (Serum bilirubin, albumin and several liver enzymes) as compared with physiological jaundice .

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