Effects of Hyperbilirubinemia on Hepatic Parameters, Electrolyte Level and Oxidant-Antioxidant Status of Neonates

Haq Nawaz1*, Muhammad Aslam Shad, Humaira Yasmin1, Amna Aslam2, Vania Amin1, Muhammad Shakir3, Ammara Zaib4, Muhammad Amir5

1Department of Biochemistry, Bahauddin Zakariya University, Multan-60800, Pakistan
2University Medical and Dental College Faisalabad, Punjab, Pakistan-38000, Pakistan
3Resident Peads Medicine, Sheikh Zayed Hospital and Medical College, Rahim Yar Khan, Pakistan
4Resident Gynecology and Obstetrics, Sheikh Zayed Hospital and Medical College, Rahim Yar Khan, Pakistan
5Department of Pediatrics, Nishtar Medical College and Hospital, Multan, Pakistan

Corresponding Author: hagnawaz@bzu.edu.pk

Abstract

The present study reports the consequences of hyperbilirubinemia on neonatal health at an early age. The study included a control group (n=10) and a study group consisting of hyperbilirubinemia neonates (n=24) subdivided into four groups (n=6/group) based on age (5, 10, 15, and 20 days). The sera obtained from the participant’s blood samples were analyzed for hepatic biomarkers, electrolyte level, and oxidant-antioxidant status. The age-dependent variations in the studied parameters were analyzed by regression analysis. Hyperbilirubinemia significantly (p<0.05) affected the serum bilirubin level and antioxidant potential of the neonates. However, the liver function enzymes and serum electrolytes remained unaffected. The hyperbilirubinemia neonates showed an age-dependent variation in free radical scavenging capacity, metal chelating activity, linoleic acid reduction capacity, and total antioxidant activity that were linearly correlated with the serum bilirubin level of the neonates. The study would provide useful information regarding the consequences of hyperbilirubinemia on neonatal health.

Keywords: Neonatal Hyperbilirubinemia, Oxidant-antioxidant status, Hepatic parameters, Electrolytes level, Serum total bilirubin

1. Introduction

Hyperbilirubinemia is a physiological condition characterized by elevated levels of serum bilirubin, a product of hemoglobin metabolism (Ostrow, Jandi, & Schmid, 1962). Total serum bilirubin (STB) levels in newborns increase from 17 to 100 mmol/L during the first 72 hours and return to normal after 7–10 days (Monaghan et al., 1999). 1% of babies have severe hyperbilirubinemia (up to 20 mgdL-1) with complex clinical features. Although most cases of elevated bilirubin levels in neonates are benign (Bhutani, Johnson, & Keren, 2004). These dosages have the potential to harm the brain, leading to mental disability, hearing loss, and naturalization (Yang et al., 2015). Increased bilirubin production, poor bilirubin-albumin binding, insufficient liver absorption, and enhanced bilirubin-intestinal circulation are the main causes of the majority of recent serious cases of hyperbilirubinemia deliveries. Babies with erythrocyte-enzyme or structural issues, newborns from diverse ethnic backgrounds, and children with anomalous blood types all produce higher levels of bilirubin. It is unknown if there is a pattern of hyperbilirubinemia among ethnic groups. Hepatic bilirubin absorption may be inadequate in Gilbert syndrome, leading to pathological hyperbilirubinemia (Al-Saedi, 2002).

Previously, studies have been reported on the potential effects of elevated levels of serum bilirubin on various physiological functions of humans. (Boon, Bulmer, Coombes, & Fassett, 2014). Severe hyperbilirubinemia, if not managed properly, has been reported to cause various abnormalities, including neonatal encephalopathy and deterioration of neurodevelopment in the nervous system in neonates (Suresh, Martin, & Soll, 2003). Physiological jaundice in infants with bilirubin encephalopathy has been associated with relatively lower levels of ceruloplasmin, antioxidant enzymes, lipid peroxidation, and total antioxidant capacity and elevated levels of malondialdehyde content and superoxide dismutase activity (Hilderbrand, Fahim, James, & Fahim, 1974; Shahab, Kumar, Sharma, Narang, & Prasad, 2008). The most frequent outcome in newborns with Rhesus-negative immunity and
acute hyperbilirubinemia is kernicterus. Survivors experienced sensorineural hearing loss, visual impairment, biliary abnormalities, and occasionally mental retardation (Hansen, 2000). Hemodynamic stability, acute renal failure, cardiovascular depression, immunological intervention, and bleeding can all result from bilirubin-induced systemic inflammatory response syndrome (Al-Saedi, 2002).

On the other hand, the effects of bilirubin on the liver parameters, antioxidant capacity, and oxidative stress in neonates are not well documented in the literature. The current study looked at the effects of newborn hyperbilirubinemia on the oxidant-antioxidant balance and liver markers in newborns. It will be simpler to deploy early preventive interventions and comprehend how hyperbilirubinemia affects infants thanks to this study.

2. Materials and methods
2.1 Subjects
Overall, 34 neonates admitted to the pediatric ward of Nishtar Hospital Multan, Pakistan, were included in the study. All of the neonates participating in the study (as per the study design) were screened for hepatic, cardiovascular, and renal problems based on physical examination and symptomatic observations by a physician. The neonates with positive signs of cardiovascular, renal, neurological, and gastric issues were excluded from the study. The neonates with normal STB levels were taken as control, while those showing relatively elevated levels of STB were included in the hyperbilirubinemia group.

2.2 Experimental design
The study plan was approved by the Advanced Studies and Research Board and Institutional Ethical Committee, Bahauddin Zakariya University, Multan, Pakistan under approval No.: Biochem/110/2018. Written consent was obtained from the parents of the neonates, and a questionnaire regarding the history of the neonates was filled out before sample collection. The study included a control group consisting of normal neonates (n=10) and a study group composed of hyperbilirubinemia neonates (n=24) having elevated levels of STB (>2.5 mgdL⁻¹). The hyperbilirubinemia group was subdivided into four groups (n=6 per group) based on the age of neonates (5, 10, 15, and 20 days). The sera obtained from the participants’ blood samples were analyzed for their hepatic parameters, electrolyte level, and oxidant-antioxidant status (Figure 1).

Figure 1 Study design
STB: Serum total bilirubin, SDB: Serum direct bilirubin, STP: Serum total protein, LDH: Lactate dehydrogenase, GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase and ALP: Alkaline phosphatase, MDA: Malondialdehyde content, FRSC: Free radical scavenging capacity, DPPHRSC: DPPH radical scavenging capacity, HRSC: Hydroxyl radical scavenging capacity, SORASC: Superoxide radical scavenging capacity, c) FICA: Ferrous ion chelating ability, LARA: Linoleic acid reduction ability, and TAOA: Total antioxidant activity.
2.3 Sampling and Clinical Investigation

Blood samples (2 mL) were collected from the participants, and the sera were obtained after centrifugation at 4000×g for 20 minutes and preserved at -4°C in the dark. The sera were subjected to analysis of hepatic parameters, including serum bilirubin, serum protein, liver function enzymes, electrolytes including Na⁺ and K⁺ ions, oxidative stress in terms of malondialdehyde content, and antioxidant potential in terms of free radical scavenging capacity, ferrous iron chelating activity, linoleic acid reduction capacity, and total antioxidant activity.

2.4 Hepatic parameters

The levels of STB and SDB were determined by the previously reported Jendrassik and Vander-Bergh method as described earlier (Ullah, Rahman, & Hedayati, 2016) following the protocols given in the commercially available bilirubin kit (Randox, Germany). The results were reported as mgdL⁻¹ serum, and the degree of hyperbilirubinemia was expressed in terms of elevation in STB.

The levels of serum total protein (STP) and liver function enzymes including lactate dehydrogenase (LDH), glutamic oxaloacetate transaminase (GOT), glutamic pyruvic transaminase (GPT), and alkaline phosphatase (ALP) were determined by the previously described methods as recommended by the International Federation of Clinical Chemistry (Schumann, Bonora, Ceriotti, Clerc-Renaud, et al., 2002; Schumann, Bonora, Ceriotti, Féroid, et al., 2002; Schumann et al. et al., 2011; Siekmann et al., 2002) following the protocols described in the commercially available kits (Cobas, Roche Diagnostics, Germany). The TSP and the activity of liver function enzymes were calculated as gdL⁻¹ and UL⁻¹, respectively.

2.5 Serum electrolytes

The serum levels of sodium and potassium ions were determined by previously described precipitation and potentiometric methods, respectively (R. J. et al Henry, 1974; R. Henry, 1974; Tietz. N. W, 1976). The concentration of Na⁺ and K⁺ was calculated as mmolL⁻¹.

2.6 Oxidant-antioxidant status

The oxidative stress was determined in terms of malondialdehyde (MDA) production using the thiobarbituric acid (TBA) method based on the formation of MDA-TBA adduct as described earlier (Nawaz, Ali, Rehman, & Aslam, 2020; Weitner, Inić, Jablan, Gabričević, & Domijan, 2016).

The antioxidant potential was determined in terms of free radical scavenging capacity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH) and superoxide SO radicals, linoleic acid reduction capacity (LARC), ferrous ion chelating activity (FICA), and total antioxidant activity (TAOA). The DPPH, OH, and SO radical scavenging capacities (%) were determined by the standard methods as described earlier (Nawaz et al., 2020; Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998). The FICAc and LARC (%) and gallic acid equivalent TAOA mgdL⁻¹ were determined by the ferrozine, thiocyanate, and phosphomolybdenum assays, respectively, as described previously (Nawaz et al., 2020; Prieto, Pineda, & Aguilar, 1999).

2.8 Statistical analysis

Sample size was calculated using the statistical program G-power. The research group and control group reported their results as the standard deviation of the replicates, plus or minus the mean, as recommended by the study design. To ascertain whether there were statistically significant differences between the control and hyperbilirubinemia groups, one-way analysis of variance (ANOVA) was employed. To ascertain the age-dependent impact of hyperbilirubinemia on newborn measures, regression analysis was employed. The experimental data were projected onto regression equations and plotted against the experimental data in order to assess the suitability of the regression models for the factor and responses. Plotting the TSB level against the response parameters showed a significant departure from the control, which indicated a correlation between the tested reactions and hyperbilirubinemia.
Table 1: Experimental data on the hepatic parameters, electrolyte level, oxidative stress, and antioxidant status of the control and hyperbilirubinemia neonates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (5-20 days)</th>
<th>HB* (5-20 days)</th>
<th>Age of HB neonates (days)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepatic parameters</strong></td>
<td></td>
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<tr>
<td>STB (mg dL⁻¹)</td>
<td>0.43±**</td>
<td>9.65±</td>
<td>8.35±</td>
<td>13.53±</td>
</tr>
<tr>
<td>SDB (mg dL⁻¹)</td>
<td>0.25±</td>
<td>4.22±</td>
<td>2.85±</td>
<td>2.92±</td>
</tr>
<tr>
<td>TP (g dL⁻¹)</td>
<td>0.08±</td>
<td>0.82±</td>
<td>0.68±</td>
<td>0.55±</td>
</tr>
<tr>
<td>LDH (UL⁻¹)</td>
<td>6.68±</td>
<td>6.45±</td>
<td>6.07±</td>
<td>6.25±</td>
</tr>
<tr>
<td>GPT (UL⁻¹)</td>
<td>0.67±</td>
<td>0.59±</td>
<td>0.69±</td>
<td>0.54±</td>
</tr>
<tr>
<td>GPT (UL⁻¹)</td>
<td>258.66±</td>
<td>301.71±</td>
<td>329.83±</td>
<td>292.17±</td>
</tr>
<tr>
<td>GOT (UL⁻¹)</td>
<td>49.50±</td>
<td>44.17±</td>
<td>48.00±</td>
<td>45.00±</td>
</tr>
<tr>
<td>GOT (UL⁻¹)</td>
<td>17.04±</td>
<td>14.12±</td>
<td>13.39±</td>
<td>15.97±</td>
</tr>
<tr>
<td>GPT (UL⁻¹)</td>
<td>48.83±</td>
<td>34.63±</td>
<td>29.83±</td>
<td>43.67±</td>
</tr>
<tr>
<td>ALP (UL⁻¹)</td>
<td>17.83±</td>
<td>14.38±</td>
<td>9.52±</td>
<td>20.85±</td>
</tr>
<tr>
<td>ALP (UL⁻¹)</td>
<td>219.50±</td>
<td>297.63±</td>
<td>287.00±</td>
<td>307.33±</td>
</tr>
<tr>
<td><strong>Electrolytes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mmol L⁻¹)</td>
<td>130.33±</td>
<td>130.25±</td>
<td>135.67±</td>
<td>133.00±</td>
</tr>
<tr>
<td>K⁺ (mmol L⁻¹)</td>
<td>7.39±</td>
<td>11.49±</td>
<td>16.89±</td>
<td>8.53±</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MDA (Abs. at 562 nm)</td>
<td>0.103±</td>
<td>0.10±</td>
<td>0.092±</td>
<td>0.103±</td>
</tr>
<tr>
<td><strong>Antioxidant status</strong></td>
<td></td>
<td></td>
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<tr>
<td>DPPHRSC (%)</td>
<td>36.12±</td>
<td>31.40±</td>
<td>29.48±</td>
<td>25.87±</td>
</tr>
<tr>
<td>HRSC (%)</td>
<td>5.85±</td>
<td>7.78±</td>
<td>3.38±</td>
<td>4.94±</td>
</tr>
<tr>
<td>SORSC (%)</td>
<td>68.72±</td>
<td>72.80±</td>
<td>74.52±</td>
<td>69.73±</td>
</tr>
<tr>
<td>FICA (%)</td>
<td>15.69±</td>
<td>13.59±</td>
<td>13.79±</td>
<td>19.51±</td>
</tr>
<tr>
<td>LARC (%)</td>
<td>52.63±</td>
<td>42.28±</td>
<td>35.62±</td>
<td>38.14±</td>
</tr>
<tr>
<td>TAOA (mg dL⁻¹)</td>
<td>10.76±</td>
<td>9.11±</td>
<td>5.43±</td>
<td>3.21±</td>
</tr>
<tr>
<td>(mg dL⁻¹)</td>
<td>44.21±</td>
<td>26.26±</td>
<td>27.35±</td>
<td>28.26±</td>
</tr>
<tr>
<td>(mg dL⁻¹)</td>
<td></td>
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</tbody>
</table>

HB: Hyperbilirubinemia; STB: Serum total bilirubin; SDB: Serum direct bilirubin; TP: Total protein; LDH: Lactate dehydrogenase; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamate pyruvate transaminase; ALP: Alkaline phosphatase; Na⁺: Sodium ion; K⁺: Potassium ion; MDA: Malondialdehyde assay; DPPHRSC: Diphenylepicrylhydrazyle radical scavenging capacity; HRSC: Hydroxyl radical scavenging capacity; SORSC: Superoxide radical scavenging; FICA: Metal chelating activity; LARC: Linoleic acid reduction capacity; TAOA: Total antioxidant activity.

The results are shown as mean± SD of the replicates, and the values followed by different alphabets in each row are significantly different at a 95% confidence level (p<0.05) using Duncan’s multiple range test.

3. Results

The experimental values of the hepatic parameters, serum electrolytes, oxidative stress, and antioxidant potential of the control and hyperbilirubinemia neonates are presented in Table 1. The levels of STB and SDB of the control group were found to be 0.43±0.28 and 0.25±0.08 mgdL⁻¹ and those of the hyperbilirubinemia group of neonates were found to be 9.65±4.22 and 1.98±0.82 mgdL⁻¹.
respectively. One-way analysis of variance of the data showed a statistically significant (p<0.05) difference in STB and SDB of hyperbilirubinemia neonates. The hyperbilirubinemia neonates showed comparatively higher levels of STB and SDB than the control group (Figure 2a).

The level of serum total protein (STP) of the control and hyperbilirubinemia neonates was found to be 6.68±0.67 and 6.45±0.59 g dL⁻¹, respectively. The serum levels of the studied liver function enzymes, including LDH, GOT, GPT, and ALP of the control group were found to be 258.67±57.58, 49.50±17.04, 48.83±17.83, and 219.5±40.24 UL⁻¹ while those of hyperbilirubinemia groups were 301.71±90.73, 44.17±14.12, 34.63±14.38, and 297.63±71.59 UL⁻¹ respectively. The serum levels of the electrolytes, including Na⁺ and K⁺, of the control group were found to be 130.33±7.39 and 3.00±0.53 mmol L⁻¹ and those of hyperbilirubinemia neonates were 130.25±11.49 and 3.28±0.86 mmol L⁻¹ respectively. Statistically, no significant variation (p>0.05) was observed in the levels of serum total protein, the studied liver function enzymes, and serum electrolytes of the control and hyperbilirubinemia neonates (Figure 2b-d).

The MDA level (in terms of absorbance of the reaction mixture at 562 nm) of the control and hyperbilirubinemia neonates was found to be 0.103±0.005 0.09±0.01, respectively. One-way analysis of variance of data showed a statistically no significant variation (p>0.05) in the MDA level of the control and hyperbilirubinemia neonates (Figure 3a).

The DPPHRSC, HRSC, SORSC, FICA, LARA, and TAOA of the control group were found to be 36.13±5.85%, 68.72±15.69%, 52.63±10.76%, 44.21±8.08%, 7.73±2.13%, and 1.39±0.62 mgdL⁻¹ while those of hyperbilirubinemia neonates were 31.40±5.85, 72.80±13.59%, 42.28±9.11%, 26.26±8.25%, 8.21±3.12% and 0.79±0.44 mgdL⁻¹ respectively. One-way analysis of variance of the experimental data showed a statistically significant (p<0.05) variation in DPPHRSC, SORSC, FICA, LARA, and TAOA and a non-significant variation in HRSC of the control and hyperbilirubinemia neonates (Figure 3b, c).

4. Discussion

In neonates, hyperbilirubinemia is common due to immature liver and hepatic dysfunction. At an earlier age of the first week, the serum level of unconjugated bilirubin is elevated due to impaired conjugation of bilirubin with glucuronic acid catalyzed by UDP-glucuronon transferase (Wolkoff, 2014). It was hypothesized that liver malfunction and hyperbilirubinemia may affect the associated liver functions and physiology. Therefore, the correlation of hyperbilirubinemia with some hepatic biomarkers and oxidant-antioxidant status of the neonates at an age of 5-20 days was investigated in the present study.

The elevation in the STB level (>2.5 mg dL⁻¹) of the study groups differentiated the hyperbilirubinemia neonates from the control ones (Figure 2a). The elevated levels of STB in the hyperbilirubinemia group may be attributed to the relatively lower activity of hepatic UDP-glucuronon transferase, an enzyme responsible for bilirubin glucuronidation, due to immature liver (Wolkoff, 2014). The elevation in the SDB level of the hyperbilirubinemia group from that of the control group may also be attributed to the elevated level of STB. However, the non-significant variation in the TSP, LDH, GOT, GPT, and ALP level of the hyperbilirubinemia neonates from those of the control ones (Figure 2b, c) suggest that hyperbilirubinemia is not associated with these hepatic parameters. The levels of serum electrolytes, including Na⁺ and K⁺, of the control and study groups were also found to be statistically similar, indicating no significant association between hyperbilirubinemia and electrolyte level (Figure 2d).

Previous studies showed that the MDA level is negatively correlated with neonatal hyperbilirubinemia, indicating relatively lower oxidative stress (Kumar, Pant, Basu, Rao, & Khanna, 2006). In the present study, the MDA content of the control and study groups also showed a statistically non-significant variation that suggested no significant association between hyperbilirubinemia and oxidative stress (Figure 3a). However, the observed variation in antioxidant status of the control and hyperbilirubinemia neonates in terms of DPPHRSC, SORSC, FICA, LARA, and TAOA showed a significant association between hyperbilirubinemia and antioxidant status as proved by their linear correlations with STB (Figure 3b, c, Figure 5a-e).

The association of hyperbilirubinemia with the studied parameters showing significant variation between the control and hyperbilirubinemia groups was studied by analyzing their age-dependent response and correlation with the STB level. The regression analysis of the experimental data showed an age-dependent significant polynomial variation (p<0.05) in the serum bilirubin level of
hyperbilirubinemia neonates (Figure 2e, f) with relatively higher values of the regression coefficient ($R^2 = 0.9861, 0.8906$). The STB and SDB levels were found to be elevated in the first two weeks, reached their peak on the twelfth and thirteenth day, respectively, and then decreased to normal at the end of the third week of the neonatal age. The elevation in the serum bilirubin level in the first two weeks and then a decline in the third and fourth weeks may be attributed to the immaturity of the liver, followed by its improvement (Bartlett & Gourley, 2001).

The markers of antioxidant status of the neonates, including DPPHRSC, FICA, and TAOA of hyperbilirubinemia groups, were also found to be changed as a polynomial function of neonatal age (Figure 3d, f, h) with relatively higher values of the regression coefficient ($R^2 = 0.9047-0.940$). The DPPHRSC and TAOA were found to be decreased at the earlier age of 10 days of hyperbilirubinemia neonates and started and continued to increase in the following period of study. The SORSC was exponentially increased while LA decreased linearly in response to the rise in neonatal age and the studied responses. The regression equations with calculated values of sensitivity coefficients and exponent values are presented in Table 2.

Table 2 Regression analysis of the age-dependent effect of hyperbilirubinemia on hepatic parameters and antioxidant status of neonates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regression Equation</th>
<th>$RC^*$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic parameters</td>
<td>STB (mg dL$^{-1}$) = $-2.825x^2 + 12.999x - 1.66$</td>
<td>0.9861</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>SDB (mg dL$^{-1}$) = $-0.0213x^2 + 0.5347x - 0.5875$</td>
<td>0.8906</td>
<td>0.00</td>
</tr>
<tr>
<td>Antioxidant status</td>
<td>DPPHRSC (%) = $0.0973x^2 - 1.7879x + 35.497$</td>
<td>0.9396</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>SORSC (%) = $28.952e^{0.0334x}$</td>
<td>0.962</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>FICA (%) = $-0.0974x^2 + 2.0294x + 19.155$</td>
<td>0.9047</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>LARA (%) = $-0.3008x + 11.97$</td>
<td>0.9744</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>TAOA (mg dL$^{-1}$) = $0.0111x^2 - 0.213x + 1.73$</td>
<td>0.940</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$^*$RC: Regression coefficient. STB: Serum total bilirubin, SDB: Serum direct bilirubin, DPPHRSC: 2, 2-Diphenyl-1-pircylhydrazyl radical scavenging capacity, SORSC: Superoxide radical scavenging, FICA: Ferrous ion chelating activity, LARC: Linoleic acid reduction capacity, TAOA: Total antioxidant activity.
Figure 2 Serum bilirubin levels, hepatic parameters, and electrolyte levels of the control and hyperbilirubinemia neonates and the age-dependent behavior of serum bilirubin level.

a) LDH: Lactate dehydrogenase, GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase and ALP: Alkaline phosphatase, b) Total protein, and c) Electrolytes, d) STB: Serum total bilirubin and e) SDB: Serum direct bilirubin

Figure 3 a-c) Oxidative stress and antioxidant status of the control and hyperbilirubinemia neonates and d-h) Age-dependent variation in antioxidant status of the hyperbilirubinemia neonates; MDA: Malondialdehyde content, DPPHRSC: DPPH radical scavenging capacity, HRSC: Hydroxyl radical scavenging capacity, SORASC: Superoxide radical scavenging capacity, c) FICA: Ferrous ion chelating ability, LARA: Linoleic acid reduction ability, and TAOA: Total antioxidant activity
Figure 4 Agreement between the actual and predicted values of bilirubin and antioxidant parameters
a) Serum total bilirubin, b) serum direct bilirubin, c) DPPH radical scavenging capacity, d) Superoxide radical scavenging capacity, e) Ferrous ion chelating activity, f) Linoleic acid reduction ability, g) Total antioxidant activity

Figure 5 Correlation between age-dependent variation in serum total bilirubin level and that in the antioxidant parameters
a) DPPH radical scavenging capacity, b) Superoxide radical scavenging capacity, c) FICA: Ferrous ion chelating activity, d) Linoleic acid reduction ability, and e) Total antioxidant activity
The age-dependent variation in DPPHRSC, SORSC, and TAOA of the hyperbilirubinemia group was inversely correlated with that in STB (Figure 5a, b, e), suggesting a negative association between hyperbilirubinemia and antioxidant status in terms of free radical scavenging and total antioxidant potential of neonates. However, FICA and LARA showed a direct correlation with the STB level of hyperbilirubinemia neonates, advocating the positive association between hyperbilirubinemia and antioxidant status (Figure 5c, d).

5. Conclusion
Serum bilirubin levels were generally greater in neonates with hyperbilirubinemia than in those without the condition. Up to their second week of life, neonates with hyperbilirubinemia displayed higher STB and SDB levels. By the third week of life, their levels returned to normal. In comparison to normal neonates, neonates with hyperbilirubinemia exhibited statistically similar levels of liver function enzymes, total blood protein, serum electrolytes, and malondialdehyde. These compounds' concentrations were contrasted with that of typical babies. Compared to control neonates, newborns with hyperbilirubinemia had significantly reduced levels of antioxidant activity, iron chelation, lipid reduction, free radical scavenging, and total antioxidant activity. Neonates with hyperbilirubinemia exhibited lowered antioxidant activity and free radical scavenging capacity by the first week of life. The second week of life saw an increase in these levels. A correlation between the antioxidant status of infants and hyperbilirubinemia is suggested by the linear relationship seen between the antioxidant properties of the hyperbilirubinemia group and serum bilirubin levels. The results of this study should advance our understanding of how hyperbilirubinemia affects newborns.

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Conflict of Interest
The authors declare no conflict of interest regarding this study.

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