Interleukin 1β Level in Human Colostrum in Relation to Neonatal Hyperbilirubinemia

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Breast-fed infants have higher bilirubin levels than formula-fed infants which is of undetermined etiology. The cholestatic effect of cytokines (e.g. IL-1β, IL-6) is believed to result from the repression of genes that normally mediated the hepatic uptake, metabolism, and biliary excretion of bile salts and bilirubin. The present study aimed to assess the level of interleukin 1β (IL-1β) in early milk of nursing mothers and its relation to neonatal jaundice. Sixty full term neonates and their mothers were included. They were classified into two groups; group I included Forty neonates patients with physiological jaundice and group II included twenty age and sex matched controls neonates without jaundice. Milk samples were collected from mothers for estimation of serum IL-1β level by ELISA, and blood samples were collected from the neonates for measuring total serum bilirubin (TSB) level. A significant difference ($P < 0.01$) in the level (pg/dl) of IL-1β was found in early milk between group I (10.25 ± 4.23) and group II (3.75 ± 2.07). The total serum bilirubin level was higher in group I than group II (10.91 ± 3.25 mg/dl versus 3.88 ± 0.78 mg/dl) ($P < 0.01$). A significant positive correlation was found between of IL-1β in breast milk and total serum bilirubin levels ($r = 0.494$, $P < 0.001$). It is concluded that Elevated levels of IL-1β in the colostrum provide additional data to understand one of path physiologic mechanisms of breast milk jaundice.
Group II (Control)

Included 20 full term neonates without jaundice and their mothers.

A) Neonates
Twenty full term neonates (37-41 weeks) were included. Their gestational age, sex, weight were recorded.

B) Mothers
Their age and antenatal history were recorded.

Inclusion Criteria
A) Mothers
1- 18 years old or more.
2- No history of inflammatory diseases or medications prior to and at the time of enrollment.
3- Uncomplicated pregnancy, delivery and purperium.
4- Exclusively breast-feeding of their newborns was eligible.

B) Neonates
1- Healthy full term neonates, their gestational age range (37-41 weeks).
2- Not having any signs of birth injuries, congenital infections, poor suckling, respiratory distress or signs of neonatal sepsis.

Exclusion Criteria
A) Mothers
1- Medical diseases during pregnancy as Diabetes Mellitus or Hypertension.
2- Taking anti-inflammatory Medications at the time of enrollment and in the previous one month.
3- Complicated delivery and purperium.
4- Previous neonates with a history of neonatal jaundice.
4- Not exclusively breast-feeding to their newborns.

B) Neonates
1- Preterm infants.

2- Neonates having any signs of birth injuries, congenital infections, poor suckling, respiratory distress or signs of neonatal sepsis.

Data Management and Analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0. for windows; SPSS Inc, Chicago, IL, 2001). Numerical data were expressed as Mean, Standard deviation (SD), Minimum and maximum values (range). Non-numerical data were expressed as Frequency and percentage. Analytical statistics including Independent-Samples T, Correlation analysis (using Pearson's method) the correlation coefficient denoted symbolically "r", Chi-Square test. P< 0.05: Significant

Results

The data in this study are formulated as tables and figures. In the present study, mean total serum bilirubin in group I was 10.9±3.25mg/dl while was 3.88±0.78mg/dl in group II (figure 1). Mean Interleukin-1β level in group I was 10.35±4.23pg/dl and was 3.75±2.1pg/dl in group II (figure 2). Comparing the studied and control groups, significant differences were found regarding Interleukin-1β level (P=0.013) and total serum bilirubin (P=0.001) while no significant differences between the two groups regarding gestational age (P=0.357), neonatal weight (P=0.707), direct bilirubin (P=0.513), hemoglobin (P=0.27), RBCs (P=0.22), WBCs (P=0.87) and platelets (P=0.517) (table 1). Significant positive correlations between IL-1β level in colostrum and both total serum bilirubin (P=0.0001, r=0.722) (figure 3, table 2) and direct bilirubin (P=0.001, r=0.588) levels in group I (table 2).
Table 1. Comparison of Laboratory findings between neonates with jaundice and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n = 40)</th>
<th>Group II (n = 20)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>38.725</td>
<td>38.65</td>
<td>NS</td>
</tr>
<tr>
<td>Interleukin-1β in Colostrums (pg/ml)</td>
<td>10.35</td>
<td>3.75</td>
<td>0.013</td>
</tr>
<tr>
<td>Weight of neonate(kg)</td>
<td>3.1</td>
<td>2.97</td>
<td>NS</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>10.91</td>
<td>3.88</td>
<td>0.0001</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.62</td>
<td>0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>15.09</td>
<td>14.9</td>
<td>NS</td>
</tr>
<tr>
<td>Red blood cells (×10^{12}/l)</td>
<td>4.71</td>
<td>4.75</td>
<td>NS</td>
</tr>
<tr>
<td>White blood cells (×10^9/l)</td>
<td>10.26</td>
<td>10.88</td>
<td>NS</td>
</tr>
<tr>
<td>Platelets (×10^9/l)</td>
<td>236.13</td>
<td>256.75</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P ≤ 0.01: is significant. Ns: not significant

Table 2. Correlation study results between IL-1β (pg/ml) and the studied variables in group I

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Interleukin-1β in Colostrums (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td>38.7</td>
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<tr>
<td>Weight of neonate (kg)</td>
<td>3.008</td>
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<tr>
<td>Total bilirubin (mg/dl)</td>
<td>10.91</td>
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<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.623</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>15.09</td>
</tr>
<tr>
<td>Red blood cells (×10^{12}/l)</td>
<td>4.709</td>
</tr>
<tr>
<td>White blood cells (×10^9/l)</td>
<td>10.26</td>
</tr>
<tr>
<td>Platelets (×10^9/l)</td>
<td>236.13</td>
</tr>
</tbody>
</table>

*P ≤ 0.01: is significant. Ns: not significant
Figure 1. Mean serum IL1β levels in both studied groups.

Group I = neonates with physiological jaundice.  Group II = controls.

Figure 2. Mean total serum bilirubin levels in both studied groups.

Group I = neonates with physiological jaundice.  Group II = controls.
Discussion

Many factors are associated with the occurrence of neonatal jaundice (NJ). Regarding gender, Seidman et al., 1999 reported that after controlling for race, birth weight, gestational age (GA), sepsis, and in a multiple regression model, the males had significantly higher bilirubin than females agreeing with the study proved significant increase in IL-1β in the colostrum of mothers of jaundiced breast-fed neonates compared with breast-fed neonates without neonatal jaundice (Zanardo et al., 2007). Ghadeer et al., 2009 found that there is a highly statistical significance difference between jaundiced breast fed neonates and those breast fed infants without neonatal jaundice regarding level of IL-1β in breast milk. Another study suggesting that the mammary gland immunologic function through altering cytokine levels may affect jaundice development in neonates. The identification of elevated IL-1β concentration in colostrum of mothers with jaundiced neonates and the clinical consequences of breast-feeding initiated through this immunological bas (Zanardo et al, 2007). Also, may be explained by sequestration and protection of cytokines and chemokines until they reach the intestine (Calhoun et al, 1999) which also supported by the hypothesis that interleukin-1 receptor antagonism improves gastric emptying.
Although cytokines are known to play a critical role in the intestinal absorption, the molecular basis remains unclear (Michael et al., 1999). Constitutively enterocytes can express pro-inflammatory cytokines and this response is up-regulated by inflammatory stimuli such as endotoxin and IL-1β (Zanardo et al., 2007). With excessive pro-inflammatory cytokine production (IL-8) an inflammatory stimulation after interaction between immature human enterocytes and excessively released cytokines (IL-1β, lipopolysaccharides), causing destruction of enterocyte barrier (Defranco et al., 1998; Nanthakumar et al., 2000). Additionally, inflammatory cytokines such as TNF-α, interferon (IFN)-γ, and IL-1β, produced by epithelial cells and cells of the innate and adaptive immune systems contribute to dysfunction of epithelial barrier enterocytes (Van Haver et al., 2009).

Another theory of relative distal expression of cytokines (IL-1β and IL-6) in the small intestine and different bacterial species increasing the expression of pro-inflammatory cytokines. IL-1β and growth factors and altering vascular smooth muscle differentiation changing from a contractile to a proliferative phenotype, which in turn leads to a change in the expression levels of contractile proteins such as actin and myosin heavy chains, causing force generation reduction (Shirkey et al., 2006) supported by the suggestion that IL-1β inhibits the initial Ca²⁺ dependent contraction of intestinal smooth muscle (Hsieh et al., 2000; Wenhui et al., 2009). Also, diminished intestinal motility enables more bilirubin deconjugation by the luminal enzyme β-glucuronidase, allowing more reabsorption of unconjugated bilirubin (James et al., 2007).

Studying the correlation between level of IL-1β in colostrum and level of neonatal TSB, Ghadeer et al., 2009 reported that there is a highly statistical significant positive correlation between level of IL-1β in colostrum and level of neonatal TSB, but this was in contrast with the opinion that neither IL-1β concentrations in colostrum nor other studied cytokines (IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α) were correlated to serum bilirubin concentrations of neonates with NJ(1). Factors that may contribute to the development of neonatal jaundice include exposure to various breast milk components such as 3-alpha-20-beta pregnanediol, nonesterified fatty acids, and glucuronidase, or injurious agents (e.g. drugs, hormones, proinflammatory cytokines,) results in alteration in bilirubin pathway, changes that may maintain and contribute to appearance of jaundice. In addition, the suppression of genes that normally mediates this bilirubin pathway and excretion (Zanardo et al., 2007). Regulatory mechanisms that affect bile acid transporter, expression and function will help to develop novel rational options to treat cholestasis and chronic cholestatic liver diseases (Sertac et al., 2004). Depending on IL-1β assay only in colostral milk without putting in consideration that concentrations of IL-1β in the early, transitional and mature milk are different may make a limitation of our study. Milk composition is affected with several factors including delivery weather normal or instrumental, multiple parturition, and with day time, at each feeding, also milk proteins produced and secreted in the mammary gland are constitute the major percentage of human milk proteins and have more postpartum variation compared with other serum proteins that are passively transferred into milk (Londerdal et al., 1995).

We conclude that elevated levels of IL-1β in the colostrum provide additional data to understand one of path physiologic mechanisms of breast milk jaundice and immunologic role of mammary gland in the occurrence of neonatal jaundice.
References


