Laser Nephelometry of Orosomucoid in Serum of Newborns: Reference Intervals and Relation to Bacterial Infections

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Orosomucoid was evaluated by laser-nephelometry in 1790 sera collected from 1170 newborns. Within-run precision (CV) was 2.1 to 4.2%, between-run 2.9 to 5.2%. Results correlated well with radial immunodiffusion \( r = 0.989 \). Results can be obtained within 1 h. Orosomucoid concentrations in serum at birth range from 130 to 200 mg/L and are influenced by gestational age during the first two days of postnatal life. Thereafter, the values increase very rapidly in the first week of life, concentrations being the same as in adults by about 10 months. In 66 of 78 cases of severe bacterial infections, orosomucoid concentrations were above normal. Evidently, serum orosomucoid constitutes an useful index in diagnosis and monitoring of bacterial infections in the neonatal period.

Additional Keyphrases: acute-phase reactants · inflammation · effects of prematurity.

Orosomucoid (\( \alpha_1 \)-acid glycoprotein) is a glycoprotein of low molecular mass (1), one of the acute-phase reactants. As is true of these reactants (which include C-reactive protein, haptoglobin, and \( \alpha_1 \)-antitrypsin), the circulating orosomucoid increases in the presence of inflammation and other related disease states, including cancer, pneumonia, or severe surgical stress.

Serum orosomucoid concentrations in the neonatal period have received little attention. In 1973, Gotoh et al. (2) measured orosomucoid by radial immunodiffusion (RID), and reported increased values in newborns with bacterial infections. We confirmed these results (3, 4). However, the reference intervals have not been precisely evaluated, especially in premature infants. The present study was carried out to evaluate the variation of serum orosomucoid concentrations as a function of gestational and postnatal age in normal and sick neonates.

Because the RID method for orosomucoid determination requires at least 24 to 48 h, we developed a quicker method, laser nephelometry, for its measurement. Our data suggest that orosomucoid measurement by laser nephelometry constitutes a quick, precise, and useful tool for detection and followup of bacterial infections in newborns.

Materials and Methods

Blood Samples

We collected 1790 blood samples from 1170 infants, which were classified into three groups according to their clinical status.

**Group I: Control group.** We analyzed 411 sera from 352 control pre- and full-term infants and children for orosomucoid. Of these 352 infants, 55 had sera sampled more than once. Although some of these control infants or children were hospitalized, it was for social rather than medical reasons. We confirmed the absence of infection by clinical, bacteriological, and virological examination of each infant. Specimens from this group included 323 sera from 264 newborns (167 pre- and 97 full-term infants), whose ages ranged from one to 32 days, and 88 sera from 88 infants and children from one month to nine years of age.

**Group II: Infected neonates.** Subgroup II-1, bacterial infections: We collected 477 sera from 148 neonates (51 pre-term and 97 full-term infants) with clinical and biological infections, as diagnosed by positive culture from the blood of 58 infants (274 sera), from the cerebrospinal fluid of 10 infants (34 sera), from the brain abscess of one infant (8 sera), from the peritoneum of nine infants (26 sera), from the stools of 22 infants (37 sera), from the urines (more than 10⁶ organisms/L) of seven infants (15 sera), and from the skin of 27 infants with severe cutaneous infections (60 sera). Infection was also diagnosed in 14 additional infants (23 sera), for which the same organism was detected in at least three of these different areas (5).

Subgroup II-2, viral and parasitological infections: These infections were diagnosed by direct isolation of the organism, or by a serodiagnosis, or both. We analyzed 46 sera from 23 infants with viral infections (nine cytomegalovirus infections, eight viral pneumonia (three myxovirus influenzae, two myxovirus parainfluenzae, three others), two congenital rubella, two mumps meningitis, and two viral hepatitis (virus A)) and 56 sera from seven infants with parasitological infections (four with congenital toxoplasmosis, two with candidiasis, and one syphilis) for orosomucoid.

**Group III: Sick neonates without infections.** This group consisted of 640 infants (801 samples). It included 51 cases of respiratory disease syndromes; 137 of neonatal asphyxia; 86 small-for-date infants; 186 infants with congenital malformations, jaundice, or digestive syndrome (of these 186 infants, virological examination has been performed to exclude a viral etiology); and 10 cases of inflammatory diseases (six cases of necrotizing enterocolitis). This group also included 170 infants thought to have an infection, but which was not in fact confirmed by clinical, bacteriological, and virological findings.

Methods

We collected 500-µL samples of blood in a polystyrene microtube without anticoagulant, by heel prick (or by venous puncture, in the case of older children), and removed the serum after centrifugation.

For laser nephelometry we used the Behring Laser Nephelometer module 1 (Behringwerke, D-3550 Marburg/Lahn, F.R.G.). Samples, standards, and antisera were diluted with sterile isotonic saline solution and 100 µL of 101-fold diluted sample were mixed in a microwell with 200 µL of a fivefold diluted anti-orosomucoid antiserum (LN serum anti-orosomucoid SAW; Behringwerke). The cuvettes were shaken briefly and allowed to stand for 1 h at room temperature, and the light scattered by the resulting antigen–antibody complexes was measured (in volts) with the nephelometer. A
calibration curve was prepared by use of an 800 mg/L standard solution of orosomucoid, diluted to give concentrations of 40, 20, 10, 5, 2.5, and 1.25 mg/L. The blank values (i.e., the light scattered by the empty cuvettes) were negligible (80-150 mV).

Results of laser nephelometry were compared with those by radial immunodiffusion on M-Partigen TBX plates for orosomucoid determination (Behringwerke).

IgM concentration was evaluated by laser nephelometry with an anti-IgM antiserum (OSAT; Behringwerke), with the same procedure as outlined for orosomucoid.

Fibrinogen was estimated according to Von Clauss (6).

Statistical methods used were the Student’s t-test and the nonparametric Mann/Whitney U-test.

Results

Methodological Aspects

Calibration curve and analytical range: An example of a calibration curve is shown in Figure 1. This curve corresponds to a third-order polynomial; thus, unknown orosomucoid concentrations in sera can, if desired, be determined with an appropriate calculator. The analytical range is wide: 120 to 3900 mg/L for a 101-fold diluted serum.

Within-run precision, between-run precision: These data are summarized in Table 1. The coefficients of variation were always <6%.

Correlation of laser nephelometry with radial immunodiffusion: Orosomucoid in 90 sera was measured both by laser nephelometry (y) and RID (x). The correlation was excellent ($r = 0.989$) and the least-squares regression equation is $y = x - 0.013$.

Clinical Aspects

Reference intervals were established according to gestational and postnatal age. Control infants from one to 32 days of age were classified into three groups according to gestational age: two groups for pre-term infants (26-32 weeks, inclusive, and 33-37 weeks, inclusive) and one group for full-term infants (38-41 weeks, inclusive). The values do not follow a gaussian distribution (Figure 2), some orosomucoid concentrations being too high. Nevertheless, the mean ± 2SD can be considered in practice as the upper limit of the reference interval for serum orosomucoid.

Figure 3 shows the change in the mean ±1 SEM (standard error of the mean) in orosomucoid concentration with postnatal age (1-32 days) in pre-term and full-term infants. For all groups, serum orosomucoid values at birth are much lower than for adults. In full-term infants, orosomucoid shows a rapid postnatal increase, from 180 mg/L at birth to 520 mg/L at five or six days, and the difference is highly significant ($p < 10^{-4}$); thereafter the value remains constant for up to one month. In pre-term infants (26-32 and 33-37 weeks gestation, inclusive) the increase is more progressive between the first

**Table 1. Precision of the Nephelometric Assay**

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Within-run</th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Mean</td>
<td>350</td>
<td>820</td>
</tr>
<tr>
<td>SD</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>CV, %</td>
<td>4.2</td>
<td>2.4</td>
</tr>
</tbody>
</table>

n = 30 for each concentration.

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Fig. 1. Example calibration curve for the assay of orosomucoid by laser nephelometry

Fig. 2. Frequency distribution of serum orosomucoid observed in 63 control neonates (subgroup 33-37 weeks, day one)

Fig. 3. Evolution of serum orosomucoid concentration with postnatal and gestational age in control infants

Gestational ages: · · · , 26-32 weeks; · · · , 33-37 weeks; —, 38-41 weeks. Bar shows mean ± 1 SEM.
Table 2. Reference Values for Serum Orosomucoid (One Month through Nine Years)

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>x</th>
<th>SD</th>
<th>SEM</th>
<th>x + 2 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 mo</td>
<td>12</td>
<td>515</td>
<td>243</td>
<td>70</td>
<td>1000</td>
</tr>
<tr>
<td>2-3 mo</td>
<td>6</td>
<td>580</td>
<td>250</td>
<td>102</td>
<td>1080</td>
</tr>
<tr>
<td>10 mo</td>
<td>25</td>
<td>820</td>
<td>200</td>
<td>40</td>
<td>1220</td>
</tr>
<tr>
<td>2 yr</td>
<td>25</td>
<td>940</td>
<td>215</td>
<td>43</td>
<td>1370</td>
</tr>
<tr>
<td>3-4 yr</td>
<td>14</td>
<td>840</td>
<td>177</td>
<td>47</td>
<td>1200</td>
</tr>
<tr>
<td>5-9 yr</td>
<td>6</td>
<td>880</td>
<td>208</td>
<td>85</td>
<td>1300</td>
</tr>
</tbody>
</table>

and the second day of postnatal life; thereafter it parallels the course for full-term infants.

Prematurity influences orosomucoid concentrations. Measured on the same postnatal day, mean concentrations at days 1 and 2 were significantly (p < 0.05) lower in the 26-32 weeks group than in full-term infants. The difference is also significant between the 33-37 weeks of gestation and full-term groups at day 2, but not at day 1, owing to the more rapid increase of orosomucoid at day 2 in full-term infants than in pre-term 33-37 week infants. From day 3 the influence of gestational age on orosomucoid values is not significant. Consequently, we made no distinction between pre-term and full-term infants from one month to nine years of age (Table 2). By 10 months, orosomucoid values are the same as in adults.

Orosomucoid in Infected Infants

In the following studies, the concentrations of orosomucoid in a particular infant were analyzed and compared with the reference values for normal infants of the same gestational and postnatal age.

Bacterial infections: Figure 4 shows our results for infants with verified bacterial septicemia, meningitis, and peritonitis. Of 78 patients, 66 (44/52 for full-term and 22/26 for pre-term infants) had increased (i.e., >2 SD from the mean) orosomucoid concentrations at the time of the infection; the range was 80-3300 mg/L.

Of the 11 cases of meningitis (a case of brain abscess included), 10 had supranormal orosomucoid concentrations; the one case with normal values was an infection due to Proteus mirabilis. One case of peritonitis, of the nine cases studied, had a very low value (80 mg/L), and the infant died on the eight postnatal day. Of the 10 (out of 58) septicemia cases with normal orosomucoid values, four were caused by Streptococcus (two viridans, two beta-streptococcus), two by Proteus mirabilis (with one death), two by Staphylococcus epidermidis, and two by Escherichia coli.

In addition, serum orosomucoid concentration was abnormal in six of seven cases of urinary tract infections, in 20 of 27 cases of severe cutaneous infections (74%), and in 16 of 22 cases of bacterial diarrhea (73%). Of the 14 infants with germs at three different areas, 10 had increased serum concentration of orosomucoid.

Overall in this group, of the 148 infected infants 118 (80%) had high serum orosomucoid values.

Figure 5 illustrates the variations of serum orosomucoid in eight cases of septicemia and in seven cases of meningitis in relation to the time since presumed onset of the infection. These examples show that the increase of serum orosomucoid was very rapid after the beginning of the infection; nevertheless, in three cases of septicemia the initial value for orosomucoid was within the normal range, but an increase oc-

Fig. 4. Serum orosomucoid in bacterial septicemia, meningitis, and peritonitis in (left) full-term and (right) pre-term infants

© Staphylococcus, △ Streptococcus, ♦ E. coli, ⊗ Proteus, ● Klebsiella, ☆ Salmonella, □ Listeria, † others; o two different germs or more. → infants who died. Dashed line indicates upper limit of reference values (x + 2 SD).
curred subsequently. For uncomplicated cases of septicemia and under antibiotherapy the maximum orosomucoid concentrations were observed from three to six days since onset of infection, and the normalization of serum concentration of orosomucoid occurred after about seven to ten days since onset. This normalization could take up to three weeks for complicated cases. In our experience, the peak and normalization of orosomucoid during meningitis appeared a little later than during septicemia. In 11 cases the normalization was parallel to clinical recovery. In the four other cases studied, two infants died with high orosomucoid concentrations; for the other two, determinations were not made until complete recovery.

Orosomucoid in viral and parasitological infections: Concentrations of serum orosomucoid in viral and parasitological infections are presented in Figure 6. Increased values were observed in 16 of the 30 cases studied (53%), ranging from 180 to 2960 mg/L. The most increased values were observed in cytomegalovirus infections.

Orosomucoid in Sick Neonates without Infections

In seven of the 51 infants (14%) with respiratory distress syndrome and in 43 of the 137 asphyxiated neonates (31%), orosomucoid concentrations were increased.

Serum orosomucoid concentrations were increased in 17 newborn infants in the group of 86 small-for-date infants (20%) and in 56 neonates in the group of 186 infants with congenital malformations, jaundice, or digestive syndromes (30%).

In eight of 10 cases (80%) of inflammatory diseases, high orosomucoid values were observed, but this increase was expected because orosomucoid belongs to the acute-phase proteins, which increase in concentration in inflammations.

Of the 170 infants with suspected infections only 42 (25%) had abnormal serum orosomucoid values.

Except for those with inflammatory diseases, only 26% of the infants of this group without documented infection had higher serum orosomucoid concentrations than those of the control group.

Among the 778 determinations when orosomucoid was increased, the increase was slight (only six values >1500 mg/L). Higher values were generally noted in the first and the second day of life following a complicated delivery; thereafter, orosomucoid concentration fell rapidly to normal values within the first postnatal week.

Orosomucoid vs Fibrinogen and IgM Concentration

In the group of infants with bacterial infections, fibrinogen and IgM concentrations were evaluated with orosomucoid in 58 and 49 cases of various bacterial infections, respectively.

Fibrinogen was increased in 40 of the 58 cases (69%), whereas in the same series orosomucoid was higher than normal in 48 cases (80%). In 18 cases there was a discrepancy between changes in fibrinogen and orosomucoid concentrations: in 13, orosomucoid was increased and fibrinogen normal; in the other five, the opposite was true.

IgM concentrations were increased (7) in only 25 of the 49 cases (51%), whereas an orosomucoid increase occurred in 36 cases (73%). In two cases only serum IgM was increased while serum orosomucoid was normal, but in 10 cases the opposite situation was observed.

Discussion

The need for a quick and safe guide to direct the diagnosis and treatment of infections is obvious in the neonatal period.
newborn infants according to gestational and postnatal age. In the neonatal period, an increase of serum orosomucoid concentrations from causes other than infection occurred in only 28% of the cases studied. Furthermore, these increases were always slight and brief.

Serum orosomucoid determination was not very useful in viral and parasitological infections. Only 53% of these infants had increased orosomucoid values, and these were in cases of viral infections accompanied by inflammation.

On the other hand, a high orosomucoid concentration in combination with clinical symptoms is highly suggestive of bacterial infection, which must be confirmed by bacteriological findings. However, one must be particularly aware of the postnatal variation of serum orosomucoid values when diagnosing bacterial infection. The capacity to synthesize orosomucoid occurs quite early, and the increase of orosomucoid, which can be as high as in adult inflammations, is a very sensitive parameter because normal values are very low (so orosomucoid values observed during infections can be 15-fold greater than normal values). The variation of these orosomucoid concentrations is of great interest in the monitoring of bacterial diseases; especially, it could help increase the efficiency of treatment. In our experience, it allowed us to reduce the time of antimicrobial therapy.

In the present investigation, 15% of false negatives were observed in severe bacterial infections: sepsis, meningitis, and peritonitis, which is quite similar to the findings of Sabel and Wadsworth (15) with regard to C-reactive protein. It is noteworthy that most of the cases with normal orosomucoid concentrations were infected with Streptococcus and that clinical evolution of these infants was not very satisfactory. Four infected infants with normal orosomucoid values died (an important one to note is the case of peritonitis with an orosomucoid concentration of 80 mg/L).

These last data support the findings of Philip (18), showing that orosomucoid appears to have a protective role in the ability to combat infections. Further studies are required to know whether these false-negative results have prognostic significance and whether the ability of the liver to synthesize a large quantity of orosomucoid is an indication of the capacity of newborn infants to fight infections.

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References