INFLAMMATORY MARKERS IN MECONIUM INDUCED LUNG INJURY IN NEONATES AND EFFECT OF STEROIDS ON THEIR LEVELS: A RANDOMIZED CONTROLLED TRIAL

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Abstract

Purpose: To determine the levels of TNFα and IL-1β in tracheal aspirates of neonates with meconium aspiration syndrome (MAS) and to ascertain whether the use of steroids by systemic or nebulized routes suppresses the levels of these inflammatory markers. Methods: This was a double blind, randomized, controlled, prospective, interventional study done over one year period in the neonatal unit of the Lady Hardinge Medical College. Fifty-one babies of MAS which were randomly distributed into three groups; control, systemic and nebulized steroids; were included in the study. Methyl prednisolone was given intravenously in the dosage of 0.5 mg/kg/day in two divided doses while nebulized budecort was given in a dosage of 50 mcg/dose twice daily. Tracheal aspirates were taken on day 1, 3 and 4 and were analyzed for TNFα and IL-1β by ELISA technique. Results: TNFα in tracheal aspirates showed an increasing trend in babies of MAS in first four days, thereby signifying an inflammatory process underlying the condition. The levels of TNFα were suppressed by use of steroids. Higher levels of TNFα were associated with longer stay in hospital. IL-1β did not show any significant correlation. Conclusions: TNFα is associated with meconium-associated inflammation. Its level is suppressed with the use of steroids and can also be used to assess prognosis of neonates with MAS.

Key words: Meconium aspiration syndrome, tracheal aspirates, steroids, TNFα, IL-1β.

Meconium aspiration syndrome (MAS) is an important cause of respiratory emergencies in term newborn babies. The overall frequency of MAS varies between 5-25% (median 14%).1 MAS occurs in around 10% of babies born through meconium stained amniotic fluid (MSAF). Infants born through MSAF are 100 times more likely to develop respiratory distress compared to their counterparts born through clear amniotic fluid.1 Chemical pneumonitis is a known consequence of meconium aspiration into the lung, but limited information is available on the cellular mechanism by which meconium produces the cascade of inflammatory reactions described in MAS. Airway epithelial cells have been shown to release several products capable of modulating a variety of cellular functions within the respiratory tract including interleukins, thromboxane B2 and TNFα.1 Interleukin-1β and tumor necrosis factor -alpha (TNFα) are two important inflammatory markers that have been extensively studied.1

Meconium induced lung inflammation was first described by Clark and Duff2 who showed the effect of meconium on neutrophil function by inhibiting oxidative burst and phagocytosis. Kojima and colleagues3 have found that meconium induces lung injury by activating alveolar macrophages and generates increased production of superoxide anion in these cells. Increased levels of inflammatory cytokine (like TNFα, IL-1β, IL-8) are produced as a result of meconium injuries.4,5

In the present study, the levels of TNFα and IL-1β were estimated in the tracheal aspirates of neonates with MAS and it was ascertained whether the use of steroids by systemic or nebulized routes alter the levels of these markers. The levels of markers among babies with longer duration of stay vis-à-vis the babies with shorter duration of stay were assessed. Further it was ascertained whether the levels of markers had any prognostic significance.

Materials and Methods

Place and time of study

A prospective, double blind, randomized, interventional study was carried out over one year period in neonatal ward of the Lady Hardinge Medical College (LHMC) and associated Kalawati Saran Children’s hospital (KSCH) and Smt. Sucheta Kriplani Hospital (SSKH) New Delhi. The study was approved by the institutional ethical committee.

Inclusion criteria

All full term babies with birth weight more than two kilograms, in whom meconium was aspirated from below the vocal cords on endotracheal suction at birth with respiratory distress, were included in the study.

Exclusion criteria

Exclusion criteria consisted of preterm and intrauterine
growth retarded babies, out-born babies, babies with congenital malformations and denial of consent for the trial by the parents.

Outcome measures

The patients were divided into three groups: A-control, B-systemic steroids and C-nebulised steroids.

Various parameters were compared between the three groups:

- Comparison of values of inflammatory markers
- Comparison of inflammatory markers on day 1, day 3 and day 4 in the three groups.
- Comparison of values of inflammatory markers in babies with MAS on day 1, day 3 and day 4.
- Comparison of values of inflammatory markers in babies with MAS who had a longer duration of stay.
- Comparison of duration of stay in babies of MAS who had higher values of inflammatory markers.

Sample size

A total of 51 babies were enrolled in the study, of whom 17 were in group A, 17 in group B and 17 in group C. The sample size of 17 babies in each group was calculated from the study of literature with the power of 80% and an alpha error of 5%.

Randomization

After informed parental consent infants were randomly assigned to three groups (by pulling a slip from randomization box).

Masking

The observer and the caregiver were not aware of the group distribution. The infants of group A and B were nebulized with normal saline while those of group A and C were given shots of 5% Dextrose intravenously to ensure blinding.

Interventions

Doses of steroids used

Standard regimens of methylprednisolone (Solu-medrol, Pharmacia N.V./S.A Rijksweg 12 2870 Puurs-Belgium) i.e, 0.5 mg/kg /day divided every 12 hours for seven days intravenously and nebulized budesonide (Budesonide respirator suspension 0.5 mg, Cipla Ltd.) i.e., 50 microgram in 2.5 mL normal saline by nebulization every 12 hours for seven days. Nebulization was done by a jet nebulizer system (Eastneb Neo™ Eastern Mediket Limited, Delhi, India). First dose of steroids was given after the first sample of tracheal aspirate was taken.

Tracheal aspirate schedule and method

First sample was obtained between 18-24 hours, second 48-72 hours and the third between 72-96 hours. Tracheal aspirate was obtained by a standardized technique. While the infant was supine with the head in midline, 1 mL of sterile saline was instilled intratracheally, three to five breaths were delivered with a self inflating resuscitation bag, the endotracheal tube was suctioned using a 6 or 8 French IG tube connected to a Dee Lee’s mucus trap (Mucus extractor, Romsons, Agra, India) which was connected to a automated suction apparatus. The procedure was repeated with a second 1 mL saline instillation. The lavage fluid was stored in deep freeze at -70°C till processed.

Assay of inflammatory markers

Estimation of TNFα and IL-1β was done by Enzyme Linked Immunosorbent Assay method (BioSource TNFα EASIA kit manufactured by BioSource Europe SA and BioSource IL-1β EASIA kit manufactured by BioSource Europe SA). The serum sample of groups A, B and C were arranged randomly and each sample was assayed in duplicate for TNFα and IL-1β and the mean value was calculated for each sample for the respective marker.

Standard care

Besides the above intervention, the management of all the neonates was done according to the standard protocol of the neonatal ward (which included normothermia, normoglycemia, treatment of sepsis and ventilation if required) and it was consistent during the study period.

Statistical analysis

Data was analysed by Microsoft excel 2000 and SPSS software applying standard statistical tests like Student t test, Mann Whitney U test, ANOVA f test and Wilcoxon Signed rank test.

Results

The subjects in the three groups were comparable in terms of mode of delivery, birth weight, APGAR scores and HIE staging (Table 1). All infants were discharged after a variable stay in the neonatal ward and no baby was excluded from the study after randomization.

The mean levels of TNFα in tracheal aspirate were determined in each group on the three days. The levels on day one were comparable among the three groups (P=0.45 by ANOVA f test). But they were significantly higher on day three and day four in group A, compared to groups B and C. There was no significant difference in the levels of TNFα in tracheal aspirate between group B and group C on any of the three days (Table 2).

On comparing the levels of inflammatory markers (mean
Table 1: Baseline parameters of the subjects in the three study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n=17)</th>
<th>Group B (n=17)</th>
<th>Group C (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of babies delivered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NVD</td>
<td>6</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>LSCS</td>
<td>11</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>No. of babies with HIE</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Birth weight (kgs)</td>
<td>2.64 ± 0.34</td>
<td>2.63 ± 0.45</td>
<td>2.80 ± 0.37</td>
</tr>
<tr>
<td>APGAR score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 minute</td>
<td>4.25 ± 2.28</td>
<td>3.65 ± 2.09</td>
<td>2.28 ± 2.0</td>
</tr>
<tr>
<td>5 minute</td>
<td>6.53 ± 1.77</td>
<td>6.94 ± 1.25</td>
<td>6.12 ± 1.02</td>
</tr>
<tr>
<td>10 minute</td>
<td>7.65 ± 1.17</td>
<td>7.94 ± 1.09</td>
<td>7.41 ± 1.42</td>
</tr>
</tbody>
</table>

P-value for mode of delivery 0.748. P-value for birth weight 0.741

Table 2: Comparison of levels of inflammatory markers in tracheal aspirates

<table>
<thead>
<tr>
<th>Days after birth</th>
<th>Levels of inflammatory markers in pg/mL (±S.D)</th>
<th>Group A (n=17)</th>
<th>Group B (n=17)</th>
<th>Group C (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNFα (n=17) IL-1β (n=17)</td>
<td>TNFα (n=17) IL-1β (n=17)</td>
<td>TNFα (n=17) IL-1β (n=17)</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>40.41 ± 35.39 54.26 ± 38.02</td>
<td>34.27 ± 21.93 44.33 ± 35.92</td>
<td>40.2 ± 32.74 56.6 ± 29.19</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>89.60 ± 30.6 44.33 ± 22.80</td>
<td>67.63 ± 34.4 39.0 ± 36.5</td>
<td>69.21 ± 47.17 42.0 ± 33.35</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>138.62 ± 40.91 84.18 ± 35.40</td>
<td>116.9 ± 51.5 88.0 ± 39.69</td>
<td>108.1 ± 56.27 75.10 ± 52.74</td>
<td></td>
</tr>
</tbody>
</table>

P-value for TNFα in groups A, B and C: Day1-0.43, Day 3-0.055, Day 4-0.033. IL-1β in groups A, B and C: Day 1-0.26, Day 3-0.70, Day 4-0.69

for all the three days) in the three groups, it was observed to be maximum in Group A and the difference was statistically significant. The difference was not statistically significant between groups B and group C (Fig. 1). On comparing the average values of inflammatory markers (for all the three groups) on the three days i.e., day 1, 3 and 4, the TNFα values showed an increasing trend along the three days and the difference was statistically significant (Fig. 2).

Estimation of interleukin 1β

The values of IL-1β were compared among the three groups on all the three days, IL-1β level was found to be higher in Group A as compared to Group B and C on day 3 and day four, but the difference was not significant. On comparing levels of IL-1β of all the three days, in the three groups it was observed to be maximum in Group A and minimum in Group C but the difference was not statistically significant (P ≥ 0.05). On comparing the average levels of the IL-1β on the three days in all the groups, the values showed a dip at third day followed by a rise again on the fourth day. The changes observed were statistically not significant.

Comparison of duration of stay with the values of inflammatory markers

The average duration of stay of group A, B and C was 19.59 ± 12.77, 13.29 ± 7.48 and 12.18 ± 6.22 days respectively. TNFα and IL-1β concentrations in tracheal aspirate of babies with longer duration of stay (longer than the average duration of stay of the respective group i.e., group A, B or C) was compared with the TNFα and IL-1β levels in babies with shorter duration of stay (less than the average duration of stay of the respective group).

Figure 1: Comparison of levels of inflammatory markers (mean for all three days) amongst groups A, B and C

Figure 2: Comparison of average values of inflammatory marker (for all three groups) on days 1, 3 and 4

P-value - For TNFα - 0.048, for IL-1β -0.748
concentration of babies with shorter duration of stay (shorter than the average duration of stay of the same group i.e., group A, B and C). The values for TNFα were found to be significantly higher in the group with longer duration, while there was no significant difference for IL-1β (Table 3).

In the same way, the effect of levels of TNFα and IL-1β on the duration of hospitalization was assessed. The duration of stay of babies whose inflammatory marker values were higher, were compared with duration of stay of babies with low levels of inflammatory markers (the average values for TNFα and IL-1β are depicted in Fig. 1. Inflammatory marker values above average were considered as high and below average were considered as low for the respective groups. It was observed that the group with high TNFα levels had a longer duration of stay as compared to those with lower TNFα levels. The difference was statistically significant. On the other hand, the duration of stay was almost similar irrespective of the levels of IL-1β (Table 4).

Discussion

The chemical pneumonitis seen in MAS is related to the physical and chemical properties of meconium, which results in lung injury; by mechanisms, which are not well understood. Meconium aspiration into the lungs induces intense inflammatory response with polymorphonuclear cells infiltration diffusely throughout the lungs. These cells may release chemical mediators that can adversely affect the tissues. Shabarek5 found that increased levels of inflammatory cytokines are produced as a result of meconium injuries. These substances may directly injure lung parenchyma and lead to vascular leakage resulting in an injury pattern similar to adult respiratory distress syndrome (ARDS).

The trends of inflammatory markers and the effect of steroids on them have largely been studied on animal models till date. Soukkas group in a piglet model6 demonstrated that development of pulmonary hypertension after meconium aspiration is associated with a simultaneous rise in both endothelin -1 (ET-1) and atrial natriuretic peptide. Methyl prednisolone pretreatment prevents the progressive elevation of both ET-1 and pulmonary vascular resistance (PVR). Miller and colleagues, in 1989 first suggested a role for tumor necrosis factor in the pathogenesis of ARDS.7 The role of inflammatory mediators in ARDS in neonates was further proven by Jones et al.8 They found that 40% of patients had detectable levels of inflammatory cytokines in bronchoalveolar lavage (BAL) at 0 hours and by 24-48 hours, 80% of the patients had elevated levels. They concluded that there is induction of proinflammatory cytokines in response to meconium and that such induction can occur in utero. Bélaï9 investigated the safety of BAL in 32 sick ventilated preterm and term newborn infants and tried to detect the levels of inflammatory markers in lung fluids. They inferred that deep BAL in intubated newborn infants is safe without acute morbidity and that the lavage material can provide insight into cytokine titers in the epithelial lung fluid of the neonatal lung.

Role of inhaled beclomethasone therapy was assessed by Gupta et al.10 in suppression of tracheal aspirate inflammatory mediators IL-8 and IL-1α in ventilated preterm infants at risk for bronchopulmonary dysplasia. Fewer beclomethasone infants required systemic glucocorticoid therapy or developed bronchopulmonary dysplasia. Dooy11 in his review on role of inflammation in development of chronic lung disease in neonate, extensively discussed TNFα as an important inflammatory mediator. He also observed that prophylactic dexamethasone could prevent development of chronic lung disease but owing to conflicting opinion did not recommend its routine use.

Jones and Cayabyab in 1996 obtained four sequential BAL from five patients in first 96 hours of life with acute respiratory failure due to MAS and subjected the BAL to ELISA for estimation of IL-1β and TNFα.12 He found that at 0 hour, the concentration of TNFα was 90 pg/mL, at 24 hours 100 pg/mL, at 48h the concentration was 380 pg/mL and at

| Table 3: Relationship of levels of inflammatory markers with duration of stay |
|--------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Mean value of inflammatory markers               |                 |                 |                 |                 |                 |                 |
| Longer duration of stay                           | Shorter duration of stay | P-value | Longer duration of stay | Shorter duration of stay | P-value |
| Day 1                                            | 116.07          | 28.48           | 0.001           | 59.0            | 48.82           | 0.548           |
| Day 3                                            | 130.88          | 65.50           | 0.015           | 50.15           | 35.83           | 0.322           |
| Day 4                                            | 146.55          | 102.23          | 0.057           | 94.20           | 70.84           | 0.129           |

| Table 4: Relationship of duration of stay with levels of inflammatory markers in tracheal aspirates |
|--------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Mean value of inflammatory markers               |                 |                 |                 |                 |                 |                 |
| Longer duration of stay                           | Shorter duration of stay | P-value | Longer duration of stay | Shorter duration of stay | P-value |
| Day 1                                            | 116.07          | 28.48           | 0.001           | 59.0            | 48.82           | 0.548           |
| Day 3                                            | 130.88          | 65.50           | 0.015           | 50.15           | 35.83           | 0.322           |
| Day 4                                            | 146.55          | 102.23          | 0.057           | 94.20           | 70.84           | 0.129           |

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96 hours the concentration was 800 pg/mL. In the present study, the levels of TNFα were found to be 55.42 ± 9.04 on day one, while on day three and four they were 85.26 ± 8.6 pg/mL and 114.16 ± 11.58 pg/mL respectively. The values show a similar trend though the levels were lower, probably due to the fact that Jones and Cayabyab had included more sick babies (ventilated). In addition, they had done the study on bronchoalveolar lavage, while in the present study markers were assayed in tracheal aspirate. The pattern of rise and fall of IL-1β in this study was closely comparable with Jones and Cayabyab. In the present study the levels of both TNFα and IL-1β were higher in group A (control) than the other two groups, although this difference was statistically significant only for TNFα. This may be due to the effect of steroids, which blunted the inflammatory response, thus showing their efficacy. There is no significant difference in the values of the inflammatory markers in the nebulized steroid group versus the systemic steroid group indicating that route of administration has no bearing on their effect.

On correlating the levels of inflammatory markers (TNFα and IL-1β) with the duration of stay of patients in the hospital and it was observed that babies who had higher levels of TNFα had a more protracted course and a longer stay in the hospital. On the other hand the babies with lower levels of TNFα had a shorter stay. This association was observed to be statistically significant. The levels of IL-1β were also high in the babies with a longer stay as compared to babies with a shorter stay. However, this association was statistically not significant. Hence it is inferred that inflammatory marker (TNFα) could help in predicting the course of the disease in patients with meconium aspiration syndrome.

Conclusion

The levels of TNFα in tracheal aspirates of newborns with meconium aspiration syndrome are suppressed with use of steroids. The levels of TNF α can also be used to assess the prognosis of neonates with MAS. The level of IL-1α does not show any significant correlation and it does not appear to be affected by steroids.

References


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