Original contribution

Activin βA in term placenta and its correlation with placental inflammation in parturients having epidural or systemic meperidine analgesia: a randomized study

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Abstract

Study Objective: To investigate the immunohistochemical localization of βA subunit of activin A in human term placenta, as a marker for placental infection/inflammation and elevated temperature, in parturients laboring during two analgesic regimens.

Design: Prospective, randomized controlled study.

Setting: Delivery room.

Patients: 56 healthy, ASA physical status I and II primiparous women in labor.

Interventions: Parturients were assigned to receive patient-controlled epidural analgesia (PCEA) with 0.2% ropivacaine or patient-controlled intravenous analgesia (PCA) with meperidine.

Measurements: Histologic and immunohistochemical placental evaluation for white blood cell infiltration and activin βA staining were made. Maternal temperature elevation above 37.6°C and leukocytosis above 15000/µL were recorded.

Main Results: Temperature was not significantly increased in parturients receiving PCEA over those who received PCA with meperidine (31% vs 11%, respectively; P = 0.1). There was also no association between temperature elevation during epidural analgesia and increased white blood cell count (>15 000/µL) or presence of polymorphonuclear and/or lymphocyte aggregation in the placenta.

Keywords:
Activin βA, analgesia; Epidural, obstetrical; Chorioamnionitis; Intrapartum fever; Neonatal sepsis; Placenta

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1. Introduction

Intrapartum maternal hyperthermia, which may indicate an underlying placental infection, is felt to be related to epidural analgesia [1-5]. However, epidemiologic studies have suggested that the increase in temperature was mostly related to noninfectious causes [6,7]. Other researchers have attributed its occurrence to altered thermoregulation [8-10]. Recently, maternal serum interleukin 6 (IL-6) was found to be increased in febrile parturients. However, only 31% of those having fever had evidence of placental inflammation [7].

Activin A is a dimeric glycoprotein that belongs to the transforming growth factor β family. It has been identified in various tissues of the reproductive system and has diverse physiologic activities that include both endocrine and paracrine functions, as well as effects on cellular growth, cellular differentiation, and apoptosis [11]. Activin A is a competitive antagonist of IL-6 [12]. The definite role of activin A in the inflammatory process has yet to be determined [13]. It is abundantly expressed in nasal polyposis associated with chronic infection, which is not associated with fever [13]. In animal models, blood levels of activin are increased in response to lipopolysaccharide administration [14]. However, to date, the occurrence of activin βA deposition with placental inflammation has yet to be identified.

The purpose of this study was to determine whether increased intrapartum temperature in parturients laboring during epidural analgesia was associated with placental activin A expression or placental infection. Our hypothesis was that intrapartum temperature elevation is associated with placental inflammation and increased placental staining for activin βA.

2. Materials and methods

The Edith Wolfson Medical Center’s Institutional Research Committee approved the study, and before labor, each participating patient gave her written, informed consent to participate in the study. Between February and September 2003, 60 ASA physical status I and II, primiparous women in spontaneous labor, with singleton cephalic presentation at term, were prospectively enrolled in the study. Parturients were randomly assigned to receive either patient-controlled epidural analgesia (PCEA; with the IVAC PCAM syringe pump model P 500, Alaris Medical Systems, Inc, San Diego, CA) with 0.2% ropivacaine or patient-controlled intravenous analgesia (PCA) with meperidine 10 mg per bolus and 10-minute lockout interval administered with an identical PCAM syringe pump (Alaris Medical Systems, Inc). Randomization was based on computer-generated codes, maintained in sequentially numbered opaque envelopes until just before use.

With a total sample size of 50 (~25 per group), the present study was designed to have 90% power to detect a true 80% relative difference in presence of activin staining in subjects with and without fever. Specifically, and based on a previous study [13], it was assumed that positive staining for activin βA subunit in the placenta in the group with fever would be not less than 50%, whereas in the group without fever, staining would not exceed 10%. A two-sided α of 0.05 was assumed.

Epidural analgesia was initiated at first request for pain relief by an attending anesthesiologist, according to the written protocol. First, a bolus of 500 mL intravenous (IV) 0.9% saline solution was administered, and an epidural catheter was placed at L3 through L4 or L2 through L3 using an 18-gauge Tuohy needle. After a test dose of two mL of 2% lidocaine, analgesia was achieved with 4 mL increments of 0.2% ropivacaine up to a bilateral T9 through T10 sensory level. Analgesia was maintained with PCEA of 5 mL/hr, 0.2% ropivacaine basal infusion rate, and 5 mL per bolus PCA with the same solution, with a lockout interval of 20 minutes (total of 20-mL/h limit). For patients requesting additional analgesia, a 10-mL bolus of the study solution was given in 5-mL increments using the PCEA device. This bolus was included in the 20-mL/h dose limit. Patients who failed to achieve a Visual Analog Scale (0-100) score of 30 or lower with meperidine and required crossover to epidural analgesia, were excluded from the study. Treatment regimen was blinded to the patients using a “dummy” IV saline infusion in parturients treated with PCEA or a “dummy” epidural PCEA in patients receiving meperidine. Also excluded from the study were women who entered labor with a body temperature higher than 37.4°C and those parturients who required cesarean delivery.

A more detailed description of the methodology of epidural versus PCA with meperidine administration has been published previously [2]. Our labor management protocol included amniotomy in active labor, when the fetal head is engaged and attached to the uterine cervix. Internal
electronic fetal monitoring was applied in women with fetal heart rate (FHR) decelerations. Pelvic examination was performed every two hours during the first stage of labor and every 30 minutes during the second stage. Oxytocin augmentation of labor contractions was employed whenever the rate of cervical dilatation was below one cm/h.

Maternal tympanic (core) temperature was measured hourly during the course of labor using the Mon-a-therm thermometer with a thermocouple temperature probe and Tab connector (both products of Mallinckrodt Medical Inc, St Louis, MO). Intrapartum temperature elevation was defined as a maternal temperature of 37.6°C or greater during the course of labor, which is the most conservative possible definition of fever. Maternal fever is usually defined as a temperature ≥38°C. Patients having a temperature ≥38°C on two consecutive measurements (one hour apart) were administered paracetamol 0.5 g orally and cefoperazone sodium one g IV after blood cultures were obtained.

Other recorded obstetrical characteristics included duration of rupture of amniotic membranes, duration of first and second stages of labor, duration of analgesia, number of vaginal examinations, and white blood cell count at the end of the first stage of labor. Fetal outcome was evaluated by Apgar score, umbilical arterial pH, and the need for neonatal sepsis evaluation when maternal temperature equaled or exceeded 38°C (or 100.4°F).

### 2.2. Histologic evaluation

The histologic grading system suggested by Salafia et al [15], which includes 4 grades of inflammation for amnion, chorion, decidua, and umbilical cord, was used. A grade of 2 or higher was considered to be a marker for significant placental inflammation. Grade 2 is characterized by multiple foci of 5 or more polymorphonuclear (PMN) leukocytes or one focus of 5 to 20 PMN in the subchorionic fibrin. Activin bA staining was graded from 0 to +3, as reported previously [13], where 0 denoted the lowest grade of staining, and +3, the highest grade of staining. Only grades +2 or +3 were considered positive staining.

Histologic analyses were conducted twice in a blinded fashion by an independent investigator who was unaware of the patient’s temperature or analgesia.

### 2.3. Immunohistochemistry

Paraffin-embedded sections were deparaffinized in xylene and rehydrated in graded alcohol (100%, 95%, 80%, and 60%). The immunohistochemical procedure was performed with the ABC kit (Novocastra; Newcastle–upon–Tyne, UK), allowing detection of the antigen antibody reaction with a biotin avidin amplification and a peroxidase

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**Table 1** Patient demographics and obstetric and neonatal characteristics with the two types of analgesia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Epidural group a (n = 29)</th>
<th>Meperidine group a (n = 27)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.7 ± 4.7</td>
<td>26.4 ± 4.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163 ± 5</td>
<td>162 ± 6</td>
<td>0.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77 ± 12</td>
<td>76 ± 13</td>
<td>0.5</td>
</tr>
<tr>
<td>Cigarette smoking (%)</td>
<td>10</td>
<td>9</td>
<td>0.4</td>
</tr>
<tr>
<td>Gestation (wk)</td>
<td>39.6 ± 1.7</td>
<td>39.7 ± 2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Cervical dilatation at epidural application (cm)</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>0.5</td>
</tr>
<tr>
<td>Duration of membrane rupture (min)</td>
<td>346 ± 267</td>
<td>407 ± 383</td>
<td>0.5</td>
</tr>
<tr>
<td>Oxytocin augmentation (%)</td>
<td>33</td>
<td>29</td>
<td>0.5</td>
</tr>
<tr>
<td>Duration of labor (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First stage</td>
<td>259 ± 41</td>
<td>255 ± 113</td>
<td>0.9</td>
</tr>
<tr>
<td>Second stage</td>
<td>53 ± 56</td>
<td>65 ± 75</td>
<td>0.5</td>
</tr>
<tr>
<td>Duration of epidural or meperidine treatment (min)</td>
<td>306 ± 171</td>
<td>221 ± 137</td>
<td>0.07</td>
</tr>
<tr>
<td>Increased intrapartum temperature (≥37.6°C) (%)</td>
<td>9(31)</td>
<td>3(11.1)</td>
<td>0.1</td>
</tr>
<tr>
<td>Increased intrapartum temperature (≥38°C) (%)</td>
<td>7(24)</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>Increased white blood cell count (&gt;15 000/μL) during labor (%)</td>
<td>10(34.5)</td>
<td>9(33.3)</td>
<td>1.0</td>
</tr>
<tr>
<td>No. of vaginal examinations</td>
<td>9.5 ± 4.3</td>
<td>8.9 ± 3.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Intrauterine pressure monitoring (%)</td>
<td>5</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>Fetal weight (g)</td>
<td>3167 ± 613</td>
<td>3107 ± 491</td>
<td>0.7</td>
</tr>
<tr>
<td>Apgar score (one min)</td>
<td>8.6 ± 0.04</td>
<td>8.7 ± 0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Apgar score (5 min)</td>
<td>9.8 ± 0.06</td>
<td>9.9 ± 0.02</td>
<td>0.2</td>
</tr>
<tr>
<td>Umbilical arterial blood pH</td>
<td>7.35 ± 4.6</td>
<td>7.31 ± 6.6</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a Results are expressed as means ± SD or percentages.

b Samples for white blood cell count were collected at the end of the first stage of labor.
detection system using the substrate diaminobenzidine tetrahydrochloride (code no. D-5905; Sigma, Rehovot, Israel) for the brown color or the chromogen aminoethyl carbazole (code no. 00-1111; Zymed, San Francisco, CA) for the red color. Different primary antibodies were used: a monoclonal antibody (MAb; mouse IgG2b) antihuman α subunit of activin (diluted 1:50, code no. MCA950S [Serotec; Oxford, UK]; MAb [mouse IgG2a]); antihuman subunit of activin (diluted 1:10, code no. MCA951S, Serotec); mouse control (diluted 1:50, code no. MCA691, Serotec); MAb mouse anti–smooth muscle actin, ready to use (code no. 08-0106 [Zymed Laboratories, San Francisco, CA]); rabbit antihuman fibronectin (diluted 1:200, product no. F3648 [Sigma]); mouse antihuman B cells, clone L-26 (Zymed); and MAb mouse antihuman T cells (UCHL1; Diagnostic Products, Los Angeles, CA) [13].

Tissue sections from patients without the first antibody were used as controls for each group studied.

2.4. Data analysis

Analysis of data was carried out using SPSS statistical analysis software (SPSS, Inc, Chicago, IL). For continuous variables such as age and laboratory parameters, descriptive statistics were calculated and reported as means ± SD. Normalcy of distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Normally distributed continuous variables were compared by group and the presence of fever using the t test for independent samples. Continuous variables with distributions deviating significantly from normal were compared by group and presence of fever using the Mann-Whitney U test. Categorical variables such as the presence of specific medical conditions were described using frequency distributions. The $\chi^2$ test was used to detect differences in categorical variables by group (positive placental findings) and the presence of fever. Leukocytosis was modeled using logistic regression analysis with a backward, stepwise approach, and odds ratios with 95% confidence intervals were estimated. All tests were two-sided and considered significant at $P \leq 0.05$.

3. Results

After 4 exclusions (three cesarean deliveries performed for nonreassuring FHRs and one parturient in the meperidine group who demanded epidural analgesia), the final study group included 56 parturients. Of these, 29 received epidural analgesia, and 27, PCA with meperidine. Comparison of obstetrical and neonatal characteristics between the two groups is presented in Table 1. There were no differences between the groups in regard to these variables. Parturients who received epidural analgesia did not have a significantly increased intrapartum temperature or an
increase in white blood cell count. Other variables that did not differ between the two groups were duration of labor, duration of rupture of membranes, and number of vaginal examinations. A total of 44 parturients from both groups had temperature below 37.6°C; 12 had 37.6°C or higher; and of these 12, 7 had temperature above 38°C (Table 2). Obstetrical variables such as duration of labor, duration of epidural, number of vaginal examinations, and increase in white blood cell counts did not differ between the two groups with different temperature (≥37.6°C vs <37.6°C). Neonatal characteristics were also similar between the groups. There was no significant difference between the two groups in umbilical artery pH values (Tables 1 and 2). None of the neonates had evidence of severe acidemia (pH <7.00). The correlation between maternal temperature changes and placental finding is shown in Table 3. Intrapartum temperature increase was not significantly correlated with increased white blood cell count or with placental histopathological finding, suggesting a placental inflammatory process (ie, localization of leukocytes or lymphocyte infiltration). We did not observe such a correlation, even in the 7 parturients who received epidural analgesia and developed temperature exceeding 38°C, nor were signs of clinical chorioamnionitis found in this subgroup.

In both study groups, some patients, including the 7 with epidural analgesia who developed fever exceeding 38°C, had negative activin βA staining (Fig. 1). Other patients with or without increased body temperature had positive activin βA subunit staining (Fig. 2), localized in the syncitiotrophoblast, cytotrophoblast, and placental vascular endothelium. There was no correlation between degree of staining and maternal temperature, nor with leukocyte or lymphocyte infiltration of the placenta.

4. Discussion

In the present study, increased maternal temperature during term labor was not significantly associated with placental localization of βA subunit of activin A or with leukocyte or lymphocyte infiltration. The reason for fever during labor is controversial. Epidural analgesia for labor is associated with mild increases in temperature [5,8,9,16] or with overt maternal fever [17,18]. However, many of these reports were based on observational, uncontrolled studies. A clinical consequence of this elevated temperature is that women having epidurals for labor are given antibiotics more frequently. In addition, the neonates are more commonly treated for sepsis [3,17]. The correlation between epidural analgesia and intrapartum fever was confirmed in a prospective, randomized trial by Philip et al [3]. They also found that nulliparity and dysfunctional labor were significant cofactors in the development of the fever attributed to labor epidural analgesia. So far, there is no evidence for a specific cause that might provoke the epidural-related hyperthermia. Some research groups claim that administration of the epidural itself may be the cause of hyperthermia [3,17,19]. We found an insignificant difference in intrapartum temperature in both of our study groups. One explanation for this finding is the relatively short duration of labor (5.3-5.9 hrs) in our patients, as compared with labors longer than 5 hours and associated with fever [17]. The difference in labor duration may be related to active obstetrical management strategy, that is, deliberate rupture of amniotic membranes and/or oxytocin administration. Other risk factors that might have contributed to the increase in fever during labor were similar in both study groups (Table 1). Similar results were observed when comparing the 44 patients who had a temperature below 37.6°C with those 12 parturients (11%) who had a temperature of 37.6°C or higher, including the 7 who had a temperature above 38°C (Table 2).

Maternal fever during labor is defined as a temperature of 38°C (or 100.4°F) or higher, and it occurs in up to 7% of term births [18]. Intrapartum fever is an important criterion for diagnosing clinical placental inflammation. Approximately 60% of clinical placental infections show histologic evidence of placental infection [7]. Fifty percent of parturients at term with histologic chorioamnionitis demonstrate a positive microbial blood culture [20]. Intrapartum maternal fever is a marker for neonatal group
B streptococcal infection [21]. Intrapartum maternal fever can be a nonspecific indicator of maternal or neonatal infection [22].

Dashe et al [23] showed that epidural analgesia is associated with fever during labor only in the presence of placental inflammation. Studies exploring the use of adjunct markers to improve understanding of the processes of inflammation, infection, and fever during labor have focused on preterm deliveries. Smulian et al [7] examined maternal serum and umbilical blood levels of IL-6 (one of the major circulating pyrogens contributing to the development of fever that directly acts on the brain and enters it by active transport). They also performed histologic placental analysis in term parturients and found that elevated IL-6 was strongly associated with maternal fever. However, only 30% of their parturients had histologic evidence of placental inflammation [7]. This finding may suggest that maternal intrapartum fever is caused largely by noninfectious factors. In another prospective, randomized trial [24], elevated maternal and fetal serum IL-6 levels were associated with fever in the absence of neonatal infection, thus suggesting that the maternal compartment is the primary origin of inflammation. In still another study [25], regional anesthesia, duration of labor, and exogenous prostaglandin administration were able to modulate the peripartum IL-6 response and, subsequently, the physiological effects of this cytokine.

Our study did not show any association between intrapartum fever and placental evidence of inflammation. No leukocyte or lymphocyte infiltration was found in the placenta of patients who developed fever or in those who had no fever. Maternal fever is usually defined as temperature above 38°C and is associated with epidural analgesia in labors extending longer than 5 hours. Our definition of intrapartum fever was set at 37.6°C or higher, which is the most conservative possible definition of fever, as the duration of the first labor in our delivery ward is usually less than 5 hours. However, this setting cannot explain our negative results in regard to placental inflammation, including our 7 parturients in the epidural group who had temperatures above 38°C to 38.5°C. The lack of association between intrapartum fever and histologic evidence of placental inflammation has been documented previously [7,18,23] and may imply that nonspecific, noninfectious causes might be responsible for intrapartum fever that develops in parturients laboring during epidural analgesia.

Another limitation of our study is the use of maternal leukocytosis above 15000/µL as a marker of infection. It is well known that the leukocyte count may increase during pregnancy to levels as high as 18000/µL and even more during and after labor. This high leukocyte level is associated presumably with stress and does not necessarily represent true infection.

Considering that intrapartum fever during epidural analgesia may be associated with placental inflammation [23], we sought to investigate placental cellular localization of the immunoreactive activin βA subunit in placental tissue of term parturients with and without intrapartum temperature elevation and to correlate its presence and localization with possible evidence of placental inflammation. Activin A is secreted by the fetoplacental unit and reaches its highest levels at term [26,27]. As activin A levels are not normally altered throughout spontaneous or induced labor, it was presumed that activin A may not play an active role in initiation or regulation of human parturition [28]. Localization of activin βA subunit in human placental cells shows positive staining in both syncytiotrophoblastic and cytotrophoblastic cells at all gestational ages. It was suggested that placental cells serve as a source of activin βA subunit and that they may play the role of an endocrine/paracrine regulator of fetomaternal interaction during pregnancy [29]. To date, the association between activin βA localization in the placenta at term and any possible placental inflammation (ie, chorioamnionitis) has not been investigated.

Our results show activin βA localization in the syncytiotrophoblast, cytotrophoblast and vascular endothelium. However, such localization was comparable in women with or without temperature elevation. In our cases, amniotic membranes were not stained for activin. This finding is in agreement with previous data reported in normal term placentas [29].

Another limitation of our study was the systemic use of meperidine, an antishivering agent used in the control group. The antishivering effect of meperidine may have prevented the increase in temperature, but it did not inhibit activin or PMN cell expression in the placenta in case of true inflammation.

In this study, there was no significant differences between the two analgesic techniques with regard to maternal temperature elevation. This finding may suggest that the increase in temperature during labor may not be attributed to epidural analgesia. Intrapartum temperature elevation also was not associated with histologic signs of placental inflammation or with expression of activin βA in the placenta. It is suggested that mechanisms other than epidural administration may be involved in the etiology of temperature elevation during labor.

References


