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The Effects of Colloid Pre-loading on Thromboelastography Prior to Caesarean Delivery: Hydroxyethyl Starch 130/0.4 versus Succinylated Gelatine

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This prospective, randomized, double-blind study compared the effects on thromboelastography (TEG) of pre-loading with two different colloid fluids prior to spinal anaesthesia for caesarean section. Healthy full-term parturients received either 500 ml 6% hydroxyethyl starch 130/0.4 (HES, *n* = 25) or 500 ml 4% succinylated gelatine (GEL, *n* = 25) prior to spinal anaesthesia. TEG parameters including reaction time (r-time), clot formation time (k-time), clot formation rate (α-angle) and maximum amplitude (MA) were measured immediately before and after pre-loading. Both groups had significantly shorter r-time and lower MA after pre-loading. The α-angle was significantly decreased after pre-loading with HES but not with GEL. No significant differences in k-time were induced pre-loading. In conclusion, pre-loading with HES or GEL was associated with a mild hypocoagulable effect in healthy parturients presenting for elective caesarean section; however, all TEG parameters in both groups remained within or very close to the normal range after pre-loading.

KEY WORDS: Colloid pre-loading; Thromboelastography; Caesarean section; Spinal anaesthesia

Introduction

Neuraxial anaesthesia is a popular choice for obstetric surgery. Side-effects of neuraxial anaesthesia include hypotension and bradycardia,[1,2] which may result in nausea, vomiting and, more importantly, fetal acidosis due to reduced uteroplacental blood flow.[3 – 5] Fluid pre-loading is advocated to reduce the incidence and severity of hypotension following neuroaxial anaesthesia, with colloids considered to be more effective than crystalloids;[6,7] however, this procedure may affect direct or indirect coagulation pathways.[8,9]

Screening tests allow interpretation of several phases of the coagulation cascade. Thromboelastography (TEG) is a real-time monitor of whole-blood coagulation that measures the viscoelastic properties of blood, as well as coagulation factors and platelet activity, from a single blood sample. TEG is more reliable and provides more comprehensive information than other coagulation tests.[10 – 12]

Although pregnancy is associated with hypercoagulability, little is known about the effects of fluid pre-loading on coagulation in pregnant patients. The aim of the present study was to evaluate the effect on coagulation of fluid pre-loading with either 6% hydroxyethyl starch (HES) or 4% succinylated gelatine (GEL), prior to spinal anaesthesia.

Subjects and methods

SUBJECTS AND INCLUSION CRITERIA

In this prospective, randomized, double-blind trial, pregnant women at term (37 – 41 weeks' gestation) who were American Society of Anesthesiologists physical status I – II (i.e. normal healthy patients or patients with mild systemic disease) and were scheduled to undergo elective caesarean section at the Uludag University Teaching Hospital, Bursa, Turkey, between May 2008 and January 2010, were enrolled sequentially into the study. Women with significant comorbidities (including liver disease, hypertension, diabetes mellitus, pre-eclampsia or pre-existing coagulation disorders) were excluded, as were those receiving aspirin or anticoagulant therapy.

The study protocol was approved by the Ethics Committee of Uludag University, Bursa, Turkey and written informed consent was obtained from all participants.

STUDY TREATMENT AND TEG ANALYSIS

Randomization was carried out using a computer-generated schedule. Each subject was assigned to receive pre-loading with 500 ml of either 6% HES 130/0.4 (Voluven®; Fresenius, Bad Homburg, Germany) (HES group), or 4% GEL (Gelofusine®; B. Braun Melsungen AG, Melsungen, Germany) (GEL group).

Haematological tests (haemoglobin level, platelet count, prothrombin time, international normalized ratio and activated prothrombin time) were performed for all patients prior to fluid pre-loading. Subjects were not pre-medicated. All subjects were monitored using non-invasive blood pressure monitoring, pulse oximetry and electrocardiography. An 18-gauge intravenous cannula was inserted into a forearm vein. The initial 3 ml of blood was discarded to avoid tissue contamination, and a further 1-ml blood sample was obtained for analysis of baseline TEG values. Fluid pre-loading was performed over 20 min through a 20-gauge intravenous cannula inserted into a hand vein. A second blood sample was then obtained from the forearm cannula, using the sampling technique previously described.

The TEG analysis was performed by another anaesthesiologist (T.Y.) who was blinded to patient randomization. Within 3 min of blood sampling, 1 ml of whole blood was placed into a vial containing 1% kaolin. After mixing by inversion seven to 10 times, 360 µl of kaolin-activated blood was put into a plastic cup in a pre-warmed TEG analyser (37 °C). Standard TEG parameters were recorded using a Thrombelastograph® 5000 analyser (Haemoscope Corp., Niles, IL, USA). The reaction time (r-time) was defined as the time from the start of recording until initial fibrin formation and represented the function of clotting factors. The k-time was defined as the time from r-time until a fixed level of clot firmness and reflected the dynamics of clot formation. The α-angle was defined as the slope of the TEG trace from r to k and represented the rate of fibrin build-up and cross-linking. The maximum amplitude (MA) reflected the ultimate strength of the fibrin clot.

After the second blood sample was obtained, spinal anaesthesia was performed

with the patient in the left lateral decubitus position, and a 27-gauge Quincke needle (Spinocan®; B. Braun Melsungen AG) was inserted at the L3 – L4 or L4 – L5 intervertebral space. A mixture containing 8 mg hyperbaric bupivacaine and 20 µg fentanyl (2 ml total volume) was injected into the subarachnoid space. The patient was then shifted to the supine position for the remainder of the procedure. All spinal needles were inserted by the same anaesthesiologist (G.T.) who was blinded to the patient randomization. Sensory blockade was evaluated using the pinprick test with a hypodermic 20-gauge needle, and dermatomal levels were tested every 60 s on the midline until the level stabilized (defined as the same result in four consecutive tests). Heart rate (HR), systolic and diastolic arterial blood pressures (SAP and DAP, respectively) and peripheral oxygen saturation (SpO_2) were measured and recorded at 5-min intervals, intraoperatively. Bradycardia (HR < 50 beats/min) or intraoperative hypotension (SAP < 20% of baseline) were recorded and treated with fluid bolus or intravenous ephedrine or atropine. Apgar scores for newborns were recorded at 1 and 5 min after delivery. The Apgar score comprises 5 components, each of which is given a score of 0, 1, or 2: HR; respiratory effort; muscle tone; reflex irritability; and colour.

STATISTICAL ANALYSES

Data are presented as mean ± SD or median (range). Statistical analyses were performed using the SPSS® statistical package, version 13.0 (SPPS Inc., Chicago, IL, USA) for Windows®. A group size of 25 was calculated, based on 90% power, to be able to detect a 30% difference in r-time between study groups. Demographic and intraoperative data were analysed using Student's *t*-test. TEG parameters before and after pre-loading for each group were assessed using Wilcoxon's signed-rank test. The independent *t*-test or Mann–Whitney *U*-test was used, where appropriate, for intergroup comparisons of changes in TEG variables. Nominal non-parametric data were analysed using Fisher's exact test. A *P*-value < 0.05 was considered to be statistically significant.

Results

Fifty pregnant women at full-term were recruited into the study: 25 were randomized to each of the HES or GEL groups, and all subjects were included in the analyses. The two groups were comparable with respect to age, weight, height, gestational age, haemoglobin levels, platelet counts and coagulation tests (Table 1). No subject had any pre-operative coagulation abnormalities or thrombocytopoenia.

There were no significant differences between the groups with respect to intraoperative findings for HR, SAP, DAP or SpO_2 (data not shown). Analysis showed no significant differences between the two groups with regard to the highest level of sensory block, total crystalloid volume infused, number of subjects who developed hypotension, total ephedrine dose used and neonatal Apgar scores (Table 2). No subject developed bradycardia during surgery.

There were significant decreases in r-time in both groups after pre-loading ($P < 0.001$ for both groups; Table 3). There was a significant decrease in α-angle with HES, but not GEL ($P < 0.05$; Table 3). MA was significantly decreased after pre-loading in both groups ($P < 0.01$), but no differences in k-time were observed (Table 3). The changes in TEG parameters after colloid pre-loading, although significant, remained within the normal range or just below the lower limit of the normal range (for r-time) in both groups,[13] and there

TABLE 1:
Demographic characteristics and pre-operative haematological results for parturients receiving either 6% hydroxyethyl starch 130/0.4 (HES) or 4% succinylated gelatine (GEL) before spinal anaesthesia for caesarean section

	HES (n = 25)	GEL (n = 25)
Age, years	29 ± 4	29 ± 4
Weight, kg	71 ± 13	74 ± 13
Height, cm	162 ± 4	161 ± 5
Gestational age, weeks	38 ± 0.9	38 ± 0.9
Pre-operative haematological tests		
Haemoglobin, g/dl	11.9 ± 1.7	11.7 ± 1.4
Platelet count, × 10^3/mm^3	282 ± 77	266 ± 70
Prothrombin time, s	12.9 ± 1.4	12.7 ± 1.6
Activated prothrombin time, s	25.2 ± 3.0	24.6 ± 4.0
International normalized ratio	1.2 ± 0.4	1.1 ± 0.3

Data presented as mean ± SD.
No statistically significant between-group differences ($P > 0.05$); Student's t-test.

TABLE 2:
Intraoperative outcomes for parturients receiving either 6% hydroxyethyl starch 130/0.4 (HES) or 4% succinylated gelatine (GEL) before spinal anaesthesia for caesarean section

	HES (n = 25)	GEL (n = 25)
Highest level of sensory block, thoracic	5 (4 – 6)	5 (3 – 6)
Total crystalloid fluid infused, ml	1188 ± 286	1260 ± 233
Patients with hypotension	2	4
Total ephedrine used, mg	2.5 ± 4.2	4.4 ± 6.8
Newborn Apgar score		
1 min	8.1 ± 2.0	8.4 ± 1.1
5 min	9.6 ± 0.8	9.4 ± 0.7

Data presented as mean ± SD, median (range) or n of patients.
No statistically significant between-group differences ($P > 0.05$); Student's t-test.

were no intergroup differences in any parameter at any time (Table 3).

Discussion

The present randomized, double-blind, *in vivo* study demonstrated that both 6% HES and 4% GEL caused a significant reduction in r-time and MA in healthy pregnant women undergoing caesarean delivery under spinal anaesthesia; however, MA remained within the normal reference range.[13] No clinical signs of abnormal coagulation were observed after pre-loading in any subject. Median r-time values were just below the lower limit of the normal reference range after pre-loading. It appeared that the two colloids had similar slight effects on coagulation.

Physiological alterations during pregnancy result in increased platelet aggregation, fibrinogen concentration and coagulation factors, decreased endogenous anticoagulants (proteins C and S) and modified fibrinolytic capacity,[14] resulting in a hypercoagulable state. This is consistent with the present study, where baseline MA

TABLE 3:
Thromboelastographic data before and after colloid pre-loading in parturients receiving either 6% hydroxyethyl starch 130/0.4 (HES) or 4% succinylated gelatine (GEL) before spinal anaesthesia for caesarean section

Thrombo-elastographic measure	HES (n = 25)		GEL (n = 25)	
	Before pre-load	After pre-load	Before pre-load	After pre-load
r-time, min	6.3 (2.2 – 11.8)	3.8 (1.5 – 9.9)***	6.0 (2.3 – 10.6)	3.7 (1.9 – 9.2)***
k-time, min	1.8 (1.0 – 3.8)	1.9 (1.2 – 3.5)	1.8 (1.3 – 4.6)	1.9 (1.3 – 2.8)
α-angle, °	65.2 (34.9 – 76.4)	61.1 (44.1 – 72.5)*	60.9 (41.7 – 73.3)	59.9 (47.8 – 71.5)
MA, mm	76.1 (55.6 – 91.9)	70.5 (53.5 – 83.6)**	76.8 (56 – 82.7)	70.2 (58.4 – 81.9)**

Data presented as median (range).
*P < 0.05, ** P < 0.01, *** P < 0.001 versus before pre-load measurements (Wilcoxon's signed-rank test).
r-time, reaction time (normal range 4 – 8 min); k-time, clot formation time (normal range 1 – 4 min); α-angle, clot formation rate (normal range 47° – 74°); MA, maximum amplitude (normal range 55 – 73 mm).

values were above the upper limit of the normal reference range in both the HES and GEL groups.

Fluid pre-loading to reduce hypotension during spinal anaesthesia for caesarean delivery is now routine, and colloids may be more effective than crystalloids.[15,16] There is, however, little data on the changes in coagulation following colloid treatment in pregnant women. Previous TEG studies have shown that haemodilution of > 20% with HES affects coagulation in non-obstetric patients,[17 – 19] and that mild-to-moderate HES haemodilution decreases circulating factor VIII and von Willebrand factor, inhibiting platelet function.[20] Low molecular weight HES, with a low degree of molar substitution, has less effect on haemostasis than equivalent high molecular weight HES with higher molar substitution.[17,21] The differences between the individual pharmacokinetic features of HES preparations may therefore be important; in the present study, a low molecular weight HES with lower molar substitution (500 ml 6% HES 130/0.4) was used, which may have had less of an impact on haemostasis compared with other HES preparations.

Gelatine solutions may have fewer negative effects on coagulation than HES,[19,22,23] although not all studies agree.[8,24] These studies were conducted under *in vivo* or *in vitro* conditions in non-obstetric populations, and different preparations from those used in the present study were administered. Butwick and Carvalho[13] compared TEG changes after pre-loading with 500 ml 6% HES and 1500 ml lactated Ringer's prior to caesarean delivery. They found that r-time and k-time were significantly prolonged in the HES group, but MA values were similar, and no significant differences in TEG values were seen in the group that received lactated Ringer's. Pre-loading with 500 ml HES was associated with a mild hypocoagulable effect in healthy parturients undergoing caesarean delivery, but TEG parameters after HES pre-loading remained within a normal reference range.[13] Based on the above, a control group was not included in the present study, as it was assumed that crystalloid solutions would have no significant effect on any TEG parameters. The lack of a control group limits the usefulness of the present study. The finding that r-time and MA were decreased

after pre-loading with either HES or GEL did not agree with that of Butwick *et al*.,[13] although both studies found only minimal alterations in TEG parameters.

Ruttmann *et al*.[25] reported that mild and moderate haemodilution increased the coagulability of whole blood *in vitro*, but that saline haemodilution had a more marked effect on final clot strength than a modified gelatin colloidal solution (Haemaccel®). It is possible that this hypercoagulable state is normally seen in the perioperative period, as a result of the acute-phase response to surgery.[13] *In vivo* coagulation studies are necessary to assess potential associations between perioperative fluid regimens and clinical outcomes. The decrease in r-times observed in both the HES and GEL groups in the present study may be due to this mild-to-moderate haemodilution and pregnancy-associated hypercoagulation.

In conclusion, pregnant parturients who received fluid pre-loading with 500 ml of 6% HES 130/0.4 or 4% GEL did not experience arterial hypotension induced by spinal anaesthesia prior to undergoing elective caesarean sections. Both colloid fluids produced a mild hypocoagulable state, but TEG parameters after pre-loading remained within or just below a normal reference range. Further studies are necessary to assess the effects of other fluid pre-loading regimens on coagulation and clinical outcomes in pregnant women.

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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