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ORIGINAL ARTICLE

Evaluation of diluted amniotic fluid effects on histological changes of intestine of rabbit fetus with gastroschisis

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Abstract Amniotic fluid exchange is a method for prevention of intestinal damage in gastroschisis, but its techniques are different in studies. We investigated the effects of amnioinfusion exchange on histological changes of intestine and feasibility and safety of amniotic fluid exchange through central vein catheter (CVC) placed in pregnant rabbit uterus. A total of 15 pregnant New Zealand white rabbits were selected. Fetuses were randomly divided into three groups (case, control, sham). On gestational day 25, under general anesthesia with midline laparotomy, the graved bicornuate uterus was exposed. In controls, fetus abdomen was opened by a transverse incision in right lower quadrant region and intestines were eviscerated. In cases, after intestine evisceration, a central venous catheter was passed from mother skin and uterus and fixed to uterus wall. In shams, fetus was delivered on gestational day 32 and its abdomen was opened. In case group, after operation, 1–2 cc of warm saline solution was replaced through catheter every 6 h. On gestational day 32, fetuses of case and control groups were delivered. Mucosal and serosal thickness, muscle thickness, fibrin deposition, serosal collagen and ganglia were compared. Ten fetuses as shams, 7 fetuses as controls and 7 fetuses as case group were studied. Serosal thickness was 4.5 ± 3.6 μm in shams, 64.2 ± 28.7 μm in controls and 6 ± 4.1 μm in cases. Serosal thickness in control group was higher than sham ($P < 0.001$) and case ($P < 0.002$) groups. In case group, infiltration of inflammatory cells with mild edema without fibroblast infiltration was seen. Application of the CVC technique was found to be a simple procedure that effectively decreased serosal inflammatory response of intestine in gastroschisis.

Keywords Gastroschisis · Amniofusion · Rabbit · Fetus

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Introduction

Gastroschisis is a congenital anomaly in which the bowel protrudes through a full—thickness defect in the abdominal wall directly adjacent to the umbilicus The herniated intestine of neonates born with gastroschisis may present with different degrees of damage at birth, varying from normal-looking gut to matted edematous bowel loops [1]. Intrauterine intestinal damage remains the major cause of early morbidity in gastroschisis and failure to control damage is one of the most crucial problems in managing of these patients. In gastroschisis, bowel is usually covered with a thick fibrinous peel. Some investigators have explained the appearance of peel on the basis of gestational age [2], abdominal wall defect [3] and changes of amniotic fluid electrolyte composition [4] with the onset of fetal kidney function.

Although amniotic fluid exchange [5–7] and amnion infusion [8, 9] are among therapeutic and prophylactic strategies to prevent or limit intestinal damage, but the concept that amniotic fluid dilution prevents intestinal damage is not widely accepted. In some studies, effects of amnion infusion on intestinal damage of chick embryos were studied [5–7], but its feasibility in human is not established. In Midrio et al. [10] study, serial amnioexchange did not modify the biochemical or inflammatory

status of amniotic fluid nor appeared to prevent injury to the herniated gut. Furthermore, repeated amniotic fluid exchange is a painful technique.

In this study, we investigated amnion fluid exchange effects on histologic changes of intestine by a new method using central vein catheter placed in uterus of pregnant rabbits.

Methods

A total of 15 pregnant New Zealand white rabbits (Pasteur institute, Tehran, Iran) were selected. The rabbits were housed in a controlled environment with an ambient room temperature of 24–26 c for at least 2 days before operation. Abdominal X-ray (at 18th day of conception) and ultrasonography (at 12th day of post conception) were taken and repeated serially if necessary. In each rabbit, the number of fetus in each uterine horn was determined. On gestational day 25, after a 12 h fast, each doe was premedicated with xylazine (5 mg/kg) intramuscularly. Cefazolin sodium (100 mg/kg) and indomethacin suppository were given half an hour prior to operation. General anesthesia was induced with ketamine (35 mg/kg) and Acepromazin (1.2 mg/kg). After induction, animals were placed in the supine position throughout the experiment. After completing the surgical preparation of the mother animal, a maternal midline laparotomy was performed and the graved bicornuate uterus was exposed. Using needle-tip electrocautry a 1-cm linear hysterotomy was made through myometrium, chorion and amnion to expose the fetal abdomen. For determination of fetus position, we palpated vertebral column and head of fetus. The uterus and exposed fetus were continually moistened with warmed saline solution. Operated fetuses were randomly divided into three groups: case, control and sham groups. In sham group, fetus was delivered and its abdomen was opened, then fetus was fixed in 10% Formalin on gestational day 32. In control group, fetus abdomen was opened by a 10-mm transverse incision in right lower quadrant region and intestines were eviscerated by gentle pressure on the fetal back of abdomen. Intraoperatively, we replaced the uterus amniotic fluid with 2 ml of warmed sterile saline according to Langer et al. [11] study. Then uterus was closed with 4-0 vicryl in a running fashion. In case group, after intestine evisceration, an Arrow single-lumen central venous catheter (14 Ga) was passed from mother skin and uterus and fixed to uterus wall and skin, then uterus was closed as such as control group. Therefore the control group was gastroschisis with no amniotic fluid exchange, and case group was gastroschisis and Arrow catheter placement for amnion exchange. Maternal laparotomies were closed in three layers, does were awakened and returned to their cages. Post operatively; antibiotic and indomethacin were used at 6-h interval. Estimated volume of amniotic fluid in uterus of pregnant rabbit is 0–4 ml [12].In a preliminary study, we used 3–4 ml normal saline for fluid exchange on five rabbits, but two of them died.In autopsy, we found uterus suture line leak and perforation. Therefore we used 1–2 ml of saline according to size of uterus. In case group, after operation, 1–2 cc of warm saline solution was replaced through catheter every 6 h. Rabbits were followed and if any of delivery signs were observed (blood spotting, contractures, etc.), cesarean section was performed immediately. On gestational day 32 (normal 31 to 33-day gestation), fetuses were delivered by cesarean section. Fetuses were killed by cardiac puncture. The intestinal tract was removed and fixed in 10% Formalin. Specimens were stained with hematoxylin and eosin for histological examination. For comparison, mucous and serosal thickness, muscle thickness, fibrin deposition, serosal collagen and ganglia were evaluated. For statistical analysis, we used ANOVA and P less 0.05 was considered significant.

Results

In this study, we evaluated 10 fetuses as shams, 7 fetuses as controls and 7 fetuses as case group. Intestinal wall thickness was 285 ± 49.6 μm in shams, 319.28 ± 86.8 μm in controls and 289 ± 39.6 μm in cases (P = NS). Muscular thickness was 55 ± 13.2 μm in controls and 58 ± 16.43 μm in cases (P = Ns). Serosal thickness was 4.5 ± 3.6 μm in shams, 64.2 ± 28.7 μm in controls and 6 ± 4.1 μm in cases. Serosal thickness in control group was higher than sham ($P < 0.001$) and case ($P < 0.002$) groups. There were no significant differences in ganglion cells histology in groups. In histological evaluations (Fig. 1) infiltration of fibroblasts in serosal layer was seen in control group accompanied by some degree of fibrin and collagen deposition. In case group, infiltration of inflammatory cells with mild edema without fibroblast infiltration was seen.

Discussion

Intrauterine intestinal damage remains the major cause of early morbidity in gastroschisis, and failure to control damage is one of the most crucial problems in managing these patients. It has become apparent that prolonged and extensive exposure of the serosal surface of intestine with amniotic fluid causes intestinal damage characterized by bowel wall thickening, intestinal dilation, mesenteric shortening and fibrous peel formation [14, 15]. Macroscopic changes of the exposed intestine were attributed to the changes of amniotic fluid composition such as

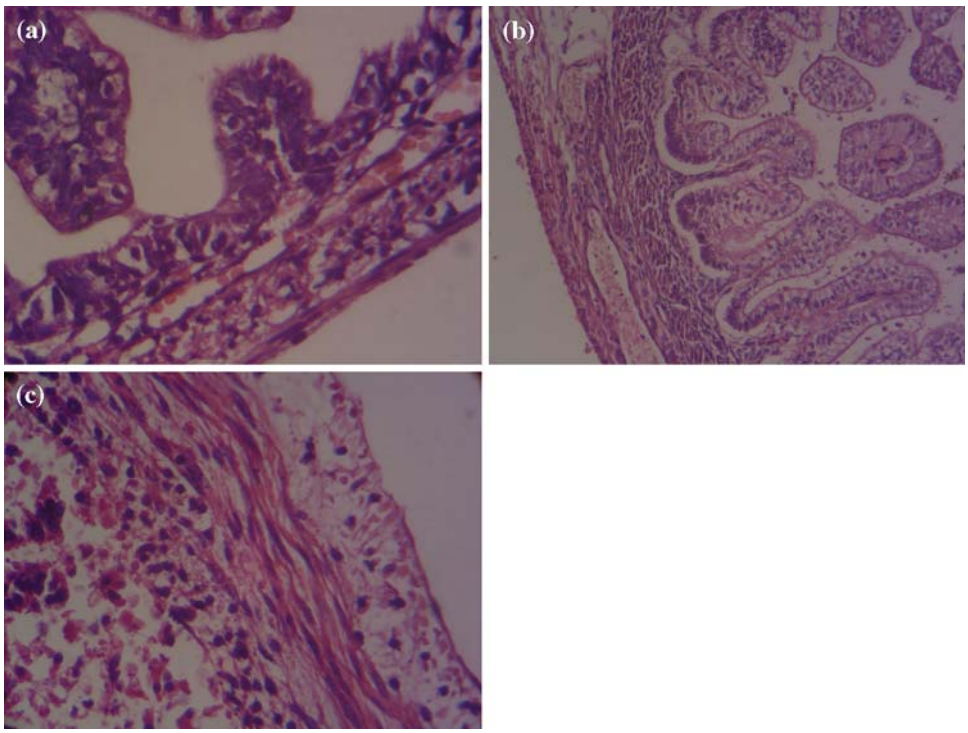

Fig. 1 Histological findings in **a** shams, **b** controls, **c** cases

increasing of urinary or gastrointestinal waste products [7, 13]. Therefore, some studies were performed antenatally in an effort to prevent intestinal damage [5–7]. Amniotic fluid exchange and amniotic infusions are among such therapies.

Experiences with amniotic fluid exchange on human with gastroschisis are limited. In a recent study by Midrio et al. [10], serial amnioexchange did not modify the status of amniotic fluid nor appeared to prevent injury to the herniated gut. Burc et al. [4] showed that infusion of as much as 300 ml repeated 2–3 times during each amniotic fluid exchange, for a total of 600–900 ml was safe in human and lack of efficacy of amniotic fluid exchange in Midrio study [10] might be related to the low amount of fluid infused in their patients. Sencan et al. [1] evaluated amnio-allantoic fluid exchange on bowel contractility in chick embryos with gastroschisis.The authors found that gastroschisis did not affect intestinal ganglia morphology, but the number of ganglion cells decreased. Dean et al. [14] stated that amniotic fluid injection into the peritoneal cavity of fetal rabbits did not produce intestinal changes. However this article was just a case series and in their report five cases of gastroschisis had no intestinal peel. Dommergues et al. [15] showed that serial transabdominal amnioinfusion is effective for management of gastroschisis with sever oligohydramnious. This findings are also showed in other studies [16–18], however in Sapin et al. [17] study the neonates were born prematurely and it is known that intestinal damage develops after gestational age 30. In Turkota study [18] cases had only one amniotic fluid exchange.

Amniotic fluid exchange is performed by repeated transabdominal amniocentesis. However the need for repeated needle passing through skin is one of the disadvantages of this procedure. Furthermore, such method may give arise to intraabdominal or intrauterine infection.

In our study, we had two purposes. The primary goal of this study was to evaluate the histological changes of intestine of fetus with gastroschisis treated by amniotic fluid exchange. Our results showed decreased serosal thickening, decreased inflammatory infiltrate and no collagen/fibroblast deposition in case group versus controls.

We also showed feasibility and safety of central vein catheter placement in uterus for amniotic fluid exchange. To our knowledge, this is the first animal study with gastroschisis in which the benefit of amniotic fluid exchange through catheter placement was investigated. Our findings demonstrated that it is possible and safe to instill 1–2 ml of saline into the uterus prenatally through catheter. This technique is remarkably easy but requires special training. However there are some cautions in this method. Catheter placement can cause uterus contraction and preterm delivery; therefore tocolysis should be performed with considerable attention.

In our first operations, we have two death that were related to excessive fluid exchange, but in other operations ,it was showed that 1–2 ml of fluid exchange per 6 h can prevent intestinal damage effectively.

In summary, our experiences showed that the application of central venous catheter technique for amniotic fluid

exchange was a simple and safe method that effectively decreased serosal inflammatory response in gastroschisis.

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