PHYSIOLOGY

Maternal Serum C-Reactive Protein Level Does Not Change Significantly After Fetal Reduction: It Could Be Used as an Indicator of Chorioamnionitis

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Purpose: This study was aimed at investigating the diagnostic value of maternal serum C-reactive protein (CRP) in the recognition of chorioamnionitis in patients undergoing fetal reduction.

Methods: Seventy-one gravidas with high-order multifetal pregnancies, including 46 with triplets, 18 with quadruplets, and 7 with quintuplets, who underwent transabdominal fetal reduction to twins during the 10th–14th gestational week were recruited. The subjects were followed up clinically and ultrasonographically 1 week and 1 month after fetal reduction for signs of infection, premature uterine contraction, and premature rupture of the membranes. CRP levels were measured prior to fetal reduction and at follow-up examinations, and were compared.

Results: Among the 71 mothers, 65 (92%) were normal after fetal reduction. The CRP levels were not significantly different prior to the procedure (0.27 ± 0.26 mg/dL), and 1 week (0.23 ± 0.24 mg/dL) and 1 month (0.24 ± 0.20 mg/dL) later. There was no correlation between the number of fetuses reduced and the CRP levels. Six (8%) experienced leakage of amniotic fluid after fetal reduction. Three patients had normal CRP levels at that time and at the following tests. The pregnancies continued smoothly after conservative treatment. The other three patients had elevated CRP levels when leakage of amniotic fluid occurred. Fever and uterine

irritability developed subsequently despite parenteral antibiotics and tocolytic therapy. Daily checks showed increasing CRP levels. The pregnancies were aborted, and the histology of the placental membranes revealed chorioamnionitis with infiltration of acute inflammatory cells.

Conclusions: The absorption of inactive gestational tissue after fetal reduction did not affect CRP levels. CRP may be used as a marker of intrauterine infection after fetal reduction.

KEY WORDS: Chorioamnionitis; C-reactive protein; fetal reduction.

INTRODUCTION

The use of ovarian stimulation alone or in combination with assisted reproductive techniques (ART) for treatment of infertility has increased the incidence of multifetal pregnancies (1). High-order multifetal pregnancy with three or more fetuses poses potential hazards to both the mother and the fetuses. Fetal reduction in the first trimester is an alternative to decrease these hazards (2,3). It has the advantages of increasing the likelihood of greater gestational age, maturity, and weight of the remaining fetuses at birth and decreases the likelihood of complications and discomforts in the mother (4). The possibility of miscarriage after fetal reduction is of great concern to mothers who have suffered long periods of infertility.

Infection has been reported as a major cause of miscarriages after fetal reduction (5). Puncturing of the gestational sacs may introduce bacteria into the uterine cavity resulting in chorioamnionitis (6). An

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early recognition of this condition is crucial for management. Serum C-reactive protein (CRP), an acute-phase protein produced by hepatocytes following infection, inflammation, tissue damage, and necrosis (7), has been widely used as an indicator of chorioamnionitis in premature rupture of the membranes (PROM) or preterm labor (8–10). However, whether CRP levels change or not after fetal reduction has not been investigated, and the clinical value of CRP levels in the detection of intrauterine infection after the procedure is not clear.

In this study, the serum CRP levels prior to fetal reduction were measured and compared 1 week and 1 month after fetal reduction. During follow-up examinations, special attention was paid to the clinical symptoms and signs of amniotic fluid leakage and intrauterine infection. The purpose of this study was to evaluate the role of CRP levels in the detection and management of chorioamnionitis after fetal reduction.

MATERIALS AND METHODS

Subjects

Between July 1996 and June 1999, 71 gravidas in the first trimester with high-order multifetal pregnancies, who had been referred to our institute for selective fetal reduction, were recruited for this study. Among these patients, 6 had conceived with ovulation induction alone, 9 with ovulation induction plus artificial insemination, 2 with gamete intrafallopian transfer, 14 with in-vitro fertilization (IVF) plus embryo transfer (ET), 23 with IVF plus tubal embryo transfer (TET), 7 with intracytoplasmic sperm injection (ICSI) plus ET, and 10 with ICSI plus TET. The mean age of the mothers was 31 years, ranging from 20 to 44. Forty-six conceptions were triplets, 18 quadruplets, and 7 quintuplets. After detailed counseling, selective termination was performed during the 10th–14th gestational week. Informed consent was obtained as approved by the Institutional Review Board.

Fetal Reduction

The procedures used in fetal reduction have been previously described in detail (4). In brief, the mother was put in a supine position, and the skin of the abdomen was disinfected with alcoholic povidone iodine. Under ultrasonographic guidance, a fine needle (gauge 22) was inserted into the fetal heart through the maternal abdominal wall. A small amount

(0.2 mL) of potassium chloride (KCl; 2 mEq/mL) was injected into the fetal cardiac chamber to induce fetal asystole. The number of living fetuses was reduced to two in all cases. No anesthesia was used for this procedure, and all of the mothers tolerated it well. After fetal reduction, each pregnancy was followed up at 1 week and 1 month for clinical and ultrasonographic examinations. Blood samples for analysis of CRP level were taken from mothers immediately prior to fetal reduction and at follow-up examinations.

CRP Measurement

Blood samples were allowed to clot, and aliquots of serum were collected. CRP was measured nephelometrically using a Beckman immunochemistry analyzer and a reagent's kit for CRP (Beckman Instruments, Fullerton, CA, USA). The intraassay coefficient of variation was $\leq 5\%$ and the interassay variation was $\leq 8\%$. A CRP value > 2.0 mg/dL was defined as abnormally elevated (8). All of the patients in this study had no recent history of viral or bacterial infections and no history of collagen diseases.

Clinical and Ultrasonographic Follow-up Examinations

The patients' temperature, pulse rate, and respiration rate were examined. Special attention was paid to symptoms of uterine contraction, vaginal bleeding, and leakage of amniotic fluid. The size and cardiac activity of the fetuses and the amount of amniotic fluid were estimated by ultrasonogram. For those patients with leakage of amniotic fluid, PROM was confirmed by positive Nitrazine test, pooling of fluid in the posterior vaginal fornix, positive ferning test, and decreased amniotic fluid pocket in the gestational sac (11). *The diagnosis of PROM was made based on the overall clinical picture.* Patients with PROM were admitted for bed rest and prophylactic oral antibiotics (Amoxycillin with Clavulanic acid; Beecham, Brentford, UK) were given. These patients were monitored daily for CRP level and white blood cell (WBC) counts, and three times daily for temperature, pulse, and fetal heart rate. Urine culture was done to detect any concurrent urinary tract infection. If amniotic fluid leakage ceased and no evidence of infection was found, the patient was discharged.

Diagnosis and Management of Chorioamnionitis

Chorioamnionitis was diagnosed in patients with PROM associated with elevated CRP levels,

increased WBC, fever, maternal and fetal tachycardia, or uterine irritability (12). The patient was treated with parenteral antibiotics (Cefoxitin; Merck, West point, PA, USA) and a tocolytic agent (Ritodrine; Duphar, Weesp, Holland). Conservative management was discontinued if infection persisted with tender uterus or foul-smelling amniotic fluid, or with advanced cervical dilatation and effacement. Labor was induced after discontinuation of conservative management.

Statistics

To test the differences of CRP levels in the groups of three different temporal time periods, statistical analysis was performed using a one-way analysis of variance with repeated measures. The between-group differences were compared using the paired *t*-tests. Values in this study were expressed as means $\pm$ SD. The difference was considered significant for $p < .05$.

RESULTS

Among the 71 mothers, 65 (92%) were normal after fetal reduction; however, six (8%) experienced leakage of amniotic fluid. In the former group, the CRP levels were not significantly different prior to the procedure (0.27 ± 0.26 mg/dL), and 1 week (0.23 ± 0.24 mg/dL) and 1 month (0.24 ± 0.20 mg/dL) later (Table I). The CRP levels at these times ranged from 0.09 to 1.5 mg/dL with a mean of 0.25 ± 0.24 . There was no correlation between the number of fetuses reduced and the CRP levels. Sixty-five mothers had successfully given birth to 64 sets of twins and 1 singleton because of an intrauterine fetal demise of twins. The mean gestational age was 36 weeks, ranging from 29 to 39.

In the latter group, the leakage of amniotic fluid happened 1–6 days after fetal reduction. The nitrazine

and ferning tests were positive for the fluid. Decreased amniotic fluid pocket in the gestational sac was detected by ultrasonogram. Urinalysis was within normal limits. WBC counts ranged from 9,600 to 14,480/ μ L. They were admitted and antibiotics treatment was given. The CRP data and outcome are presented in Table II. Three patients (P11 with triplets, P24 with quintuplets, and P37 with quadruplets) had normal CRP levels on admission and in the following 2 days. Because there was no evidence of infection, and amniotic fluid leakage had ceased, they were discharged from the hospital. The CRP values 1 week and 1 month after fetal reduction were also normal. The pregnancies were continuing smoothly, and three sets of normal twins were delivered.

Three patients (P18 with quintuplets, P53 with triplets, and P66 with quadruplets) had elevated CRP levels when leakage of amniotic fluid occurred. The CRP levels of these affected cases at 1 week after fetal reduction were significantly higher than that of controls. On admission, parenteral antibiotics were given. Unfortunately, fever and uterine irritability developed in the following days. Daily examinations exhibited rising CRP levels and WBC counts. Despite tocolytic therapy with intravenous ritodrine, cervical dilatation and effacement progressed. After counseling, the prospective parents decided to have the pregnancies terminated. Histology of the placental membranes confirmed the diagnosis of chorioamnionitis with the presence of acute inflammatory change.

DISCUSSION

The rate of pregnancy loss subsequent to fetal reduction had been reported as ranging from 11 to 35% (5,13), probably decreasing with the degree of experience and expertise of the physicians (3). Three (4%) in 71 mothers had abortion in this series and this result was comparable to those of several recent reports of 7–10% (3,6,14). Maymon *et al.* (5) estimated that 3–7% of mothers developed chorioamnionitis and septic abortion after fetal reduction. Early detection of intrauterine infection may facilitate a timely treatment to cure the disease (10,15). CRP has been established as an early diagnostic marker for chorioamnionitis in obstetrics (8,9). The results of this study demonstrated that the CRP value did not significantly change after fetal reduction per se and appeared to be unrelated to the number of fetuses reduced, although tissue absorption for the dead concepti was

Table I. The CRP Value (mg/dL) Prior to Fetal Reduction, and 1 Week and 1 Month After Reduction from Triplets, Quadruplets, and Quintuplets to Twins in 65 Uneventful Cases

Fetal reduction to twins	CRP before	1 week after	1 month after
Overall ($n = 65$)	0.27 ± 0.26	0.23 ± 0.24	0.24 ± 0.20
Triplets ($n = 44$)	0.29 ± 0.27	0.25 ± 0.27	0.23 ± 0.18
Quadruplets ($n = 16$)	0.20 ± 0.24	0.17 ± 0.15	0.25 ± 0.23
Quintuplets ($n = 5$)	0.27 ± 0.26	0.28 ± 0.26	0.30 ± 0.30

Note. Values are means $\pm$ SD. Differences are statistically non-significant for the three different temporal time periods.

Table II. The Data of Six Patients Who Experienced Leakage of Amniotic Fluid After Fetal Reduction

Patient	Pregnancy	CRP before	When leakage of amniotic fluid	1 week after	1 month after	Outcome
P11	Triplets	0.09	0.18	0.09	0.09	37 weeks, delivery
P18	Quintuplets	0.09	3.7	5.8	N.A.	16 weeks, abortion
P24	Quintuplets	0.13	0.09	0.09	0.12	34 weeks, delivery
P37	Quadruplets	0.39	0.75	0.33	0.41	36 weeks, delivery
P53	Triplets	0.76	3.11	3.87	N.A.	14 weeks, abortion
P66	Quadruplets	0.50	1.63	4.35	N.A.	15 weeks, abortion

Note. N.A. = not accessible after abortion.

proceeding. Therefore, an abnormal elevation of CRP level may reflect an intrauterine infection.

CRP is normally present as a trace constituent in maternal serum, and the level is independent of gestational age (16). The biosynthesis of CRP is induced by humoral mediators, such as interleukin-1 and interferon β_2 , which are elaborated by macrophages, monocytes, and the reticuloendothelial system (17,18). Once released, CRP becomes bound to altered or necrotic membrane structures. Its biological effects include enhancement of phagocytosis, stimulation of leukocyte motility, and opsonic effects, suggesting a specific role in tissue clearance and repair (7). The inflammatory process for the reduced fetuses may be localized in utero, and does not appear to elicit an elevation of CRP. In contrast, an intrauterine infection may have a systemic influence and results in an increase of CRP. The reference values of CRP used to define chorioamnionitis are not consistent in the literature, ranging from 0.7 to 2.0 mg/dL (8–10) and could affect the incidence of false positive and false negative results (8). In our patients with ongoing fetal reduction, all had CRP values less than 1.5 mg/dL, and we arbitrarily demarcated 2.0 mg/dL as a reference value (8). *The sensitivity and specificity of CRP tests for detection of chorioamnionitis was high (both 100% in these cases). However, the sample of affected cases was still small in this study. The findings need more data to be confirmed.* Determination of the most appropriate cutoff point that has the highest sensitivity and specificity will also require further investigation.

Lipitz *et al.* (6) reported that 13% pregnancies with triplets and 19% with quadruplets had leakage of amniotic fluid after fetal reduction. The occurrence of PROM is usually alarming, but not absolutely ominous, for the remaining fetuses (6). In our series, 6 (8%) of 71 mothers had PROM, 2 (4%) following reduction for triplets, the other 2 (11%) for quadruplets and another 2 (29%) for quintuplets. The conventional laboratory indicators used to detect intrauterine

infection are WBC counts with differential counts and erythrocyte sedimentation rate. However, these tests might be undependable because of the wide range for normal values in pregnancy and could be influenced by stress or physical activity (9). Spiking fever, an obvious clinical symptom, arises when the infection is already established. It has been shown that CRP often increases within hours of infection (7), and may precede maternal fever, uterine irritability, and increase in WBC and bands (8,10). In patients with PROM or premature uterine contraction after fetal reduction, CRP could be a sensitive marker to detect chorioamnionitis, and may be useful as an indicator to start and monitor treatment and determine prognosis.

CONCLUSIONS

CRP measurement is a rapid, noninvasive, and reproducible test for the detection of intrauterine infection. *None of the 65 controls had elevations of CRP in this study, may be due to no recent infections.* In the absence of infection, it does not significantly change with gestational age and after fetal reduction, and is not related with the number of fetuses reduced. *In addition to chorioamnionitis, an elevated CRP following multifetal reduction may also result from unrelated reasons such as urinary tract infections, upper respiratory infections, etc. These should be considered.* CRP may be a good indicator for chorioamnionitis after fetal reduction and could be used as a test during the follow-up examination. For patients with PROM or increased uterine contraction after fetal reduction, we advise admission for bed rest and examination for signs of infection including CRP and other clinical and laboratory indicators. CRP could also be used as an indicator for performing amniotic fluid culture for selecting treatment modality. Prolongation of pregnancy is imperative when infection can be excluded or detected very early in its course. In the other aspect, to reduce the high-order multifetal pregnancy

by ART, such as with adequate limitation and selection of embryos replaced according to age of patients, is preferred to divert the complications of multiple gestations and fetal reduction.

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