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자궁경부무력증에서 양수내 엑소솜, hypoxia inducible factor-1α와 inflammatory cytokine 역할에 대한 연구

The role of amniotic fluid exosome, hypoxia inducible factor-1α, and inflammatory cytokine in cervical insufficiency

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ABSTRACT

The role of amniotic fluid exosome, hypoxia inducible factor-1α, and inflammatory cytokine in cervical insufficiency

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Cervical insufficiency (CI) has been defined as painless cervical dilatation, leading to recurrent second trimester births. Physical examination-indicated cerclage (PEIC) is an obstetric salvage procedure for CI patients with dilated cervix and prolapsed membrane. Hypoxia inducible factor-1α (HIF-1α) has been reported to activate inflammatory cascade. Recently, exosomes have been known to have pivotal roles in intercellular communication. The aim of this study was to compare the concentration of amniotic fluid (AF) HIF-1α, exosomal HIF-1α,

and inflammatory cytokines such as interleukin-1α (IL-1α), interleukin-1ß (IL-1ß), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) between PEIC and control group. We also investigated the associations between biomarkers and amniocentesis-to-delivery interval and the correlations of inflammatory cytokines, HIF-1α, and exosomal HIF-1α. Case-control study was performed. Cases were defined as 16 patients who underwent PEIC and controls were 19 women who underwent amniocentesis for confirming chromosomal abnormalities. The concentration of IL-1α, IL-1ß, IL-6, TNF-α, HIF-1α, exosomal HIF-1α were measured using enzyme-linked immunosorbent assay (ELISA). Exosomes were confirmed by Tumor Susceptibility Gene 101 (TSG 101) and transmission electron microscopy (TEM). The mean HIF-1α in PEIC group was higher than control group (PEIC, 15.03 ± 9.60 pg/mL vs. control, 2.96 ± 1.99 pg/mL; $p < 0.01$). There were significant differences in inflammatory cytokines between two groups. A significant difference in exosomal HIF-1α was shown between two groups (PEIC, 27.97 ± 28.61 μg/mL vs. control, 12.42 ± 8.20 μg/mL; $p < 0.01$). HIF-1α, IL-1α, IL-6, TNF-α, and exosomal HIF-1α showed significantly negative association with cerclage-to-delivery interval. However, IL-1ß was not associated with cerclage-to-delivery interval. HIF-1α was positively

correlated with exosomal HIF-1α (rho=0.93, p<0.01). Both HIF-1α and exosomal HIF-1α were significantly associated with TNF-α (rho=0.94, p<0.01; rho=0.97, p<0.01, respectively). Both HIF-1α and exosomal HIF-1α had positive correlation with IL-1α (rho=0.96, p<0.01; rho=0.91, p<0.01, respectively). However, IL-1ß showed no correlations with HIF-1α and exosomal HIF-1α. A positive correlation between HIF-1α and IL-6 was observed (rho=0.58, p=0.01). Exosomal HIF-1α also had correlation with IL-6 (rho=0.52, p=0.03). This study demonstrated that AF HIF-1α and AF exosomal HIF-1α were higher in PEIC group than control group. AF HIF-1α and AF exosomal HIF-1α were associated with shorter amniocentesis-to-delivery interval. More importantly, they had positive correlations with AF inflammatory cytokines such as IL-1α, IL-6, and TNF-α. Our results may indicate that AF HIF-1α and AF exosomes interact with AF inflammatory cytokines and contribute to inflammatory response in complicated pregnancies.

Keywords : Cervical insufficiency, amniotic fluid, physical examination-indicated cerclage, exosome, HIF-1α, IL-1α, IL-1ß, IL-6, TNF-α

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LIST OF ABBREVIATIONS

AF: amniotic fluid

CI : cervical insufficiency

GA : gestational age

HIF-1α : hypoxia inducible factor-1α

IL-1α : interleukin-1α

IL-1ß : interleukin-1ß

IL-6 : interleukin-6

PEIC : physical examination-indicated cerclage

TNF-α : tumor necrosis factor-α

INTRODUCTION

Cervical insufficiency

Cervical insufficiency (CI) accounts for approximately 8% of preterm birth (PTB), which is defined as delivery less than 37 weeks of gestation (1, 2). CI is one of the leading problems of mid-trimester miscarriage, which is closely related with neonatal mortality and morbidity (1, 2). CI has been defined by historical criteria: painless cervical dilatation, leading to second trimester immature births in the absence of other causes (1, 2). Recent advancement of ultrasonography allows the diagnosis of CI without prior pregnancy losses (3, 4). The diagnosis of CI can be made reliably based on short CL (<25 mm), funneling, and/or cervical changes on physical examination (1, 3-5).

The causes of CI has not been fully understood (1). Cervical softening and shortening have been reported to be influenced by multifactorial causes such as inflammatory response or mechanical stretch (1, 3, 4). Risk factors of CI may be mechanical injury or congenital anomaly (1-5). Mechanical cervical injury includes dilatation and curettage (D&C), dilatation and evaucation (D&E), pregnancy termination, hysteroscopy, loop

electrosurgical excision procedure (LEEP), laser conization, and cold knife conization (1-5). Septate uterus, or biocornuate uterus are representative congenital uterine anomalies (1-5). Abnormal cervical development, resulting from in utero exposure to diethylstilbesterol (DES) may be contributory to CI (1-5). Collagen abnormalities such as Ehlers-Danlos syndrome or polymorphisim in the collagen-Iα1 and transforming growth factor-β (TGF-β) genes may be associated with CI (1-5).

The diagnosis of CI before pregnancy is difficult (1-5). Prepregnancy cervical competence examination using sonography, Hegar dilator, or balloon device cannot predict CI during pregnancy (1-5). Moreover, there is no definite laboratory test or biomarkers for the screening and diagnosis of CI (1-5). Speculum examination is crucial to confirm cervix dilatation and prolapse of membrane into the vagina (1-5). Transvaginal sonography is also effective to measure short CL and funneling to predict CI (3-5, 7). Funneling is defined as the ballooning of membrane into a dilated internal os, but with a closed external os (7, 8).

Cerclage is an obstetrical intervention for CI. Cerclage was first introduced by Drs Shirodkar and McDonald in the mid-1950s for women with a history of recurrent midtrimester losses or uterine anomaly or current cervical change or prior cervical surgery (2). Currently, there are 4 common types of cerclage as follows:

(1) History-indicated cerclage (HIC)

(2) Ultrasound-indicated cerclage (UIC)

(3) Physical examination-indicated cerclage (PEIC)

(4) Transabdominal cerclage (TAC)

HIC is a placement of cerclage in women with prior PTB or second trimester losses (3). HIC is a prophylactic vaginal cerclage (3). UIC is a cerclage placed in women with sonographically detected short cervix (<25 mm) and/or funneling before 24 weeks of gestataion (3). Berghella et al. analyzed five trials and reported that UIC significantly reduced PTB in women with short CL and/or funneling (8). PEIC is a salvage procedure, which improves pregnancy outcome in CI patient with advanced cervical change with visible bulging membrane into vagina (3). PEIC has been reported to prolong pregnancy by an average of

4-5 weeks with a 2-fold reduction of preterm birth before 34 weeks (4). The current data demonstrate that PEIC is associated with significantly better pregnancy outcome compared to bed-rest group (2-4). The live birth rate in PEIC group was 92.5%, and 89.1% of patients delivered beyond 24 weeks of gestation (2). Although PEIC may prolong latency period and prevent imminent delivery for a while, CI patients with PEIC are still high risk for preterm birth (PTB). Fluctuations of amniotic fluid (AF) inflammatory cytokines have been associated with adverse pregnancy outcome following PEIC (5). TAC is a prophylactic cerlage via abdominal approach. TAC is beneficial in women with prior trachelectomy or multiple conization histories (3, 9).

Inflammatory cytokines

It is now well recognized that inflammation may be one of key mechanism of PTB (6-8). A similar inflammatory reaction have been reported in CI, one of the causes of PTB (1-5). The non-pregnant cervix is fusiform, stiff and non-distensible organ, which has the internal and external os. Cervical ripening, which is the softening of the cervix, occurs during pregnancy. Immature cervical ripening may induce gradual opening of the internal and

external os, and result in second trimester preterm delivery. Cervical ripening and dilatation occurs when the concentration of collagen and the structural changes of extracellular matrix (ECM). (10). Cervical ripening changes, such as altered hyaluronan or collagen content may be contributory to CI (11). Hyaluronan disintegrates collagen bundles, and results in cervical softening (11).

Although the molecular mechanisms of PTB are not well understood, numerous inflammatory cytokines have been reported (12-14). Cytokines are involved in cervical softening, weakening or rupture of fetal membrane, and myometrial contraction (12-14). Cytokines produced in maternal decidua and myometrium seems to have an effect on uterine contraction. However, cytokines from fetal membrane does not transfer to maternal tissues (12-14). The biological mechanisms underlying the requirement of leukocytes for cytokine production have not been elucidated. Cytokines are produced resulting from heavy infiltration of resident and invading leukocytes into myometrium and cervix (15). These leukocytes are mainly neutropuils, macrophages, and T lymphocytes (15). In the cervix, glandular and surface epithelial cells may contribute to cytokine production (15). Although the correlation of amniotic fluid cytokines and PTB are well observed, there is little information about the exact cellular origin of amniotic fluid cytokines (15). Mononuclear phagocytes

or neutrophils in amniotic fluid appear to induce cytokines (15). Interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) have been implicated in the key mediators of PTB (13, 14). AF IL-1, IL-6, and TNF-α are elevated in PTB compared to normal term pregnancy, even in the absence of infection (13, 14). The upregulation of IL-1, IL-2, IL-6, IL-8, and TNF-α have been reported to be associated with the synthesis of prostaglandins which trigger uterine contraction (14, 16). Recently, 33 inflammatory cytokines in amniotic fluid were evaluated using multiplex assay in patients with sonographic short cervix (17). In the study, top-ranked cytokines were IL-6, IL-8, and macrophage inflammatory protein-1α (MIP-1α) (17). Another study demonstrated that amniotic fluid IL-1, IL-6, and TNF-α were significantly elevated in PEIC group compared to normal term pregnancy (18). Additionally, single nucleotide polymorphism in IL-6 gene showed association with preterm delivery resulting from CI (6).

Hypoxia-inducible factor-1α (HIF-1α)

Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor, which has been regarded as a master regulator of cellular adaptation to hypoxic stress (19, 20). HIF-1α is widely expressed in macrophages, neutrophils, and lymphocytes (19, 21, 22).

Recent work has shown that HIF-1α upregulates inflammatory cytokines and accelerates inflammatory reaction (19, 20). HIF-1α has been reported to be increased in many inflammatory diseases, such as rheumatoid arthritis, allergic rhinitis, chronic sinusitis, and prostate hyperplasia (21-23). HIF-1α activates allergic or eosinophilic inflammation of the lower airway (21). Cytokines released during inflammation, such as TNF-α, and IL-1 can upregulate HIF-1α levels (21, 22). TNF-α can contribute to HIF-1α expression in macrophages under normoxic condition, which demonstrates one common link between HIF-1α, and inflammation (21, 22). In atherosclerosis, reactive oxygen species (ROS) regulate HIF-1α protein accumulation in human macrophages (24, 25). HIF-1α transcription activity can be regulated by ROS through the mechanism of the activation of HIF-1α promoter or the regulation of hydroxylase function (24, 25). Some research has implied that HIF-1α mediated prostate enlargement under inflammatory conditions (23). HIF-1α directly activates the upregulation of inflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α) in rheumatoid arthritis (24). Conversely, HIF isoforms are upregulated by IL-1, IL-33, and TNF-α in synovial fibroblasts (25). HIF-1α has an impact on the progression of inflammation in rheumatoid arthritis synovial tissue (25). However, HIF-1α is not widely investigated in obstetric field. Relatively few studies on HIF-1α have been reported in human

and animal model (26-28). In pregnant mice model of preeclampsia, HIF-1α is increased significantly(28). In human study, HIF-1α is upregulated in preeclampsia or intrauterine growth restriction (IUGR) (26, 29). However, little research has been performed on the relationship between HIF-1α and inflammatory cytokines in amniotic fluid.

Exosome

Extracellular vesicle (EVs) had been regarded as 'cell debris' (30). However, there has been numerous reports on the function of EVs in cell-to-cell communication (30). EVs are classified into three subpopulation : (a) exosome, (b) microparticles (MPs), (c) apoptotic bodies (30). MPs are formed by passive shedding from cell surface plasma membrane with the size of 100 nm - 1μm (30). Apototic bodies are released from blebs of apoptoic cells with the size ranging from 1-5 μm (30). Exosomes originate from various cells including hematopoietic, non-hematopoietic cells, tumor cells, and microbes. Cellular proteins that are targeted for exosomes are first directed to endosomes through Ca^{2+}-dependant process (30, 31). The endosome is then directed to a multivesicular body (MVB) by endosomal sorting complex required for transport (ESCRT), which is critical for forming the MVBs and sorting the endosomal proteins (30, 31). MVBs average 600 nm in size. Therefore exosomes must be smaller

than MVB (30, 31). The MVB moves along microtubules toward the cell periphery where they fuse with the plasma membrane to release intraluminal vesicles (ILV) (30, 31). Once these ILV are released from the cell, they are regarded as exosomes (30, 31). Exosomes, 40-120 nm in size, result from an active secretion process, which is associated with micro-autophagy process in MVB, whereas MPs are generated from passive direct shedding of the membrane in response to cellular activation or stress(30). MPs have more heterogenous diameters because they originate from the cell periphery(30).

Exosome is enriched with various proteins, lipids, and nucleic acids, which have biologic function and modulate cell-cell communication (30, 31). There is increasing evidence that biologically active molecules in exosomes can modulate the redirection of signaling pathway in recipient cells (31, 32). The presence of TNF-α within exosomes from hypoxic cardiomyocytes was identified (31). HIF-1α initiated the expression of exosomal TNF-α in cardiomyocytes under hypoxia and induced apoptosis (31). Recent studies have demonstrated the presence of heat shock protein 72 and micro RNA (miRNA) on exosomes, which might reflect that stress response affects on exosomes (32).

Rapidly emerging evidence demonstrates that exosomes are associated with intercellular communication in cancer or inflammatory disease, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (32). In nasopharyngeal

carcinoma, elevated HIF-1α on exosome accelerates metastatic effects (33). Some research has implied that synovial exosomes, which contain autoantigens, are involved in the pathogenesis of RA (34). Immunostimulatory exosomes have been reported to be associated with the pathogenesis of SLE (35).

In obstetric fields, recent studies on placental exosomes have been reported in complicated pregnancy using placenta tissue or maternal serum (36-38). Placental alkaline phosphatase (PLAP) is a specific marker for placenta(36-38). The upregulation of PLAP on exosomes has been reported in preeclampsia, or gestational diabetes (GDM) (36, 37). However, little is known in the role of amniotic fluid exosome in complicated pregnancy.

Aims of the study

The hypothesis of this work was that amniotic fluid inflammatory cytokines and exosomes in CI patients increase significantly compared to term pregnancy. For this, several factors were analyzed in amniotic fluid.

Specific aims were:

-to identify amniotic fluid exosome and exosomal HIF-1α in CI and term pregnancy

-to determine the amniotic fluid concentration of well-known cytokines (IL-1α, IL-1ß, IL-6, and TNF-α) and HIF-1α in CI and term pregnancy

-to determine the possible differences in these biomarkers between CI and term pregnancy

-to investigate whether these biomarkers have correlation with pregnancy outcome

-to determine whether these biomarkers have correlation with one another

MATERIALS AND METHODS

Study design

This was a retrospective study of patients who presented between January 2010 and December 2011 at Kangnam Sacred Heart Hospital. The institutional review board approved this study. 16 patients who underwent PEIC at 17-24 weeks of gestation and 19 women who underwent amniocentesis for confirming chromosomal abnormalities were included in the study. The cases and controls were well matched in terms of age, gestational weeks, and gravidity. The inclusion criteria were a singleton pregnancy, the absence of regular uterine contraction, and no evidence of major fetal congenital anomalies. The exclusion criteria were as following: proven or suspected clinical chorioamnionitis at the time of PEIC; any vaginal bleeding or preterm labor pain; premature rupture of membranes (PROM); detected maternal diseases. All study participants were negative for vaginal culture during prenatal examination. The patient's medical records were reviewed for demographic variables, visible membrane size, and delivery outcomes.

Sample collection from patients with cervical insufficiency and control group

After disinfection of the skin with povidone-iodine solution, amniotic fluids were sampled by transabdominal amniocentesis with a 21-gauge needle under ultrasound guidance. All AF samples were collected with informed consent. For the case group, amniocentesis was performed to reduce intraamniotic pressure for replacing prolapsed membrane into uterine cavity. PEIC was performed under general anesthesia. First, amnioreduction was performed for reducing intraamniotic pressure, thus allowing easier and safer PEIC. Next, prolapsed membrane is replaced into uterus mechanically (39). Then, cerclage stitch is placed using McDonald technique. When combined with preterm labor, premature rupture of membrane, or chorioamnionitis, cerclage was removed earlier at the discretion of maternal-fetal medicine specialists.

Analysis of amniotic fluid HIF-1α, IL-1α, IL-1ß, IL-6, and TNF-α

Remaining amniotic fluids not needed for clinical analysis were transported immediately to the laboratory. Samples were centrifuged and stored in polypropylene tubes at -70 ℃ until analysis. IL-1α, IL-1ß, IL-6, TNF-α, and HIF-1α

concentration in stored AF were measured with commercially available enzyme-linked immunosorbent assay(ELISA) according to the manufacturer's instructions (USCN Life Science Inc., Houston,TX,USA). In brief, 50μl of prepared AF was added to each well and incubated for 2 h at 37 ℃ or overnight at 4 ℃. The plates were rinsed four times and incubated with 100 ml of biotinylated primary antibodies (Detection Reagent A) for 1 h at 37 ℃. After washing, the plates were treated with HRP-conjugated streptavidin (Detection Reagent B) for 1 h at ambient temperature. After washing four times, 100 ml of TMB peroxidase substrate was added and incubate for 10-20 minutes at 37 ℃. The absorbance at 450 nm was determined the optical density (OD value) of each well.

Exosome isolation and tramission electron microscope (TEM)

Exosomes were purified from 5 ml amniotic fluid according to an established protocol (40). Samples were subjected to successive centrifugations at 300×g for 5 min to remove cells, 1,200×g for 20 min and 10,000×g for 30 min to remove cellular debris, and 100,000×g for 1 h to pellet exosomes. The resulting pellet in each tube was resuspended in 50μl of isolation solution (250 mM sucrose/10 mM triethanolamine/0.5 mM PMSF/1μM leupeptin). Each pellet was washed with

isolation solution and repelleted, and then the repellet was resuspended in 50μl of isolation solution. Nearly all exosomes have typical exosomal markers (36, 41). In this study, Tumor Susceptibility Gene 101 (TSG 101) was used for exosomal marker. Exosomes originate from multivesicular bodies (MVB) and secreted into extracellular space via active exocytosis process. Endosomal-sorting complexes required for transport (ESCRT complexes) are involved in the active exocytosis (36,41). TSG 101 is one of ESTCR complexes which is essential for the formation of exosomes (36, 41). Proteins from isolated exosome (40μg/lane) were separated by 12 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto PVDF membranes. Following transfer, membranes were preincubated with 5 % non-fat milk and incubated overnight at 4℃ with anti-TSG101. After incubation with primary antibody, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:5000; Amersham) for 1 hour, washed, and then subsequently visualized with the Amersham ECL system (Amersham). The quantitative analysis of protein was detected using Photo-captMw computer software (Vilber Lourmat, France). Ultracentrifuged pellets were embedded in 2% agar solution, and then pellets were diced into 1.5mm cubes. Pellets were fixed with 4% paraformaldehyde and 0.1M PBS-buffered 2.5% glutaraldehyde (Electron Microscopy Sciences

(EMS), Hatfield, PA, USA) for 1 h and washed with PBS, followed by post-fixed in 1% osmium tetroxide (EMS, Hatfield, PA, USA) for 1 h. Pellets were then dehydrated through a graded series of ethanol and propylene oxide and were then embedded in Epon 812 kits (EMS, Hatfield, PA, USA). Ultrathin sections were cut on an RMC MTXL ultramicrotome (Tucson, Arizona, USA) and then stained with uranyl acetate and lead citrate. The sections were examined with a transmission electron microscope (TEM) (JEM-1011, JEOL, Japan).

Analysis of amniotic fluid exosomal HIF-1α

The presence of HIF-1α within exosomes was verified by a commercial ELISA kit (USCN Life Science Inc., Houston, TX, USA) as described by the manufacturer. Prior to ELISA, exosomes were sonicated to release HIF-1α within exosomes. Isolated exosome fractions were sonicated using five 2-s bursts at 35 W in a sonicator bath (Lab-line Instruments, Melrose Park, IL, USA). The samples were added to wells, and then incubated for overnight at 4℃. Detection reagent A (biotin-conjugated anti-HIF-1α antibody)was added to each well, followed by detection reagent B (Horseradish Peroxidase (HRP)-conjugated avidin), and substrate solution after washing at each step. Finally stop solution was added and plates were read at 450 nm.

Statistical analysis

Statistical analysis was performed using SPSS (IBM Corp., Armonk, NY, USA). Results are expressed either as number and percent, or mean ± standard deviation for continuous variables. Continuous variables were evaluated by Student's t-test or Mann-Whitney U-test, where appropriate. Chi-square analysis was used for categorical data. Correlations for continuous variables were assessed using either the Pearson or the Spearman test, depending on normal distribution. P <0.05 was required for statistical significance.

RESULTS

1. Patient characteristics and pregnancy outcomes in PEIC and control group

Table 1 presents maternal characteristics and pregnancy outcomes in PEIC and control group. PEIC was operatively successful in all patients. The average size of bulging membrane was 4.78 cm, ranging from 2 cm to 10 cm. There were 3 patients with full cervical dilatation (10 cm) and prolapsed membrane. There was no iatrogenic membrane rupture during PEIC. Groups did not differ for maternal demographics and gestational age at amniocentesis. However, gestational age at delivery and live birth rate were significantly lower in PEIC group compared to control group. The causes of PTB in PEIC group were preterm labor (PTL) (62.5 %, 10/16), and premature rupture of membrane(PROM) (37.5 %, 6/16). There was a significant difference in neonatal survival between two groups (75%, 12/16 vs. 100%, 19/19, p=0.02). Interestingly, all three patients with full cervical dilatation (10 cm) with bulging membrane had live birth outcome: one patient 1480 gram baby at 28.71 weeks resulting from PTL (cerclage-to-delivery interval, 67 days); second patient delivered 890 gram neonate at 25.85 weeks due to PROM (cerclage-to-delivery interval, 40 days);

third patient delivered 860 gram baby at 25.28 weeks resulting from PROM (cerclage-to-delivery interval, 39 days). The worst pregnancy outcome was shown in a patient with 4 cm prolapsed membrane who delivered 340 gram non-viable baby at 20.2 weeks (cerclage-to-delivery interval, 8 days). The most favorable pregnancy outcome was shown in a patient with 4 cm bulging membrane who delivered 2410 gram baby at 34.42 weeks resulting from PTL (cerclage-to-delivery interval, 95 days).

Table 1. Patient characteristics, and pregnancy outcomes

	PEIC group (n = 16)	control group (n = 19)	p value
age (years)	34.63 ± 2.80	35.37 ± 1.83	0.35
multiparity (%)	6/16 (37.5%)	9/19 (47.3%)	0.55
BMI (kg/m^2)	21.72	20.52	0.28
GA at amniocentesis (week)	19.83 ± 0.67	19.39 ± 0.83	0.1
GA at delivery (week)	27.02 ± 3.81	38.46 ± 1.16	<0.01*
neonatal weight (gram)	1091.88 ± 591.20	3273.16 ± 398.20	<0.01*
amniocentesis-to-delivery interval (days)	50.38 ± 26.56	133.53 ± 8.26	<0.01*
Live birth	12/16 (75 %)	19/19 (100%)	0.02*

GA, gestational age

* statistically significant

Data are presented as mean ± standard deviation (SD)

2. Concentration of amniotic fluid HIF-1α

Figure 1 demonstrates the comparison of HIF-1α between PEIC group and control group. HIF-1α was identified in all AF samples, and was significantly higher in PEIC group (mean ± SD) (PEIC, n=16; 15.03 ± 9.60 pg/mL vs. control, n=19; 2.96 ± 1.99 pg/mL; $p < 0.01$).

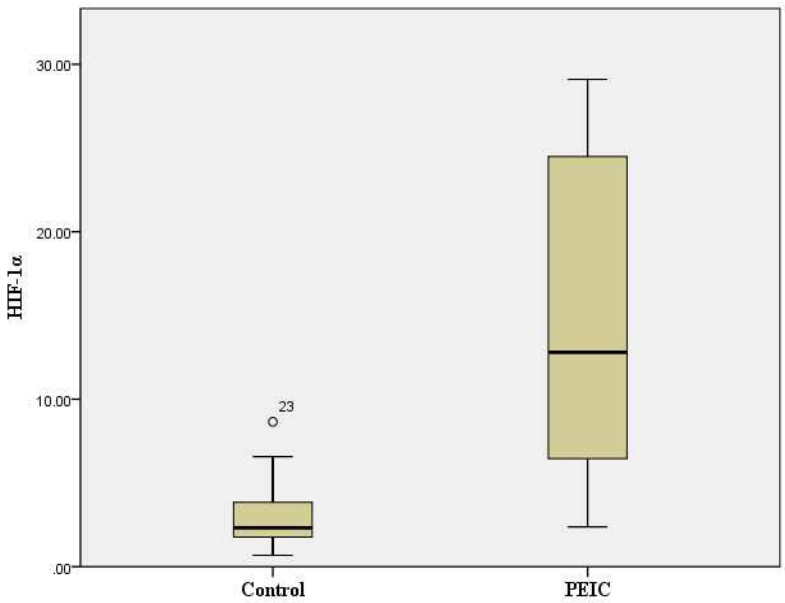
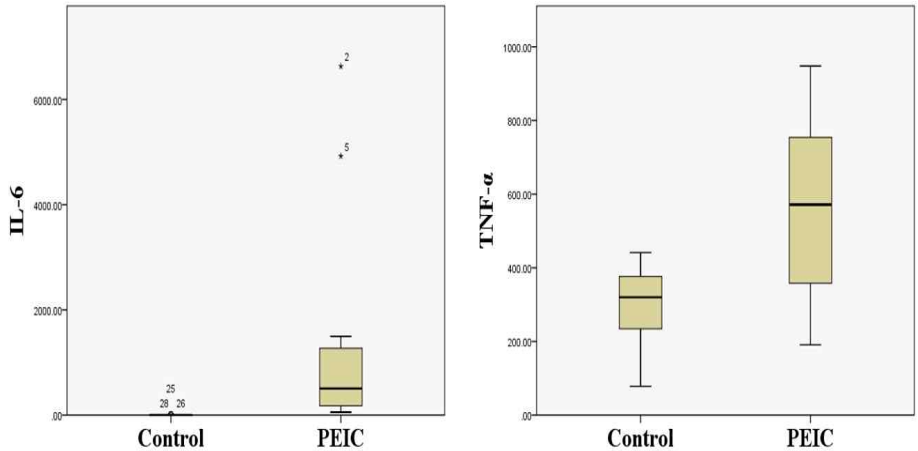


Figure 1. Comparison of HIF-1α between PEIC group and control group.

Amniotic fluid HIF-1α was measured using ELISA kits following the manufacturer's instructions. PEIC group showed significantly higher HIF-1α compared to control group ($p<0.01$).

3. Concentration of amniotic fluid IL-1α, IL-1ß, IL-6, and TNF-α

IL-6, and TNF-α were significantly higher in PEIC group (Figure 2). IL-6 in PEIC group was 278.80 ± 243.30 pg/mL, and IL-6 in control group was 3.07 ±1.84 pg/mL (p=0.009). TNF-α in PEIC group was elevated compared to control group (PEIC; 562.86 ± 234.58 pg/mL vs. control; 305.07 ± 103.14 pg/mL, $p<0.01$). Although IL-1α and IL-1ß were higher in PEIC group, there were no statistical differences between two groups. IL-1α in PEIC group and control group were 1198.88 ± 1869.34 pg/mL, and 923.28 ± 1129.97 pg/mL, respectively(p=0.59). IL-1ß in PEIC group and control group were 94.28 ± 32.72 pg/mL, and 69.79 ± 55.07 pg/mL, respectively (p=0.12). Table 2 summarizes the concentration of AF IL-1α, IL-1ß, IL-6, TNF-α, and HIF-1α.



2-a) 2-b)

Figure 2. Comparison of IL-6, and TNF-α between PEIC group and control group.

2-a. IL-6 was significantly elevated in PEIC group ($p=0.009$); 2-b. TNF-α were significantly higher in PEIC group($p<0.01$).

Table 2. Comparison of inflammatory cytokines, and HIF-1α in amniotic fluid

intraamniotic biomarkers	PEIC group	control group	p value
IL-1α (pg/mL)	1198.88 ± 1869.34	923.28 ± 1129.97	0.59
IL-1ß (pg/mL)	94.28 ± 32.72	69.79 ± 55.07	0.12
IL-6 (pg/mL)	278.80 ± 243.30	3.07 ± 1.84	0.009*
TNF-α (pg/mL)	562.86 ± 234.58	305.07 ± 103.14	< 0.01*
HIF-1α (pg/mL)	15.03 ± 9.60	2.96 ± 1.99	< 0.01*

* statistically significant

Data are presented as mean ± standard deviation (SD)

4. Transmission electron microscope (TEM) of exosome in amniotic fluid

Exosomes are secreted membrane-bound nanovesicles. Transmission electron microscopy showed abundant exosomes with size of approximately 60-120 nm (Figure 3). Exosomes had pale and dense core, and bounded by single, or double membranes. TEM demonstrated the existence of amniotic fluid exosomes in both of PEIC and control group (Figure 4). Exosomes have typical internal markers called endosomal-sorting complexes required for transport (ESCRT complexes). Tumor susceptibility gene 101 (TSG 101) and Apoptosis linked gene 2-interacting protein X (ALIX) are members of ESCRT complexes. TSG 101 were detected in all amniotic fluid samples. The vesicles were confirmed as exosomes by Western blotting, which were positive for TSG 101, a typical exosomal marker (Figure 5).

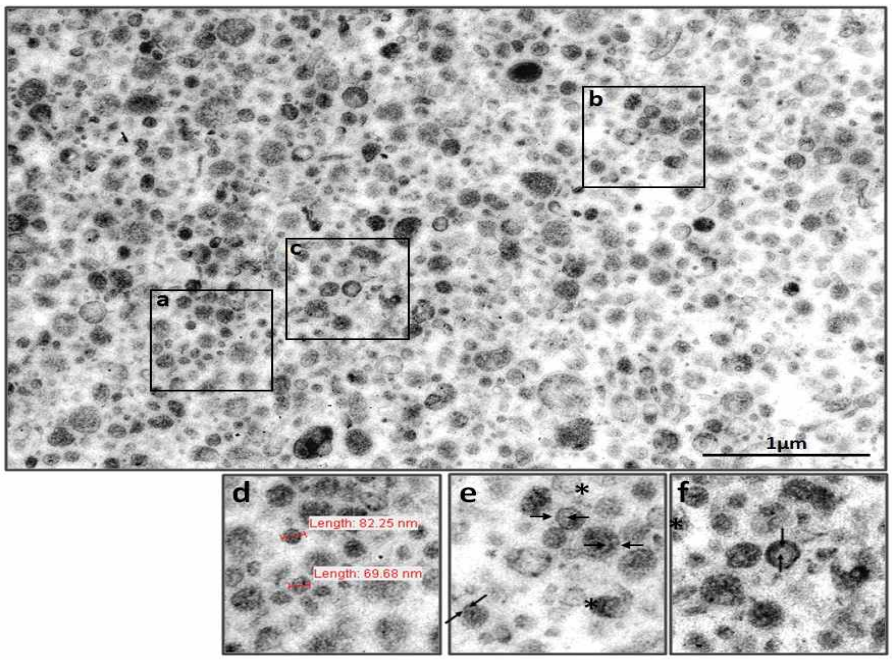
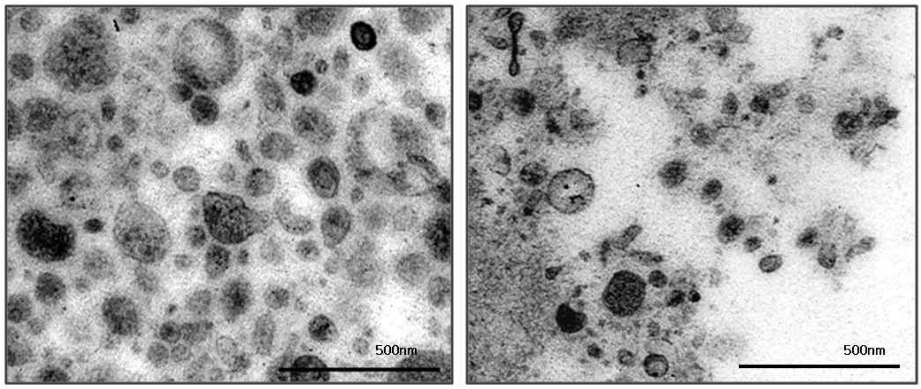


Figure 3. Transmission electron microscopy of exosomes purified from amniotic fluid.

Transmission electron microscopy of exosomes purified from amniotic fluid. Diameters of abundant nanovesicles were between 60 and 120 nm (d, enlargement of insert solid line square a). Exosomes had pale and dense core, and bounded by single (aster, e) or double membranes (arrows, e and f; enlargement of insert solid line square b and c).



a) b)

Figure 4. Transmission electron microscopy of amniotic fluid exosomes in PEIC and control group.

4-a) amniotic fluid exosome in PEIC group; 4-b) amniotic fluid exosome in case group.

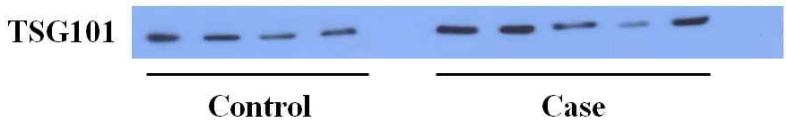


Figure 5. TSG 101, a typical exosomal marker, identified in Western blot analysis.
TSG 101, Tumor susceptibility gene 101.

5. Concentration of amniotic fluid exosomal HIF-1α

PEIC group demonstrated significantly higher exosomal HIF-1α compared to control group. The concentration of exosomal HIF-1α in PEIC group was 27.97 ± 28.61 μg/mL, and that of control group was 12.42 ± 8.20 μg/mL ($p<0.01$). The comparison of exosomal HIF-1α between PEIC group and control group were shown in Figure 6.

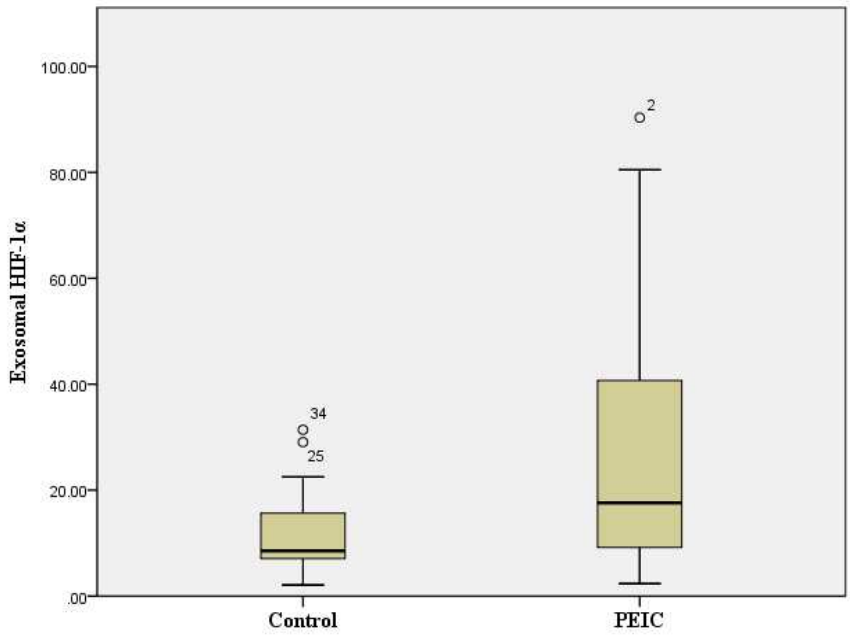


Figure 6. Comparison of exosomal HIF-1α between PEIC group and control group.

Exosomes have diverse proteins. Exosomal HIF-1α was measured using ELISA. PEIC group showed significantly higher exosomal HIF-1α than control group ($p<0.01$).

6. Spearman correlation of amniotic fluid biomarkers with cerclage/amniocentesis-to -delivery interval

HIF-1α showed significantly negative correlation with cerclage-to-delivery interval (rho=-0.96, $p<0.01$) (Figure 7). Exosomal HIF-1α also had statistically negative association with cerclage-to-delivery interval (rho=-0.92, $p<0.01$) (Figure 8). IL-1α was negatively correlated with cerclage-to-delivery interval (rho=-0.98, $p<0.01$). However, IL-1ß was not associated with cerclage-to-delivery interval (rho=-0.22, p=0.39) (Figure 9). There was significant negative correlation of IL-6 with cerclage-to-delivery interval (rho=-0.56, p=0.02). TNF-α had negative correlation with cerclage-to-delivery interval (rho=-0.95, $p<0.01$). Table 3 demonstrates the Spearman correlation with cerclage-to-delivery interval in PEIC group. Table 4 presents the Spearman correlation with amniocentesis-to-delivery interval in control group. There were no statistically significant correlations between intraamniotic biomarkers and amniocentesis-to-delivery interval in control group.

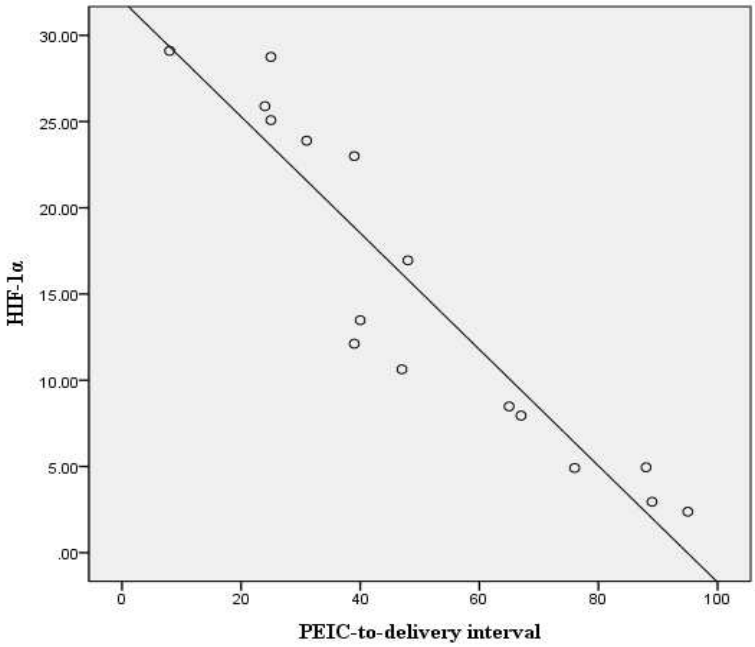


Figure 7. Spearman correlation of HIF-1α with cerclage-to-delivery interval.

HIF-1α showed significantly negative correlation with cerclage-to-delivery interval (rho=-0.96, $p<0.01$).

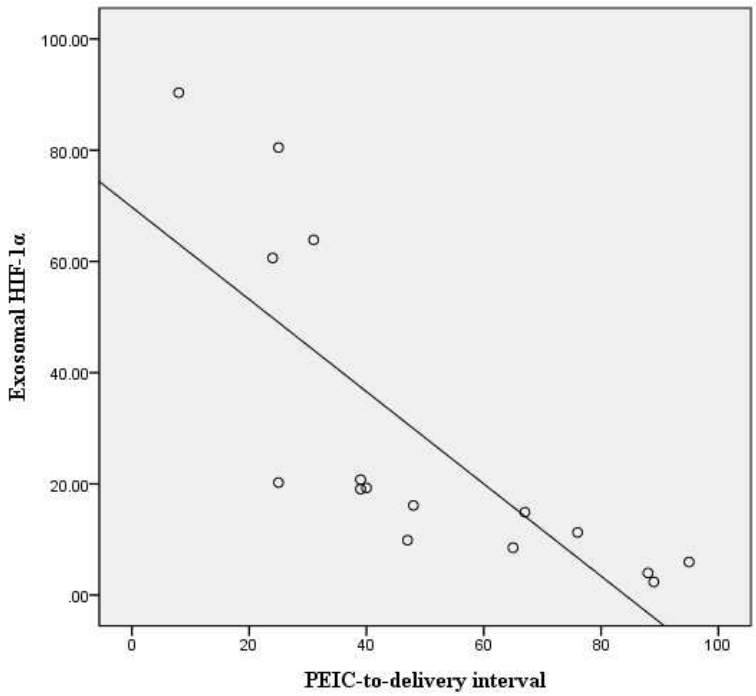
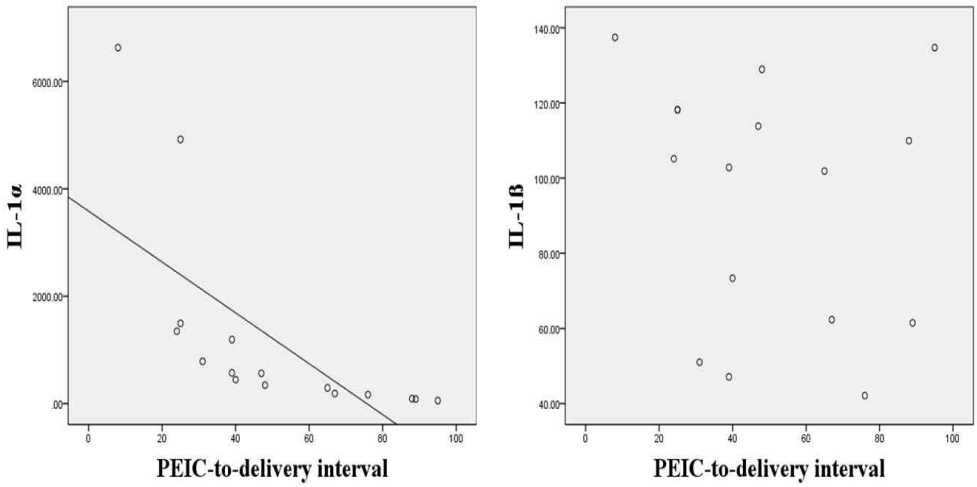


Figure 8. Spearman correlation of exosomal HIF-1α with cerclage-to-delivery interval.

Exosomal HIF-1α had statistically negative association with cerclage-to-delivery interval (rho=-0.92, $p<0.01$).



a) b)

Figure 9. Spearman correlation of IL-1α, and IL-1ß with cerclage-to-delivery interval.

9-a) IL-1α was negatively correlated with cerclage-to-delivery interval (rho=-0.98, $p<0.01$).

9-b) IL-1ß was not associated with cerclage-to-delivery interval (rho=-0.22, $p=0.39$).

Table 3. Spearman correlation of biomarkers with cerclage-to-delivery interval in PEIC group

intraamniotic biomarkers	Spearman correlation with cerclage-to -delivery interval	p value
IL-1α	-0.98	<0.01*
IL-1ß	-0.22	0.39
IL-6	-0.56	0.02*
TNF-α	-0.95	<0.01*
HIF-1α	-0.96	<0.01*
exosomal HIF-1α	-0.92	<0.01*

* statistically significant

Table 4. Spearman correlation of biomarkers with amniocentesis-to-delivery interval in control group

intraamniotic biomarkers	Spearman correlation with amniocentesis-to-delivery interval	p value
IL-1α	0.01	0.96
IL-1ß	-0.11	0.65
IL-6	-0.30	0.21
TNF-α	0.12	0.61
HIF-1α	0.14	0.57
exosomal HIF-1α	-0.24	0.32

7. Correlation of amniotic fluid HIF-1α, IL-1α, IL-1ß, IL-6, TNF-α, and exosomal HIF-1α

Table 5 demonstrates the correlation of intraamniotic cytokines, HIF-1α, and exosomal HIF-1α in PEIC group. HIF-1α was positively related with exosomal HIF-1α (rho=0.93, $p<0.01$) (Figure 10). There was significant correlation between IL-1α and HIF-1α (rho=0.96, $p<0.01$) (Figure 11-a). Significant correlations of IL-6 & HIF-1α and TNF-α & HIF-1α were also observed (rho=0.58, p=0.01; rho=0.94, $p<0.01$, respectively) (Figure 11-b, 11-c). Exosomal HIF-1α was also similarly associated with IL-1α (rho=0.91, $p<0.01$) (Figure 12-a). However, there was no correlation between IL-1ß and HIF-1α (rho=0.35, p=0.18). Exosomal HIF-1α showed no association with IL-1ß (rho=0.17, p=0.50). Exosomal HIF-1α was also positively correlated with IL-6 and TNF-α (rho=0.52, p=0.03; rho=0.97, $p<0.01$, respectively) (Figure 12-b, 12-c). A strong positive association between TNF-α and IL-1α was observed (rho=0.95, $p<0.01$) (Figure 13-a). TNF-α was positively associated with IL-6 (rho=0.52, p=0.03) (Figure 13-b). However, there were no associations between IL-1ß and other cytokines. Table 6 shows the correlation of intraamniotic cytokines, HIF-1α, and exosomal HIF-1α in control

group. There were significant correlations of IL-6 & exosomal HIF-1α, IL-6 & IL-1α, and IL-6 & IL-1ß in control group (rho=0.49, p=0.03; rho=0.47, p=0.04; rho=0.78, p<0.01, respectively) (Figure 14).

Table 5. Correlation of amniotic fluid inflammatory cytokines, HIF-1α, and exosomal HIF-1α in PEIC group

intraamniotic biomarkers	Spearman correlation (rho)	p value
TNF-α & IL-1α	0.95	<0.01*
TNF-α & IL-1ß	0.22	0.39
TNF-α & IL-6	0.52	0.03*
IL-1α & IL-6	0.49	0.05
IL-1α & IL-1ß	0.28	0.28
IL-1ß & IL-6	0.11	0.68
HIF-1α & TNF-α	0.94	<0.01*
exosomal HIF-1α & TNF-α	0.97	<0.01*
HIF-1α & IL-1α	0.96	<0.01*
exosomal HIF-1α & IL-1α	0.91	<0.01*
HIF-1α & IL-6	0.58	0.01*
exosomal HIF-1α & IL-6	0.52	0.03*
HIF-1α & exosomal HIF-1α	0.93	<0.01*
HIF-1α & IL-1ß	0.35	0.18
exosomal HIF-1α & IL-1ß	0.17	0.50

* statistically significant

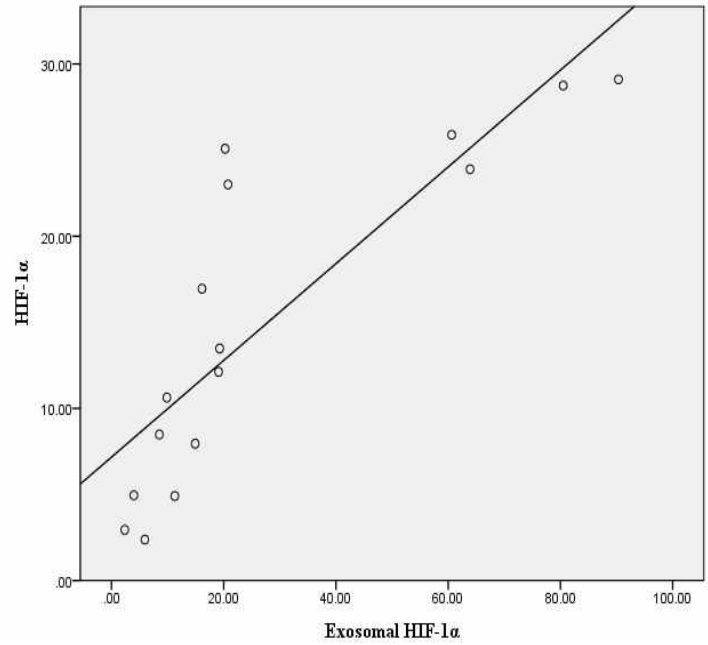


Figure 10. Scatter plot of HIF-1α, and exosomal HIF-1α in PEIC group

Exosomal HIF-1α was positively related with HIF-1α (rho=0.93, $p<0.01$).

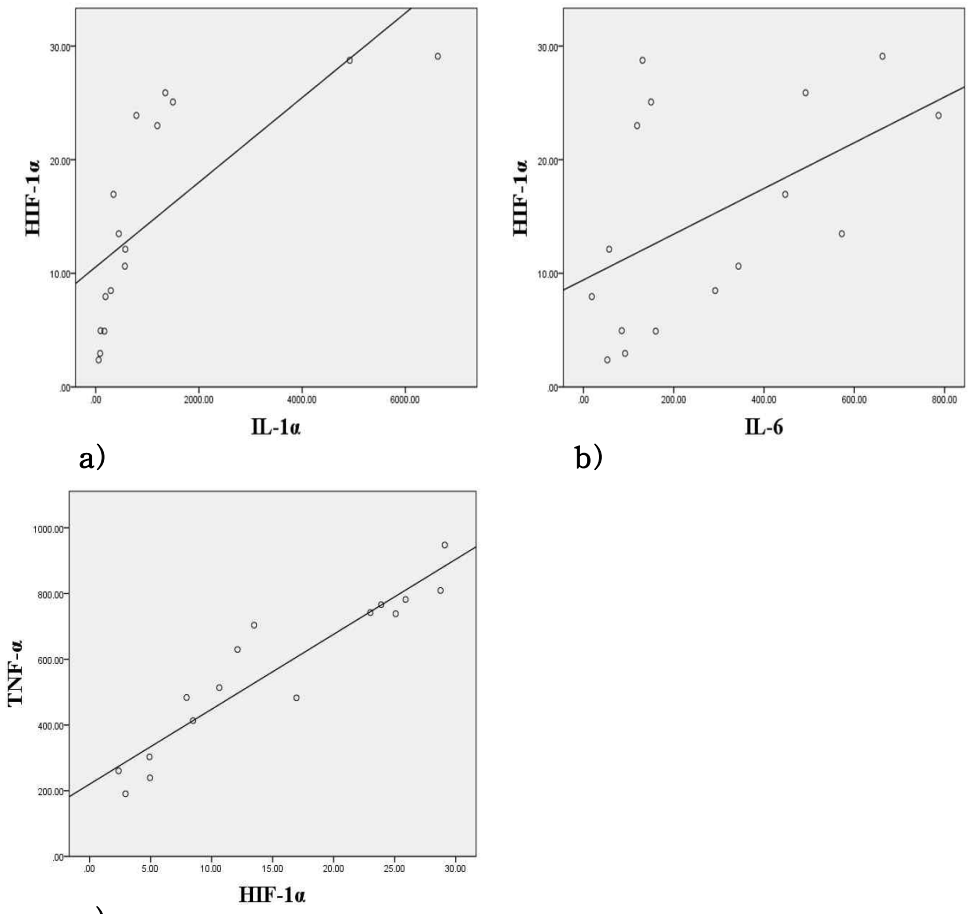


Figure 11. 11-a) Scatter plot of IL-1α, and HIF-1α; 11-b) Scatter plot of IL-6, and HIF-1α; 12-c) Scatter plot of TNF-α, and HIF-1α in PEIC group.

There was significant correlation between IL-1α and HIF-1α (rho=0.96, $p<0.01$). A significant correlation between HIF-1α and IL-6 was observed (rho=0.58, $p=0.01$). HIF-1α was positively associated with TNF-α (rho=0.94, $p<0.01$).

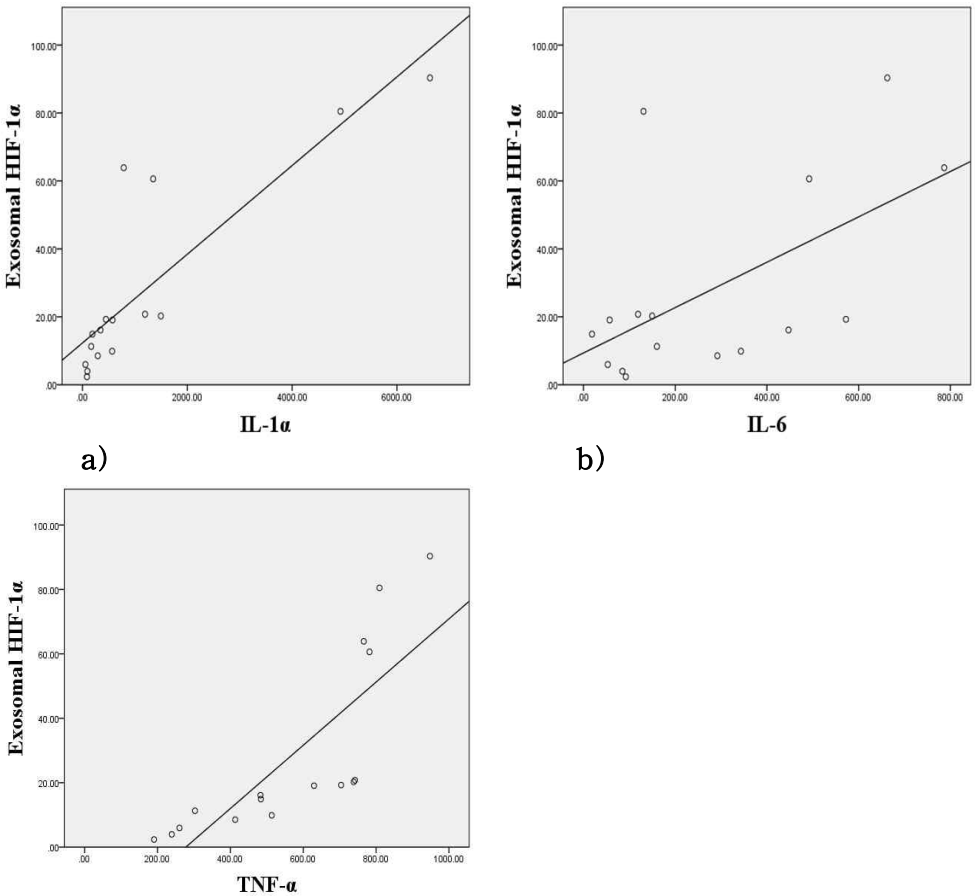
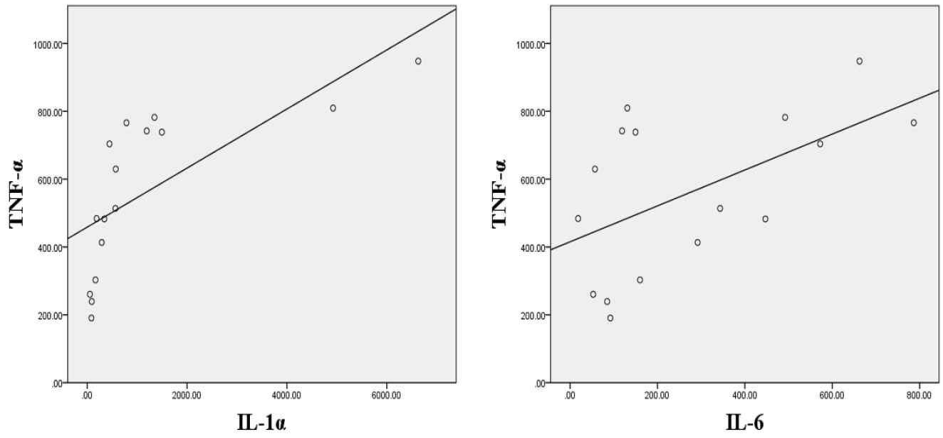


Figure 12. 12-a) Scatter plot of IL-1α, and exosomal HIF-1α; 12-b) Scatter plot of IL-6, and exosomal HIF-1α; 12-c) Scatter plot of TNF-α, and exosomal HIF-1α in PEIC group.

Exosomal HIF-1α was positively associated with IL-1α (rho=0.91, $p<0.01$). Exosomal HIF-1αwas also similarly correlated with IL-6 (rho=0.52, $p=0.03$). Exosomal HIF-1α had significant correlation with TNF-α (rho=0.97, $p<0.01$).



a) b)

Figure 13. 13-a) Scatter plot of IL-1α, and TNF-α; 13-b) Scatter plot of IL-6, and TNF-α in PEIC group.

A strong positive association between TNF-α and IL-1α was observed (rho=0.95, $p<0.01$). TNF-α was positively associated with IL-6 (rho=0.52, p=0.03).

Table 6. Correlation of amniotic fluid inflammatory cytokines, HIF-1α, and exosomal HIF-1α in control group

intraamniotic biomarkers	Spearman correlation (rho)	p value
TNF-α & IL-1α	0.30	0.22
TNF-α & IL-1ß	0.18	0.47
TNF-α & IL-6	0.39	0.10
IL-1α & IL-6	0.47	0.04*
IL-1α & IL-1ß	0.42	0.07
IL-1ß & IL-6	0.78	<0.01*
HIF-1α & TNF-α	0.30	0.22
exosomal HIF-1α & TNF-α	0.15	0.53
HIF-1α & IL-1α	0.31	0.20
exosomal HIF-1α & IL-1α	0.03	0.90
HIF-1α & IL-6	0.07	0.78
exosomal HIF-1α & IL-6	0.49	0.03*
HIF-1α & exosomal HIF-1α	0.15	0.53
HIF-1α & IL-1ß	0.11	0.63
exosomal HIF-1α & IL-1ß	0.28	0.27

* statistically significant

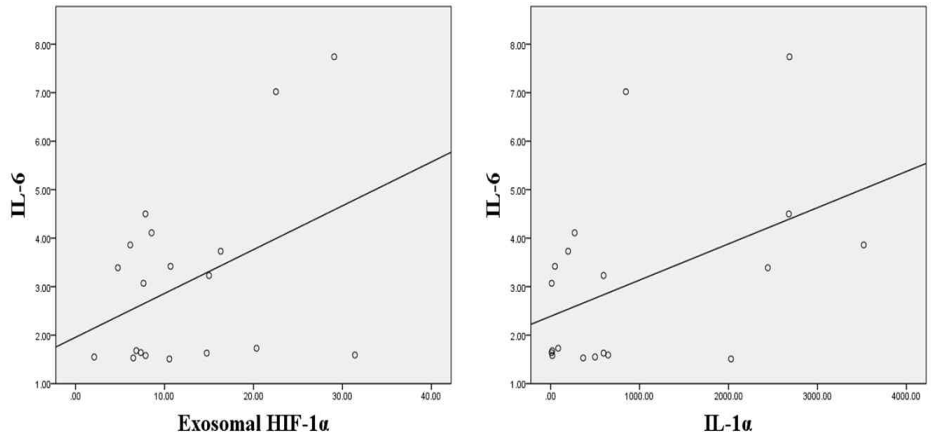


Figure 14. 14-a) Scatter plot of IL-6, and exosomal HIF-1α; 14-b) Scatter plot of IL-6, and IL-1α; 14-c) Scatter plot of IL-6, and IL-1ß in control group.

Exosomal HIF-1α was positively associated with IL-6 (rho=0.49, p=0.03). IL-1α was also similarly correlated with IL-6 (rho=0.47, p=0.04). IL-1ß had a significant correlation with IL-6 (rho=0.78, p<0.01).

DISCUSSION

This study extends our understanding of amniotic fluid biomarkers. This study demonstrated for the first time that HIF-1α is detectable in amniotic fluid. Recent researches have indicated that HIF-1α plays a major role in inflammation and immune reactions (19, 20). HIF-1α drives inflammatory reaction by activating proinflammatory cytokines (21-23). In obstetrics, relatively few studies on HIF-1α have been reported. Some researchers demonstrated that HIF-1α in placenta or maternal serum were elevated in preeclampsia (34-37). Mice model of gestational hypertension indicated that TNF-α infusion stimulated the upregulation of HIF-1α in placenta tissue (36). Human villous explants study also showed that HIF-1α might have a pivotal role in trophoblast development, and be associated with pathophysiology of preeclampsia (34, 35). Others reported that HIF-1α in maternal serum was upregulated in preeclampsia (37). My study demonstrated the presence of amniotic fluid HIF-1α in normal and complicated pregnancy. Amniotic fluid HIF-1α was significantly higher in PEIC group compared to control group, which coincides with other reports that the elevation of HIF-1α was observed in complicated pregnancy. My study implies that amniotic fluid HIF-1α may have a role in inflammatory reaction.

To my knowledge, this study is the first research, which verifies the presence of HIF-1α in aminiotic fluid exosomes. Amniotic fluid exosomes were confirmed by TSG 101, a typical exosomal marker, and electron microscopy. Exosomes are bioactive membranous nanovesicles ranging 40-120 nm in diameter, which originate from late endosomes called MVB via exocytosis (30, 36, 41). The average size of MVB is 600 nm (40). Therefore, exosomes must be smaller in size (40). ESCRT complexes, the well-characterized multiprotein network, are essential in the degradation and formation of exosomes. TSG 101 and Apoptosis linked gene 2-interacting protein X (ALIX) are members of ESCRT complexes, and are used as exosome-specific internal marker (36, 41). Exosomes are enriched with diverse cargo of proteins, and have a major function in intercellular communication (31, 36, 41). However, the mechanism of sorting proteins in exosome has not been defined (31, 36, 41). Growing evidence has shown that exosomes are susceptible to acute stressor, modulate immune system and trigger inflammatory processes by intercellular communication (31, 36, 41). Exosomes have been reported to be involved in the initiation and progression of numerous diseases, such as cancer, heart disease, infectious disease, and inflammatory disease(33, 42-44). Some research indicated that HIF-1α in exosome stimulated exosomes-mediated metastasis in nasopharyngeal cancer (33).

Others reported that exosomes actively participated in the pathogenesis of autoimmune diseases, such as rheumatoid arthrhitis (RA), systemic lupus erythematosus (SLE) and Sjogren's syndrome (SS) (32). Obstetric research on exosomes have mainly focused on placenta-derived exosomes using maternal serum, placental explants, or cultured trophoblast cells (36-38,41). Exosomes generating from placenta contain PLAP, a characteristic placenta specific marker (41). Exosomes in pregnant women were found to be 50-fold higher than non-pregnant women (41). In addition, PLAP containing exosomes were positively correlated with disease severity (36-38, 41). Recent studies indicated that PLAP containing exosomes were found to be increased in preeclampsia or gestational diabetes women compared to normal pregnancy (36, 41). However, studies on amniotic fluid exosomes are rudimentary. There has been two reports that AF exosomes might be helpful to identify fetal kidney diseases (45, 46). Pisitkun et al. demonstrated the presence of AF exosome, and suggested that AF exosome might originate from fetal kidney as fetal urine is main component of AF (45). Another research group reported that AF exosomal CD24 were generated from fetal urine production, and might be used as an indicator for fetal kidney disease (46). There has been a report on heat shock protein (HSP) containing AF exosome in midtrimester pregnant women, which demonstrated the presence

of HSP in AF exosome, but the study was not a case-control study (47). This study was a case-control study on AF exosome and showed that exosomal HIF-1α in PEIC group were greater compared to normal term pregnancy. This study implied that elevated exosomal HIF-1α might be related with disease severity or pathophysiology of CI or PTB.

In my study, I demonstrated the correlation of intraamniotic biomarkers and pregnancy outcome. PEIC group showed that higher concentration of HIF-1α, exosomal HIF-1α, and inflammatory cytokines, such as IL-1α, IL-6, and TNF-α are associated with shorter cerclage-to-delivery interval. In contrast, control group presented no correlation between intraamniotic biomarkers and latency of pregnancy. These findings may reflect that inflammatory reaction influences on complicated pregnancy resulting in PTB. Although PEIC prevents imminent delivery and allows significant prolongation of pregnancy, CI patients with PEIC are still at high risk of PTB (4). There have been many reports on the fluctuations of AF inflammatory cytokines such as IL-1, IL-6, and TNF-α in PTB patients, even in the absence of infection (13). My study verified the elevation of these well-known cytokines, and also showed higher concentration of newly detected AF HIF-1α, exosomal HIF-1α in PEIC group. I also evaluated the correlations of intraamniotic biomarkers one another. There were significant correlations between HIF-1α,

exosomal HIF-1α, and inflammatory cytokines, such as IL-1α, IL-6, and TNF-α in PEIC group. This study demonstrated that AF HIF-1α was positively correlated with exosomal HIF-1α in PEIC group. Furthermore, AF HIF-1α and exosomal HIF-1α were significantly associated with inflammatory cytokines (IL-1α, IL-6, and TNF-α). My results may inform that amniotic fluid HIF-1α and exosome are correlated with inflammatory cytokines, and affect in the pathophysiology of CI. In contrast, there was no correlation between IL-1ß and other intraamniotic biomarkers in PEIC group. Recent study reported that trophoblast exosomes induced IL-1ß by macrophages (36). Interestingly, the correlation of intraamniotic biomarkers differed in control group. There were significant correlations between IL-6 and exosomal HIF-1α, IL-1α, and IL-1ß. Although we could not clarify why there was a difference in correlation of intraamniotic biomarkers between two groups, I hypothesized that the pathophysiology of preterm delivery differs from term pregnancy. Further study is needed to evaluate the interaction of intraamniotic biomarkers in normal and complicated pregnancy. The size of prolapsed membrane was not correlated with AF biomarkers in PEIC group. The patient with the highest concentration of AF biomarkers showed the shortest cerclage-to-delivery interval (2 days), but she had 4 cm sized bulging membrane preoperatively. In contrast, all patients with full cervical dilatation (10cm) and prolapsed membrane had

favorable pregnancy outcome. These patients had lower concentration of HIF-1α, exosomal HIF-1α, IL-1α, IL-6, and TNF-α than the patient with the worst pregnancy outcome. This might explain that inflammatory reaction directly influences on the prognosis of PEIC rather than the size of cervical dilatation and prolapsed membrane. There was a report that inflammatory cytokines upregulate HIF-1α (21). Others demonstrated that inflammatory cytokines such as IL-1, TNF-α, and IL-33 increased the expression of HIF isoforms in synovial fibroblasts (25). The other study group reported that TNF-α, and IL-1ß stimulated HIF-1α in nasal inflammation (21). Conversely, some research have indicated that HIF-1α exacerbates inflammatory response by stimulating inflammatory cytokines (48, 49). HIF-1α activated the expression of exosomal TNF-α in hypoxic cardiomyocytes (36). These studies might well explain why we found a significant association of HIF-1α, exosomal HIF-1α, IL-1α, IL-6, and TNF-α in PEIC group.

There are limitations in this study. First, my study could not clarify the origin of AF HIF-1α and exosomal HIF-1α. I could not demonstrate AF biomarkers were generated from mother or fetus. Second, my study could not identify the cellular pathway of AF biomarkers. Moreover, this study could not explain the reason of the difference in the correlation of intraamniotic biomarkers between PEIC and control group. Third,

I could not clarify the exosomal pathway to stimulate and modulate inflammatory cytokines in PEIC group. Lastly, this study had a small sample size.

In summary, this study shows that HIF-1α and exosomal HIF-1α may be correlated with inflammatory cytokines. Thus these biomarkers may participate in maternal inflammatory reaction in PEIC group. Recently, animal model study showed that HIF inhibitors prevented inflammatory response (50). As studies on HIF-1α and exosomes are rudimentary in obstetrics, further basic research is warranted to evaluate the role of these biomarkers during normal and complicated pregnancy.

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국 문 초 록

자궁경부무력증에서 양수내 엑소솜, hypoxia inducible factor-1α와 inflammatory cytokine 역할에 관한 연구

서울대학교 의과대학
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자궁경부무력증은 임신 2분기에 진통 없이 자궁경부가 개대되어 조산에 이르게 하는 질환으로 염증 반응과 연관이 있을 것으로 여겨지나 명확한 병태생리가 밝혀지지 않았다. 응급자궁경부원형결찰술은 자궁경부가 개대되고, 질 내로 양막이 돌출된 자궁경부무력증 환자에서 임신 유지를 위해 시행하는 수술이다. 응급자궁경부원형결찰술을 받더라도 조산의 위험성은 매우 크다. 엑소솜은 최근 세포 간 신호전달에 매우 중요한 역할을 하는 것으로 알려진 나노미터 물질이나, 양수내 엑소솜에 대한 연구는 제한적이었다. HIF-1α는 염증 연쇄반응을 활성화 시키는 물질로 알려져 있으나, 양수 내 HIF-1α의 역할을 규명된 바 없다. IL-1, IL-6, TNF-α는 조산의 병태생리와 밀접한 관련이 있는 염증성 사이토카인들이다. 본 연구 에서는 응급자궁경부원형결찰술을 받은 자궁경부무력증

환자의 임신예후에 미치는 양수내 엑소솜과 HIF-1α, IL-1α, IL-1ß, IL-6, TNF-α의 역할에 대해 알아보고자 한다.

환자-대조군 연구를 시행하였다. 환자군은 응급자궁경부원형결찰술을 받은 자궁경부무력증 산모 16명, 대조군은 태아 염색체 이상을 검사하기 위해 양수검사를 시행한 산모 19명으로 하였다. ELISA를 이용하여 양수내 HIF-1α, IL-1α, IL-1ß, IL-6, TNF-α 농도 및 엑소솜내 HIF-1α를 측정하였다. 양수내 엑소솜은 전자현미경 및 엑소솜 마커인 TSG 101을 이용하여 확인하였다.

자궁경부무력증 환자군에서 대조군에 비해 의미 있게 높은 양수내 HIF-1α 수치를 보였다. 양수내 IL-6, TNF-α 또한 자궁경부무력증 환자군에서 의미 있게 높은 수치를 보였다. 양수내 IL-1α, IL-1ß는 자궁경부무력증 환자군이 대조군에 비해 높은 수치를 보이기는 하였으나, 통계학적 의미는 없었다. 전자현미경 및 TSG 101 마커를 통해 양수내 엑소솜을 확인할 수 있었다. 엑소솜내 HIF-1α는 자궁경부무력증 환자에서 유의하게 높았다. 응급자궁경부원형결찰술 후 분만까지의 기간과 양수 내 HIF-1α, IL-1α, IL-1ß, IL-6, TNF-α 및 엑소솜내 HIF-1α의 연관성 분석에서 HIF-1α, IL-1α, IL-6, TNF-α 및 엑소솜내 HIF-1α 농도가 높을수록 임신연장기간이 짧았다. 양수 내 HIF-1α, IL-1α, IL-1ß, IL-6, TNF-α 및 엑소솜내 HIF-1α의 상호 연관성 분석에서 HIF-1α는 IL-1α, IL-6, TNF-α 및 엑소솜내 HIF-1α와 양의 상관관계를 보였다. 엑소솜내 HIF-1α 또한 IL-1α, IL-6, TNF-α와 양의 상관관계를 보였으며, TNF-α는 IL-1α, IL-6와 양의 상관관계를 보였다.

본 연구에서는 양수내 HIF-1α, IL-1α, IL-6, TNF-α 및 엑소솜내 HIF-1α 농도와 자궁경부무력증환자의 수술 후 임신연장 기간과의 상관관계를 밝히는 한편, 양수내 HIF-1α및 엑소솜내 HIF-1α와 기존에 알려진 염증 사이토카인인 IL-1α, IL-6, TNF-α와의 상관관계에 대해서도 분석하였다. 향후 양수내 HIF-1α와 엑소솜이 자궁경부무력증 환자의 염증연쇄반응 병태생리 규명에 도움을 줄 수 있을 것으로 사료된다.

주요어 : 자궁경부무력증, 엑소솜, HIF-1α, IL-1α, IL-1ß, IL-6, TNF-α

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