

## **TREATMENT OF CERVICAL INSUFFICIENCY ABORTION BY ZAMZAM WATER ACTIVATED AUTOLOGEOUS HUMAN PERIPHERAL BLOOD MONONUCLEAR CELL, MODERN TREND**

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### **ABSTRACT**

Treatment of cervical insufficiency abortion is by cerclage which either done vaginally (Shirodkar, McDonald) or abdominally and laparoscopically. Due to many complications arise from cerclage a need for non-invasive or minimally invasive procedure is urgent.

As mononuclear cell is a potential regulator of cell proliferation, stimulate progesterone by luteal cells, promote embryo invasion, stimulate adenosine triphosphate, improving mitochondrial respiration, strong anti-microbial, hence comes its application in cervical wall injection.

***Aim of the work:*** to use for the first time in the literature zamzam water activated autologous mononuclear cell intracervically to treat repeated abortion due to cervical insufficiency.

***Subject and methods:*** 50 pregnant women at a period of gestation between 8-10 weeks to exclude fetal causes of abortion. Informed consent was taken. The mean age of women was  $30\pm 3.5$ , a mean number of abortion was  $8.2\pm 2.6$  were enrolled in the study, inclusion criteria were historical and radiological features of cervical insufficiency. Transvaginal ultrasound for measurement of absolute cervical length, cervical effacement. ***Exclusion criteria:*** previous cerclage, other causes of abortion, medical and genetic disorder, previous progestational drugs, viral hepatitis and blood diseases. Ultrasound was done before the procedure for diagnosis and then every 2 weeks after the procedure, also cervical smear for aquaporin and cervical mucous for detection of IL8, collagenase, were done before the procedure and at the time of delivery. Fetal evaluation by Apgar score and fetal weight was done. PBMCs were obtained from the patients and were cultured with 10 c.c sterile zamzam water for 3 days, and then administered in the cervical wall.

***Results:*** full term delivery occurred in 48 cases (96%), abortion 2 cases (4%), cesarean section 45 cases (90%), vaginal delivery 5 cases (10%). No fetal or maternal complication was reported.

**Conclusion:** Treatment of cervical insufficiency by cervical injection of zamzam activated autologus human peripheral blood mononuclear cell (PBMCS) is safe, effective, and cheap with positive fetal effect and no fetomaternal complications, but more cases and randomization is needed before elucidation the effectiveness of the procedure.

**Keywords:** Cervical insufficiency, Abortion, Zamzam water, PBMC, Aquaporin, IL8, Collagenase.

## INTRODUCTION

The first recognition of cervical incompetence was reported in the literature in 1658 by Cole, Culpetter and Rowland in the practice of Physick<sup>(1)</sup>. The authors noted that: "the second fault in women which hindered conception is when the seed is not retained or the orifice of the womb is so slack that it cannot rightly contract itself to keep in the seed. The fibers of the womb are broken in pieces, one from another and the inner orifice of the womb overmuch slackened". Despite the description of this condition, a surgical approach for treatment did not emerge until nearly 300 years later.

Treatment of cervical insufficiency abortion is by cerclage which either done vaginally (Schirodkar, McDonald) or abdominally and laparoscopically. Due to many complications arise from cerclage a need for non-invasive or minimally invasive procedure is urgent. Cerclage is not an innocent procedure it is associated with an increased risk of premature preterm rupture of membranes, bleeding, and intrauterine infection, cerclage may also cause severe pain and inconvenience throughout pregnancy<sup>(2)</sup>.

As mononuclear cell is a potential regulator of cell proliferation<sup>(3,4)</sup>, stimulate progesterone by luteal cells<sup>(5)</sup>, promote embryo invasion<sup>(6)</sup>, stimulate adenosinetriphosphate<sup>(7)</sup>, improving mitochondrial respiration<sup>(8)</sup>, strong anti-microbial<sup>(9)</sup>, hence comes its application in cervical injection.

## MATERIALS AND METHODS

50 pregnant women at a period of gestation between 8-10 weeks to exclude fetal causes of abortion. Informed consent was taken. The mean age of women was  $30 \pm 3.5$  years, a mean number of abortion was  $8.2 \pm 2.6$  were enrolled in the study, inclusion criteria were historical and radiological features of cervical insufficiency. Traditionally, historical features were used to make the diagnosis of cervical insufficiency as described by **Harger et al**<sup>(10)</sup>. which were (i) history of two or more second-trimester pregnancy losses (excluding those resulting from preterm labor or abruption); (ii) history of losing each pregnancy at an earlier gestational age; (iii) history of painless cervical dilatation of up to 4-6 cm; (iv) absence of clinical findings consistent with placental abruption; (v) history of cervical trauma causes by cone

biopsy; (vi) intrapartum cervical lacerations; or (vii) excessive forced cervical dilation during pregnancy termination. Transvaginal ultrasound for measurement of absolute cervical length, cervical effacement after the cervical injection was done every two weeks.

### **Exclusion criteria:**

Previous cerclage, other causes of abortion medical and genetic disorder, previous progestational drugs, viral hepatitis and blood diseases. Ultrasound done before the procedure for diagnosis and then every 2 weeks after the procedure, also cervical smear for aquaporin and cervical mucous for detection of IL8, collagenase were done before the procedure and at the time of delivery. Fetal evaluation by Apgar score and fetal weight was done.

Detection of IL8 in cervical mucus before the procedure and at the time of delivery method used as described by **Sakai et al. (2004)**<sup>(11)</sup>. Collagenase, as described by **Kleiner et al. (1993)**<sup>(12)</sup>; **Murphy et al. (1995)**<sup>(13)</sup> and **Brew et al. (2001)**<sup>(14)</sup>. Detection of aquaporins type AQP3, AQP4, AQP5 in the cervical smear cell by immunohistochemistry as described by **Procino et al. (2003)**<sup>(15)</sup>; **Hasler et al. (2006)**<sup>(16)</sup>.

### **For detection of aquaporins:**

Evaluation of staining intensity was performed by using a grading scale from 0 to 3 where 0 = no staining, 1 = faint staining, 2= moderate staining and 3 = intense staining. The number of stained cells were similar in all biopsies, two observers each unaware of the identity of slides evaluated the staining intensity the average value from the two observers was calculated (**Hildenbrand et al., 2006**)<sup>(17)</sup>.

### **Preparation of zamzam water activated autologous peripheral blood mononuclear cells:**

Blood samples were obtained from individual patients and PBMCs ( $1 \times 10^7$  cells) were isolated by Ficoll-hypaque centrifugation as described previously (**Hashii et al., 1998**)<sup>(5)</sup>. PBMCs were incubated in the presence of 10 c.c of sterile zamzam water for 72 hours. After isolating PBMCs, the cell suspension was gently administered in the cervical wall at any site of the cervix no difference whether anterior or posterior. The amount of cell suspension differ from patient to patient. The senior author introduce a new equation which is the amount of cell suspension to be injected= length of cervix x inner-inner diameter of the cervix.

### **Data analysis:**

Difference between IL8, collagenase, AQP3, AQP4, AQP5 before activated PBMC and at the time of delivery were analyzed by two tailed t-test, chi-square test was used for comparison a difference of  $<0.05$  was considered significant.

## RESULTS

50 pregnant women at a period of gestation between 8-10 weeks were subjected to zamzam water activated autologous human peripheral blood mononuclear cells injected in the cervical wall the results were summarized in the following tables.

**Table (1): Cervical mucus collagenase, IL-8 before zamzam water activated autologous human peripheral blood mononuclear cell "Z(Activated PBMC)" cervical injection and at time of delivery**

Character	Before procedure Z(Activated PBMC)	After delivery	P value
IL-8	6.11±2.1	2.2±0.5	P<0.05
Collagenase	5.21±1.5	11.9±1.8	<0.05

**Table (2): Staining intensity of AQP3, AQP4, AQP5 in cervical smear**

Character	Before procedure Z(Activated PBMC)	After delivery	P value
AQP3	4.1±2.99	2.1±1.88	<0.5
AQP4	5.2±3.18	3.1±2.99	<0.05
AQP5	6.5±4.1	2.1±1.56	<0.001

**Table (3): Obstetrics outcome after procedure Z(Activated PBMC) cervical wall injection**

	No.	%
Full term	48	96%
Abortion	1	2%
Preterm	1	2%

**Table (4): Method of delivery**

	No.	%
Cesarean section	45	90%
Vaginal delivery	5	10%
Vacuum extraction	3	60%
Normal vaginal delivery	2	4%

**Table (5): Foetal assessment**

	Mean $\pm$ SD
Birth weight	3.99 $\pm$ 0.805
APGAR score First minute	8 $\pm$ 0.1
APGAR score Fifth minute	9 $\pm$ 0.2
Prenatal loss	-

## DISCUSSION

Treatment of cervical insufficiency abortion is by cerclage which done either vaginally (Shrodkar or McDonald) or abdominally and laparoscopically.

Women with cerclage had more hospitalization during pregnancy, lower birth weight and had a higher of maternal peripheral fever **Rush et al**<sup>(18)</sup>.

**La Zan et al**<sup>(19)</sup>. Also failed to show any benefit from cerclage in a large cohort of 506 women according to the largest randomized study was performed by the medical research council/ royal collage of obstetricians and gynecologists MRC/RCOG, women who were assigned to cerclage received more tocolytic medications (34% vs 27%) than in women without cerclage, and had a higher rate of antepartum hospital admissions (37% vs 29%). Puerperal fever was also more commonly after cerclage (6% vs 3% p= 0.03).

A meta-analysis by **Drakley et al**<sup>(20)</sup> pooled the results of four trials that together summarized 2062 women. There was no difference in the total pregnancy and early (< 24 weeks) pregnancy loss rates (relative risk RR 0.86, 95%). There was no beneficial effect of cerclage RR 1.29, 95% (CI 0.67-2.49), there was also no difference in perinatal death RR 0.8, 95%, CI 0.48- 1.36) or mean gestational age between cerclage and non cerclage group.

So, in summary it appears that the use of cerclage<sup>(21,22)</sup> in women with various risk factor of spontaneous preterm birth has little clinical benefit, but is associated with more medical complication and interventions<sup>(23)</sup>.

Due to the above situation regarding cerclage vaginally or abdominally<sup>(24)</sup> we introduced for the first time in the literature a new technique based on cervical injection of autologous human peripheral blood mononuclear cell in our unpublished work we reached<sup>(25)</sup> full term pregnancy rate 85%, 10% abortion, 5% preterm birth here in our work due to activation of human peripheral blood mononuclear cell by sterile zamzam water, we reached to a full term pregnancy rate 96%, 2 % abortion 2% preterm labour which was statistically significant P< 0.05 (table 3). In our work we found cesarean section rate was 90% and vaginal delivery rate was 10% (table 4).

Mean birth weight  $3.99 \pm 0.805$  (table 5) and the mean Apgar at one minute and five minutes was statistical not significant from our previous work in which we used nonactivated peripheral blood mononuclear cell (P value > 0.05). We reported here no case of prenatal loss. To explain the results of our work a serial ultrasound were done postoperatively we found a normalize of the cervical internal assessment at the time of delivery than before zamzam activated PBMC.

The biochemical basis of our is based upon statistically diminished collagenase enzyme (table 1) at the time of delivery meaning that the technique act by stimulating a new collagen fiber and increase the number of collagen number fibers in the cervix. In unpublished work we demonstrate increase in collagen type I which is a rigid fibril collagen and diminished collagen type III which is elastic and thin and result in more compliant tissue. The ratio between collagen I and III determines the characteristics of the tissue, in our unpublished work we demonstrate increase in the ratio between collagen type I and III in cervical biopsy taken at time of delivery and also we demonstrate increase in the gap junction proteins and up regulation of useful genes and positive effect on amniotic fluid meaning that it increase the amniotic fluid in cases of oligohydramnious.

Regarding cervical mucus IL-8 we demonstrate a statistically significant decrease at the time of delivery IL-8 is a marker of infection meaning that our technique reduce the incidence of infection contrasting to cerclage operation which infection is raised, the reduction of infection is based upon the fact that (PBMC) is a major source of proteinase nexin-1 which is a strong antimicrobial together with zamzam water which is a strong antimicrobial<sup>(9)</sup>.

We studied aquaporin in the cervical smear before the procedure Z(activated PBMC) and at the time of delivery we found the statistically significant decrease in AQP3 and AQP4 and statistically highly significant decrease in aquaporin 5 meaning that our technique work by other mechanism which through the reduction of expression of AQP. This means that the cervix become more rigid and the this attributed to success of this new procedure. Again we should stress the point that there were differential expression of AQP it is less in the side of AQP5 which means that it is the major AQP responsible for the change in the elasticity of the cervix.

Again to give other explanation it was demonstrated that PBMC improved skin graft survival<sup>(27)</sup>. And PBMC is a rich source of early pregnancy factor which has an immune-suppressive and growth factor properties<sup>(28)</sup>.

The activation of PBMC by zamzam water due to peculiar of zamzam water which was discovered by radioimmunoassay, nano-technology, laser femto, crystalline electromicroscopy, specific refractive index, number single oscillator, specific dispersing of optical parametersal assay, Abbe number of zamzam water was completely different from other types of water<sup>(29,30,31)</sup>.

So, the advantage of our technique in comparable to cerclage were: nonsurgical, no need for antibiotics, no anesthesia, positive effect on fetus, stimulate progesterone, strong antimicrobial (tap and infection) express HCG receptors, cheap, learning curve, stimulate endometrial receptivity, promote fibrosis, up regulation of useful genes, stimulation of gap junction protein, stimulate adenosine triphosphate, improving mitochondrial function, positive effect on ischemia, stimulate type I collagen, increase ratio collagen I/collagen III, reduce type III collagen, positive electron microscope effect.

## CONCLUSION

Treatment of cervical insufficiency by cervical injection of zamzam activated autologous human peripheral blood mononuclear cell (PBMCS) is safe, effective, and cheap with positive fetal effect and no fetomaternal complications, but more cases and randomization are needed before elucidation the effectiveness of the procedure.

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