Treatment of Refractory (Resistant) Cerclage by Human Menstrual Blood Stem Cells(Modern Trend)

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Abstract: Introduction: Resistant (Refractory) Cerclage which is a new term we introduced which means failure of repeated vaginal Cerclage (3 times) done by expert gynecologist and with no contraindication for the operation. Human endometrial stem cells (which were isolated from menstrual blood) possess the adult stem cell like characteristics of self-renewal, high proliferative potential in vitro, and the ability to differentiate toward diverse cell lineages in induction media. These cells were directly harvested from the endometrium. No report in the literature deals with the treatment of this condition, so the aim of this work is to use Human endometrial stem cells as a new modality of treatment.**Material and Methods:**10 patients of Refractory Cerclage (Resistant) were enrolled in the study; Mean age 38 ± 3.2 years, Mean number of failure Cerclage 8.2 ± 2.6; human menstrual blood stem cells were prepared and injected in the cervix. Ultrasound, cervical IL8, collagenase, AQP3, AQP4, AQP5 was done at time of injections and 4 weeks after the injection.**Results:**70% of cases ended with full term pregnancy, 20% ended with pre-term delivery and 10% suffered abortion.Statistically significant decrease in IL8, collagenase, AQP3, AQP4 and AQP5 after 4 weeks of the injection.**Conclusion:**Treatment of Refractory (Resistant) Cerclage by cervical injection of autologus human menstrual blood stem cells (HuMenSCs) is safe, effective, and cheap with positive fetal effect and no feto-maternal complications, but more cases and randomization are needed before elucidation the effectiveness of the procedure.

Keywords: Resistant Cerclage, Refractory Cerclage, human menstrual blood stem cells, collagenase, IL8, ultrasound, AQPs.

1 Introduction

Thefirst recognition of cervical incompetence was reported in the literature in 1658[1]. 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 [2].

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Human endometrial stem cells (HuMenSCs), which were isolated from menstrual blood, possess the adult stem cell like characteristics of self-renewal, high proliferative potential in vitro, and the ability to differentiate toward diverse cell lineages in induction media [3]. These cells were directly harvested from the endometrium and first described by Gargett [4].

Thereafter, several research groups have expanded on the knowledge of HuMenSCs, which exhibit stem/progenitor cell properties in vitro and can also repair several types of damaged cells in vivo [3,4, 5,6]. It was found that HuMenSCs had high expression levels of mesenchymal stem cell surface markers, including CD29, CD44, CD49f, CD90, CD105, and CD117, and embryonic stem cell markers (Oct4 and SSEA3/4) [3,7].

On the other hand, other researchers have confirmed that HuMenSCs can be induced to differentiate into a variety of somatic cell types under special conditions, including adipocytes, osteoblasts, chondrocytes, neurons, endotheliocytes, pulmonary epithelial cells, hepatocytes, islet cells, cardiac myocytes, and insulin-producing cells [3,8,9]. Thus, a large body of evidence indicates the strong pluripotent characteristic of HuMenSCs [3, 6, 7]. HuMenSCs are more easily accessible than other adult stem cells, making them a potential donor source for stem cell therapy, hence come their use in Resistant (Refractory) Cerclage which is a new term we introduced which means failure of repeated vaginal Cerclage (3 times) done by expert gynecologist and with no contraindication for the operation.

2 Material and Methods

10 patients of Refractory Cerclage (Resistant) [a term

we introduced which means failure of repeated vaginal Cerclage (3 times) done by expert gynecologist and with no contraindication for the operation]

Mean age 38 ± 3.2 years, Mean number of failure Cerclage 8.2 ± 2.6 with ten times as a large number and 3 times as small number, gynecological examination revealed no contraindication for vaginal Cerclage, Four of them done in the same center and six done in different centers after explaining to the patient and her relatives the technique, informed consent was taken.

Preparation of human menstrual blood stem cells was done by the same method introduced by Te Liu et al 2014 which included: Cells cultured, Flow cytometry analysis, Western blotting analysis, Immunofluorescence staining [10].

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 and 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 4 weeks after the procedure [11], Collagenase[12,13,14], Detection of aquaporins type AQP3, AQP4, AQP5 in the cervical smear cell by immunohsitotchemistry [15,16] 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 smears, two observers each unaware of the identity of slides evaluated the staining intensity the average value from the two observers was calculated [17]

The amount of cell suspension differ from patient to patient. The senior author introduce a new equation for the amount of cell suspension to be injected = length of the cervix × diameter of the internal os from inner to inner × diameter of the internal os from outer to outer [18]

3 Ethics:

Informed Consent and explaining all the details of the procedure to the patients, and all procedures involving human subjects complied with the declaration of Helsinki 1975 and revised in 2000, again all the procedures accepted from the ethical committee of Heliopolis research center and Heliopolis Hospital.

Statistical analysis

Difference between IL8, collagenase, AQP3, AQP4, AQP5 before intracervical injection of HuMenSCs and at the time of normalization of cervical assessment were analyzed by two tailed test, chi-square test was used for comparison a difference of < 0.05 was considered significant.

4 Results

Ten pregnant women at a period of gestation between 8-10 weeks were subjected to autologous intracervical injection of human menstrual blood stem cells. Seven out of ten ended with full term delivery by caesarian section two ended with delivery at 32 and 34 weeks and one case abortion (16 weeks)

Table (1): Cervical mucus collagenase, IL-8 before autologous human menstrual blood stem cells "(HuMSCs)" cervical injection and after 4 weeks (n= 10).

Character	Before HuMSCs Injection	After 4 weeks	P value
IL-8	5.11±2.1	1.2±0.5	P<0.05
Collagenase	6.21±1.5	12.9±1.8	<0.05

Table (2): Staining intensity of AQP3, AQP4, and AQP5 in cervical smear. Before HuMSCs and after 4 weeks (no. 10):

(Type AQP)	Before HuMSCs Injection	After 4 weeks	P Value
AQP3	5.1±2.99	1.1±1.88	<0.5
AQP4	6.2±3.18	2.1±2.99	<0.05
AQP5	7.5±4.1	3.1±1.56	<0.001

5 Discussion

HuMSCs can overcome the ethical problems associated with the use of HuESCs and reduce the possibility of immune rejection; therefore, if solutions become available for the source limitations HuMSCs ay become an attractive source for cell therapy.

Many studies have indicated that when stem cells were transplanted into a specific microenvironment they are stimulated by the niche, and while the release of cell growth factors stimulate the surrounding tissue regeneration, the cells may also be induced to differentiate into specific tissue or organ-like cells. However, stem cell transplantation in the treatment of preconditions must be safe and effective. In this study, we transplanted a novel and special source of HuMSCs, HuMenSCs derived from the endometrium, into the cervix of resistant Cerclage or Refractory Cerclage is a new term we introduced for the first time in literature it means failure of repeated vaginal Cerclage (3 times) done by expert gynecologist and with no contraindication for the operation and in suitable place with all the essential requirements of the operation was needed, the solution of this clinical problem in reviewing the literature is to repeat the Cerclage(endless Cerclage) or some advised to do abdominal Cerclage, The method described in this study has a number of advantages over current approaches. First, the method of isolation and enrichment of HuMenSCs is very convenient. Previous studies have demonstrated that female menstrual blood contains a large number of MenSCs and that HuMenSCs

are excreted in each menstrual cycle along with blood cells.Furthermore it was found that HuMenSCs derived from the endometrium express high levels of mesenchymal stem cell biomarkers, suggesting that these cells possess the ''stemness'' of mesenchymal stem cells. The source and the method of enrichment and isolation of these cells are very simple.

Second, HuMenSCs are similar to HuMSCs derived from the other sources. Third, in our previous work we found that cervical microenvironment stimulated and induced differentiation in HuMenSCs.

HuMenSCs are advantageous over mesenchymal cells as they are derived from menstrual blood, and we found that they could be stimulated to differentiate into cervical like fibromuscular tissue in cervical microenvironment.

The amount of cells injected in the cervix is based upon equation introduced by the authors: the amount of cell injected= length of the cervix × diameter of the internal os from inner to inner × diameter of the internal os from outer to outer [18]

The technique is very easy and is done as outpatient procedure and this is added to the value of this technique we reached to full term delivery in 70% and preterm delivery (32-34 weeks) in 20% with failure in 10 % of the cases.The method of delivery in all cases is by caesarian section which was done on the bases of extensive cicatrization from the previous failed Cerclage.

Mean birth weight of full term pregnancies was 3.995 ± 0.805 kg and the mean Apgar score at 1 and 5 minutes was 8.5 ± 0.1/ 9.5 ±0.2 respectively, this means that our technique has a positive impact of fetal development.

In our work we didn't encounter pregnancy induced hypertension, again this add to the positive impact of our techniques on the feto-maternal adaptation.Normalization of cervical internal assessment was achieved after 4 weeks of the procedure.

The biochemical basis of our results is based upon statistically diminished collagenase enzyme (Table 1) at the time of normalization of cervical assessment the technique acts by stimulating a new collagen fiber and increase the number of collagen fibers in the cervix this means that HuMenSCs either transferred to collagen and muscle fibers or stimulates the collagen and muscle fiber in the cervix it was demonstrated [19] that increase in collagen type I which is a rigid fibrous 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.

Regarding cervical mucus IL-8 we demonstrated a statistically significant decrease at the time of normalization of the cervical assessment (4 weeks).IL.8 is a marker of infection meaning that our technique reduce the incidence of infection contrasting to Cerclage operation in which infection is raised, the reduction of infection is based upon the fact that (HuMenSCs) is a major source of proteinase nexin-l which is a strong antimicrobial [10, 20].

We studied aquaporin in the cervical smear for the first time in the literature before the procedure and at the time of normalization of the cervical assessment we found the statistically significant decrease in AQP3 and AQP4 and statistically highly significant decrease in aquaporin meaning that our technique works by other mechanism which through the reduction of expression of AQP. This means that the cervix become more rigid and this attributed partially 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.

It was demonstrated that HuMenSCs improved skin graft survival [21]. And HuMenSCs is a rich source of early pregnancy factor which has an immunesuppressive and growth factor properties [22].

The advantage of our technique are: outpatient, non-surgical, no need for antibiotics, and no anesthesia, positive effect on the fetus, strong antimicrobial, Cheap and easy learning curve.

6 Conclusion

Treatment of Refractory (Resistant) Cerclage by cervical injection of autologus human menstrual blood stem cells (HuMenSCs) 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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