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Case Report

Autologus Stem Cell Therapy in Congenital Endometrial Aplasia

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Summary

Primary amenorrhoea due to a congenital absence of endometrium without uterine underdevelopment is rare though a few cases have been reported. The endometrium is cyclically regenerated from stem cells residing in the stratum basalis. The bone marrow stem cells havethe ability to differentiate into endometrial lineages. In the present study we propose autologus stem cell transplantation for de novo generation of the deficient endometrium.

An 18 year old girl was diagnosed with congenital endometrial aplasia after a complete evaluation including genetic, biochemical and hysteroscopic examination for primary amenorrhoea. Bone marrow aspiration was performed under aseptic conditions from posterior superior iliac crest. Harvested autologus stem cells were transferred into uterine cavity using ET catheter on the following day. This was supplemented with cyclical hormonal therapy, Aspirin, L-arginine and Sildenafil.

Post stem cell transplantation, the patient developed normal menstrual cycles and USG confirmed the presence of a functioning endometrium.

The paper promotes the use of Stem cells transplantation as a new modality in the management of Congenital Endometrial Aplasia (CEA) and encourages further research towards developing novel therapies using autologus stem cells.

Autologus StemCell Therapyin Congenital Endometrial Aplasia

Keywords: Amenorrhoea; Autologus Stem Cell; Congenital Endometrial Aplasia; Endometrium

Synopsis

A case of Congenital Endometrial Aplasia was successfully treated using autologus hematopoietic stem cells resulting in *de novo* development of endometrium and onset of menses

Abstract

Objective

Primary amenorrhoea due to a congenital absence of endometrium without uterine underdevelopment is rare though a few cases have been reported. The endometrium is cyclically regenerated from stem cells residing in the stratum basalis. The bone marrow stem cells have the ability to differentiate into endometrial lineages. In the present study we propose autologus stem cell transplantation for de novo generation of the deficient endometrium.

Method

An 18 year old girl was diagnosed with congenital endometrial aplasia after a complete evaluation including genetic, biochemical and hysteroscopic examination for primary amenorrhoea. Bone marrow aspiration was performed under aseptic conditions from posterior superior iliac crest. Harvested autologus stem cells were transferred into uterine cavity using ET catheter on the following day. This was supplemented with cyclical hormonal therapy, Aspirin, L-arginine and Sildenafil.

Result

Post stem cell transplantation, the patient developed normal menstrual cycles and USG confirmed the presence of a functioning endometrium.

Conclusion

The paper promotes the use of Stem cells transplantation as a new modality in the management of Congenital Endometrial Aplasia (CEA) and encourages further research towards developing novel therapies using autologus stem cells.

Introduction

The case study reports for the first time, a case of primary amenorrhoea due to congenital absence of endometrium without an associated absence of uterus. The patient was treated with adult autologous stem cells for *de novo* generation of endometrial lining that resulted in restoration of normal menstrual cycle.

Endometrium aplasia is a differential diagnosis for primary amenorrhoea. Primary amenorrhoea is defined as the absence of menarche by 16-18 years of age in the presence of well-developed

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secondary sexual characters. The reported incidence [1] of primary amenorrhoea in Indian population is 2.5%. The etiology of the disorder includes congenital, metabolic, endocrine, economic (malnutrition) and infectious causes [2].

The endometrium is a dynamic, cyclically regenerating tissue that provides a unique model of physiological angiogenesis in adults. Angiogenesis results from sprouting of new vessels through recruitment of local endothelial cells from neighbouring blood vessels. Bone marrow-derived endothelial progenitors also contribute to angiogenesis of the endometrium [3]. Based on these facts, adult autologous bone marrow stem cells were utilised in this case for developing a whole new endometrial lining.

The Case

On 26th December 2012 an 18-year-old girl presented with symptoms suggestive of primary amenorrhoea. Past history revealed that the patient had developed breasts, pubic and axillary hair by the age of 14 years. Her general health had always been goodand in the past she was administered progesterone following which she had no withdrawal bleeding. Medical history and family history of patient was not significant. On examination it was noted that she was 162 cm tall and showed no physical features suggestive of being eunuchoidal. She had well-developed secondary sexual characters and her breasts and pubic hair belonged to Tanner stage IV. Vaginal examination revealed a normal sized uterus measuring 58×20×33mm with a well-developed vulva and vagina. Excess facial hair was present on cheeks for which the patient stated she was receiving electrolysis.

An ultrasound scan revealed normal size uterus with endometrium of 3 mm thickness (Figure 1A) and bilateral polycystic ovaries.

Tests showed that her dihydroepiandrosterone (DHEA) level was 184.9 ug/dl, serum free testosterone level was 0.71 mmol/l, lutenizing hormone (LH) level was 9.82 mIU/ml and follicle stimulating hormone (FSH) level was 3.97 mIU/ml. Cytogenetic examination showed that her karyotype was 46 XX. Her chest X-ray was normal.

She was advised to take tablet spironolactone 50mg twice daily for three months for the treatment of hirsuitism.

On 22nd February 2013, she was given cyclical oestrogen and progesterone therapy following which there was no withdrawal bleeding. Hysteroscopic evaluation of the uterine cavity revealed the absence of an endometrial lining with blood vessels seen through a mucous lining (Figure 2A-D). Endometrial curettage obtained no endometrial tissue. A nested polymerase chain reaction (PCR) was performed on the curettage sample to rule out the possibility of mycobacterial infection. Mycobacterial DNA was notdetected in sample, thus excluding mycobacterial infection as a possible cause for amenorrhoea. The patient was administered with 3 cycles of oral conjugate oestrogen and progesterone tablets; however, there was no increase in endometrium thickness on ultrasonographic examination during treatment, and there was no vaginal bleeding.

The case was given a confirmed diagnosis of Congenital Endometrial Aplasia (CEA) following extensive tests. The patient was advised for autologous stem cell transplantation for *de novo* development of endometrial lining.

Autologous stem cells have been used to repair cartilage and regenerate cardiomyocytes post myocardial infarction [4]. Using the same principle we attempted to restore the morphology of the endometrium using autologous haematopoietic stem cells.

The procedure was explainedin detail to the patient and her parents, and informed consent was obtained from the patient.

Bone marrow aspiration was performed under aseptic conditions from the posterior iliac crest. In total, 40 ml of autologous bone marrow was pooled and was used for abstraction, separation and culture of autologous stem cells. Autologous stem cells consisting of 2% of CD34 cells and the remaining content of CD133, CD73, CD90, CD105 cells, plasma cells and less than 1% of red blood cells which was similar to endometrial stem cells were used. The cells were separated using "Ready Cell" protocol(Sepax® Software Protocol).

On 19th November 2013, autologous stem cells transplantation was performed on the patient under general anesthesia. Labotect ET catheter attached to a 1-ml syringe, filled with 0.7 ml stem cell suspension was advanced gradually using real-time ultrasoundguidance to a point approaching but not touching the uterine fundal end. The catheter was moved to a point 5 mm from the fundal limit of the uterine cavity and the piston was gently advanced to facilitate steady flow of the stem cell suspension into the uterine cavity.

The patient was started on a drug regimen consisting of cyclical oestrogen (6mg), L-arginine sachet, Sildenafil (50mg for two days), for 21days from the day of transplantation. Aspirin (75mg) was started from the same day of transplantation and was continued until 11 days. Cyclic micronized progesterone (400mg) was added on the 11th day after the transplantation.

This drug regimen supplemented in the regeneration of the endometrium by the transplanted autologous stem cells.

An ultrasound was performed on 3rd December 2013, (13 days after transplantation) which revealed the presence of trilaminar endometrial lining with an endometrial thickness of 7.0 mm and distinct vascularity reaching sub-endometrial region (Figure 1B). There was evidence of myometrial contraction perceived as endometrial peristalsis noted in antegrade and retrograde pattern.

Vaginal bleeding occurred on 16th December 2013, two days after discontinuing cyclical oestrogen and progesterone intake. Mid-cycle ultrasound scans revealed further improvement in endometrial morphology and vascularity.

Post stem cell transplantation, hysteroscopy was not done as it is an invasive procedure and is not cost effective. Moreover, the patient had regular menstrual cycles (7 cycles until now) thereby overruling the need for repeat hysteroscopy.

Discussion

This is the first successfully treated case of CEA using autologous bone marrow derived stem cells.

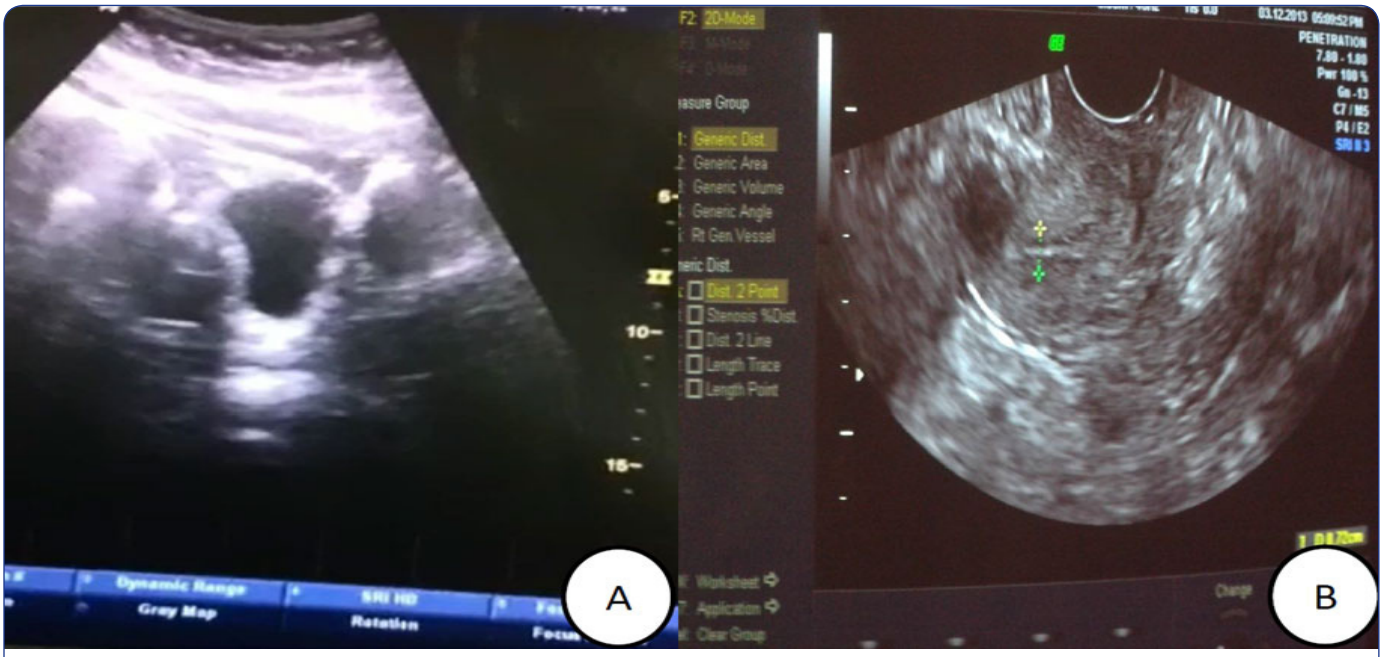

Figure 1A: USG showing no endometrial lining.1B: USG scan post autologous stem cell transplantation showing the presence ofendometrium.

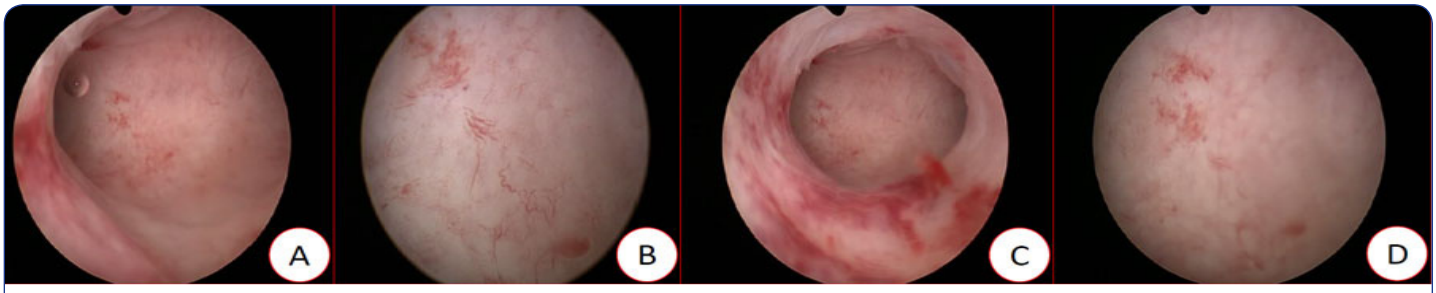

Figure 2A-D: Hysteroscopy showing a uniform absence of endometrial lining.

The patient had developed secondary sexual characters at the pubertal age. She had a normal XX karyotype with normal female levels of testosterone, LH and FSH. All these diagnostic studies confirmed the normal functioning of the hypothalamic-pituitary-ovarian axis.

The endometrial biopsy was sent for detection of mycobacterium tuberculosis DNA PCR. This test is performed by using a detection and quantification system of mycobacterium tuberculosis complex on Rotor Gene Real Time PCR based on Taqman principle.

This test measures the amount of Tuberculosis bacteria present in the sample and are reported as number of copies per milliliter (Copies/ml), if the patient is positive for Mycobacterium Tuberculosis. If the test result reported as not detected means the patient is not infected with Mycobacterium Tuberculosis or the infection may not be detected in particular simplest PCR for mycobacterium was negative in endometrial sample, thus ruling out the possibility of a mycobacterial infection.

Hysteroscopy studies confirmed the non-existence of Asherman's syndrome but depicted complete absence of the endometrium.

The 3 mm thickness of endometrial lining detected in ultrasound scan suggested only mucosal lining and not endometrial thickness which is similar to atrophic endometrial lining seen in post-menopausal women.

The evidence confirmed the diagnosis of the condition, CEA. CEA is a very rare disorder and only few cases have been reported in the literature so far. Although cyclical hormone therapy is a commonly used regimen for endometrial regeneration [5], in this case hormone treatment failed to cause any withdrawal bleeding and improvement in the endometrial morphology.

The human endometrium is a dynamic, remodeling tissue that undergoes more than400 cycles of regeneration, regression and shedding during a woman's reproductive life [6]. Histologically, the endometrium is composed of an upper functional is and a lower basal is layer. The functional is layer is sloughed off with each menstrual cycle where as the basalis layer induces rapid regeneration of the functional is layer in the proliferative phase of menstrual cycle.

Similar pattern of tissue regeneration is also evident in the bone marrow haematopoietic tissue, the epidermis and intestinal epithelium [7]. Somatic stem cells cause cellular growth and

differentiation at these sites by constantly regenerating tissues. It has been hypothesized that the basal is layer harbours these adult stem or progenitor cells which are responsible for the cyclical renewal of the functional is layer of the endometrium each month [8].

Stem cells derived from bone marrow, adipose tissue and umbilical cord have been studied extensively for the development of novel therapies [9]. Studies have indicated that the presence of donor-derived bone marrow stem cells in female endometrium can originate endometrial cells, which suggests that cells of non-uterine origin can differentiate and contribute to endometrial regeneration. [3, 10-11]. Mints *et al* [12] reported that bone marrow-derived stem cells contribute to neo-vascularisation of the endometrium.

This case is based on a study that reports the presence of a subpopulation of adherent mononuclear cells that retains expression of markers CD9, CD29, CD41a, CD44, CD59, CD73, CD90 and CD105, without karyotypic aberrations. [13]. The protocol for cellular therapy requires strict standards and control of all steps including Bone Marrow collection, processing into concentrated cells and reinjection of cells for the therapy. The cells required for stem cell therapies are Hematopoietic Cells (CD34), Progenitor Endothelial Cells (CD133) and Mesenchymal stem cells (MSCs). The Bone Marrow has a good amount of above said cells. These cells are used in various clinical applications. The harvested bone marrow of 100 ml was collected in a blood bag with pre irrigated anticoagulant solution in a ratio of 1:8. The bone marrow of the patient was processed in Sepax® an Automatic Stem Cell Processing unit, using the "ReadyCell" protocol (Sepax® Software Protocol). It is designed for routine processing of Bone Marrow to isolate, and to concentrate the nucleated cell component fraction enriched in stem cells. The 100ml bone marrow (including anti-coagulant) was concentrated to a predetermined final volume of 10ml of concentrated stem cells. The concentration was performed in 20 mins. The concentrated stem cells were evaluated and found to be 200 million cells/ml of WBC. The concentrated stem cells consisted of 2% of CD34 cells and the remaining content consisted of CD133, CD73, CD90, CD105, Plasma cells and less than 1% of Red cells.

In the present case of CEA, the endometrial progenitor stem cells present in the basal is layer were either nonexistent or had lost their ability to regenerate the endometrium. Therefore, we attempted for *de novo* growth of the endometrium by transplantation of autologous adult stem cells into the endometrium. The procedure was furthersupplemented with cyclical hormonal therapy (along with Arginine and Sildenafil) in an effort to obtain optimal vascularisation of the endometrium.

Conclusion

This is the first case of CEA treated with autologous stem cells. The authors encourage the implementation of this therapy in cases such as complete absence of endometrial lining or damaged basal is layer. This is a simple cost-effective procedure with negligible risk of malignancies and is a minimally invasive procedure. Moreover, it has a psychological and social advantage that the patient who was unlikely to conceive, can bear her own children.

To the best of our knowledge, this is the first report of *de novo* endometrial generation by adult autologous stem cell transplantation leading to onset of menstrual cycle in a patient diagnosed with primary amenorrhoea due to CEA.

Acknowledgements

The girl whose story is told in this case report has provided written consent for its publication.

Conflict of interest Statement

The authors declare that there are no conflicts of interests.

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