

## Conference Paper

# The Use of Human Dermal Papillae Conditioned Medium (HDPCM) on Aplasia Cutis Congenita in Lower Extremity due to Intrauterine Varicella Infection

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## Abstract

Aplasia cutis congenital (ACC) in lower legs is a rare disease with the absence of the skin, which commonly localized, widespread or scarred at birth. The exact pathophysiology remains unclear, and varicella infection may have essential roles in the aetiologies. Wound healing can be accelerated by using Human Dermal Papillae Conditioned Medium (HDPCM) which has multi-lineage potential differentiation and releases many growth factors. This paper reports the possibility of using human dermal papillae conditioned media to speed up the wound healing process. A 4 days old baby boy presented with ulcers on his both knees since birth and had no history of birth trauma. The mother had varicella infection during the 2<sup>nd</sup>-trimester of pregnancy. From the dermatological examination, we found defects and total skin loss on both knees. The result of the serology test showed the persistence of varicella-zoster virus immunoglobulin in both patient and his mother. The result of histopathological test supported the diagnosis of ACC. The patient was given topical therapy using 0.1 ml of HDPCM and 10 gr of water-based antioxidant gel and showed improvement after ten days of administration. Evaluation of a patient with ACC by the detailed history and examination could determine varicella as the etiology of the disease. Furthermore, work-up should be based on symptoms and clinical findings. The HDPCM contributes to neovascularization and tissue maturation which in turn could reduce inflammation, resulting in a better healing process with less scar and wound contraction, thus hasten the healing process. We conclude that the HDPCM can be used to accelerate the wound healing process in ACC due to varicella infection.

**Keywords:** Aplasia cutis congenital, Human Dermal Papillae, Conditioned Media, Varicella

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## 1. Introduction

Aplasia cutis congenital (ACC) is a rare malformation that is characterized by the total absence of all layers of skin at birth. The lesion commonly presents on the scalp, but

several studies reported the lesion was on the trunk and extremities. After birth, the diagnosis of ACC is determined from the clinical and histologic examination.

Until now, no embryologic theory supports the etiology of ACC [1, 2] and the pathophysiology of ACC is not clear. Though genetic factor plays an essential role in ACC, other factors may contribute and are responsible for the mechanism. These factors included intrauterine trauma, local amniotic adhesions, and exposure to varicella and herpes simplex infection. The teratogenic agents, for example, antithyroid drugs, valproic acid, marijuana, heroin, alcohol, and cocaine may contribute to ACC [3]. The condition also may be associated with abnormalities of chromosomal, ectodermal dysplasias, epidermolysis bullosa, specific teratogens, intrauterine infections, or other malformation syndromes like Adams-Oliver syndrome, SCALP syndrome (nevus sebaceous, central nervous system [CNS] malformations, aplasia cutis congenital, lumbal dermoid, pigmented nevus) and Opitz syndrome [3, 4]. The incidence of varicella in pregnancy remains unclear although infection may lead to severe illness in pregnancy and may associate with transplacental virus transmission and fetal infection.

The treatment of ACC remains a dilemma with no agreement upon methods. Both conservative and surgical approaches to the early management of tissue defects have been reported, with varying results [2, 5, 6]. There were 4 cases of ACC reported in the literature, the cases were caused by varicella infection in the first and second trimester of the pregnancies. The last case was reported by Gupta et al. in 2014 where the lesion appeared on the thigh to the knee of the twin [7, 8]. Management of ACC is using conservative, surgical or a combination of both. Conservative treatment for small ACC lesions using petrolatum, silver sulfadiazine or bacitracin aimed to give rise to granulation and healing with scars [2, 5]. Shirvany et al. reported that ACC repair using mupirocin took about two months, and in cases with no scalp wounds, the healing process took 38 days [4, 9]. The use of acellular dermal graft and cultured epithelial autograft has also been reported with full repair in 7 to 14 days [6].

The HDPCM is a secretion factor of stem cells obtained from dermal papilla cultures. The dermal papillae have the most stem cells compared to other hair parts. The use of HDPCM has been widely reported to accelerate wound healing in the presence of various growth factors that contribute to wound healing. These growth factors include insulin-like growth factor-I (IGF-I), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and TGF- $\beta$ 1 [10]. The use of HDPCM in the ACC case has never been reported before. We report the use of HDPCM in the ACC case which was expected to accelerate wound healing.

## 2. Case and Methods

A four-day-old baby boy was born from a 28-year woman with 35 weeks of pregnancy. He was delivered vaginally with the body weight of 2900 gram. The infant was born with the absence of the skin congenitally that involved both knee and upper thighs. The skin lesions revealed a thin translucent membrane covered the skin defect and dermal vessels were easily visible. The wound tended to exude with serous fluid and distributed symmetrical and bilateral. The healthy skin surrounding the wound appeared dry and scaling (Fig.1). No drugs were consumed during the mother's pregnancy and the baby had a non-consanguineous father. His mother suffered from varicella during the second trimester of pregnancy.

On physical examination, the neonate had a skin defect on both knees with the size of 2x2 cm and 3x1cm and had inflammation surrounding the ulcers. On laboratory examination, serologic results revealed high titers of varicella-zoster virus (VSV) immunoglobulin (IgG) 2.08 in the baby and 2.79 in his mother; those results were three times higher than reference and were interpreted as positive detection of VZV IgG. There were no abnormalities in babygram and thorax radiology. The radiologic examination of extremities showed soft tissue swelling, no bone deformities with a blastic and lytic lesion. The histologic examination demonstrated vacuolar degeneration and rete-ridges flattening, an absence of adnexal structures, edema of dermis and dilatation of vasculature, thus confirmed the ACC group VII according to Frieden's clinical classification. The affected areas were given 0.1 ml of HDPCM and 10 gr of water-based antioxidant gel; then the wounds were dressed exclusively. The dressings were changed regularly every three days. On day 10, the defect closed completely.

### 2.1. Topical HDPCM

The isolation of dermal papillae stem cells was carried out by cutting 3x3 mm<sup>2</sup> of dermis tissue, incubated at 1:1 PBS, and disposed at 4 °C for 16-18 hours. After cell suspension and neutralization with complete media, then the cells were cultured in a 60 mm Petri with an entire culture medium. Cells subculture was then carried out with warm trypsinisation techniques using EDTA trypsin 0.25%. Media from confluent cell cultures were then discarded and washed with PBS for 2x. Cells that were released from Petri culture were neutralized with complete media as much as 2x the volume of trypsin and after centrifugation, they are re-cultured in complete media or kept frozen until used for treatment. The characteristics of hair bulge stem cells were evaluated using the flow

cytometry method using anti CD59 and CD 200 antibodies according to the protocol recommended by BD.

Conditioned dermal papilla media is produced to induce folliculogenesis by improving the niche in the environment in which the dermal papilla as a supporter of hair follicles. The technique used to stimulate the secretion of bioactive metabolites from cell cultures is a three-dimensional culture technique using fibrin gel coating on Petri culture. Twenty hours after incubation, the media was collected and filtered with a 0.45  $\mu\text{m}$  Millipore filter then stored at  $-80\text{ }^{\circ}\text{C}$  until ready for use. Ten grams of antioxidant gel were used as DP-CM carriers and applied directly to the wound area.



**Figure 1:** Day 0. Skin defect on the right and left knee, then given topical HDPCM.



**Figure 2:** Day 10. Evaluation of the lesion showing a complete closure after ten-day administration of HDPCM.

### 3. Discussion

Ulceration in newborns can be associated with several conditions, and one of them is ACC [9]. Aplasia cutis congenita is a rare congenital condition [2] in the absence of skin, most often involving the epidermis and dermis [11] and in some cases involve subcutan area [9]; which usually occurs in a localized or wide area, or appears as scar

at birth [11]. Cutaneous aplasia can occur as an isolated defect, associated with other developmental abnormalities, or as a description of various abnormalities. There is no single underlying cause in the occurrence of ACC which shows the physical findings of intrauterine skin development abnormalities. Other etiologies may include genetic factors, vascular disorders, trauma, teratogens, and intrauterine infections [11].

About 15% of ACC lesions occur in the trunk and extremities [12] and are generally bilateral and symmetrical. However, asymmetrical distribution has been reported [9]. Defects involving the trunk and extremities often have more extensive lesions than the scalp, but have a better prognosis and tend to heal faster [12]. Cutaneous congenital aplasia in the lower extremities is a rare disorder [11].

Varicella-zoster virus can induce various forms of diseases, from mild to severe and can be treated satisfactorily. In pregnancy, this virus can also affect the mother and fetus, causing maternal, fetal and neonatal abnormalities [13]. Vertical transmission can be detected using polymerase chain reaction (PCR) before the 24th week of pregnancy in 24 and 8% of varicella cases. Varicella in pregnancy can cause spontaneous preterm birth, low birth weight, intrauterine growth restriction, congenital varicella syndrome or maternal varicella pneumonia and varicella in neonates [14].

From history taking, we were informed that the mother of our present patient had a history of varicella at the second trimester of pregnancy and is supported by the positive results of VZV IgG in the patient and the mother. The primary factor in the occurrence of ACC from this patient is a history of varicella infection intrauterine (during pregnancy).

The treatments for ACC are reported using conservative and surgical tissue defects. ACC skin lesions often heal gradually and leave hypertrophic or atrophic scars (Figure 1 and 2), skin contractures, and hypopigmentation or hyperpigmentation. Healing can occur spontaneously through granulation and re-epithelialization from the edges of the normal skin [2]. Handling for small ACC lesions tends to be conservative, including local wound care that will cause granulation and scar healing [3]. Local wound care is recommended for small lesions of less than 3 cm<sup>2</sup>. The advantage of conservative treatment is to avoid the risk of surgery in newborns [15]. An earlier repair surgery [13] is recommended for extensive skin defects or if the defects affecting the bone [5]. Radiological examination before surgery is needed to identify the structure of the blood vessels underlying the lesion [11]. Surgical revision for scarring that appears later in childhood or adulthood can be done electively for cosmetic improvement [14].

Eurocat, an institution that collects statistical data on congenital abnormalities throughout the world, classifies ACC as a congenital skin disorder and has a total of 177 cases of ACC registered in Poland in 1999-2009. The Polish Registry of Congenital

Malformations has collected data on 168 cases of AKK that occurred in the area surveyed in 1998-2008; 2 cases included ACC with defects in the lower extremities. Aplasia cutis congenita of the extremities is characterized by the loss of skin with well-defined lesions, most often involving the epidermis and dermis. These well-defined lesions can involve muscles and bones. Lesions can be ulcerated, with a smooth pink surface where the epidermal is lost or reduced. The dermis, if present, is thin and has no appendages. Lesions are usually small, about 0.5-3 cm in diameter [15].

In recent years, many efforts have been made to enhance the progress and modalities of wound therapy, including the stem cells and its growth factors [16]. The use of Mesenchymal Stem Cells (MSCs) is a new approach to the treatment of wounds, especially chronic wounds [17]. MSCs have their characteristics such as the ability to self-renew and have the ability to differentiate into various types of tissue with asymmetric replication into complex structures such as osteoblasts, adipocytes, chondrocytes, tenocytes, myocytes and epithelialization of the skin. Many sources of stem cells can be used to modulate wound healing responses in acute and chronic disease, such as bone marrow, cord blood, adipose tissue, skin and hair follicles [16, 18].

One of MSC populations on the skin is dermal papillae (DP), which is a stem cell with multilineage differentiation potential. The role of DP is known not only to regulate the development and growth of hair follicles but also considered as a reservoir multipotent stem cells that have the potential to differentiate into various types of cells that have important therapeutic potential. In the hair growth cycle, the role of DP begins at post-natal where hair follicles grow in several phases, resting or telogen phases, growth or anagen phases and regression phases or pathogenesis. During the catagen phase, the epithelial cells at the base of the follicle undergo apoptosis, but the DP remains intact and will migrate upwards until it ends at the bulb stem hair follicle. This situation continues during the telogen phase. In the anagen phase, DP cells at the base of the follicle begin to multiply, which results in the downward growth of the follicles and closure of the DP [19].

Meanwhile for wound healing, *in vitro* experiments reported that the use of DP stem cells for wound healing showed good results, the result seen from good wound healing without scarring and minimal wound contraction, good neovascularization and shortened inflammation time. The beneficial result is because the dermal papilla has various growth factors that contribute to the healing process of the wound [10]. Some of these growth factors include insulin-like growth factor-I (IGF-I), hepatocyte growth factor (HGF), VEGF and TGF- $\beta$ 1 [20].



## 4. Conclusion

We reported the case of aplasia cutis congenita, which involved both knees. There was the absence of the skin, and the surrounding skin appeared redness. The lesion was managed with topical HDPCM and the wounds closed completely in ten days.

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