Characteristic Morphologic Abnormality of Harlequin Ichthyosis Detected in Amniotic Fluid Cells

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We have examined cells from amniotic fluid obtained at 17 and 21 weeks' gestation and fetal skin biopsy samples from a fetus at risk of harlequin ichthyosis by light and electron microscopy. Clumps of abnormally keratinized cells that had a large number of lipid droplets in the cytoplasm were seen within both the 17- and 21-week amniotic fluid cell pellets. The cells in these clumps were similar to the thick layers of keratinized cells observed in the skin biopsy and autopsy samples. Morphologic examination of the fetal skin biopsy samples obtained at 21 weeks gestation revealed the characteristic changes of harlequin ichthyosis. The intraepidermal portions of hair canals had an excessive number of layers of keratinized cells. Normal lamellar granules were absent but abundant membrane-bound vesicles of a similar size and a number of dense bodies were observed in the cells of the upper intermediate layers of the epidermis. Autopsy skin samples of the terminated fetus at the twenty-third week of gestation showed structural changes that corresponded to those of the amniotic fluid cells and the fetal skin biopsy samples, although the peri-derm was gone in all the regions. Our findings of amniotic fluid indicate that the characteristic epidermal abnormality of harlequin ichthyosis has been expressed at 17 weeks gestation in some parts of the body or structures of fetal skin (e.g., hair canals) that keratinize before interfollicular epidermis. Moreover, the results suggest that harlequin ichthyosis can be detected in utero by morphologic analysis of amniotic fluid cells obtained by amniocentesis, Key words: prenatal diagnosis/lamellar granules/electron microscopy. J Invest Dermatol 102:210–213, 1994

Harlequin ichthyosis (HI) is a rare autosomal recessive hereditary disorder in which newborn infants are covered with a thick plate of stratum corneum. Ears and nose are underdeveloped and ectropion and eclabium are severe [1]. Although these clinical features are consistent in all HI cases, varying structural and biochemical findings have been reported [2–6]. Buxman et al. [2] reported increased triglyceride content in the epidermis in HI and suggested that a lipid defect may be related to the pathogenesis of HI. Further investigation revealed in HI cases could be classified into three distinct types on the basis of protein expression and ultrastructural characteristics of the epidermis. The common morphologic abnormalities include hyperkeratosis, accumulation of lipid droplets within corneocytes, and absence of normal lamellar granules [5]. The thick scale of newborn infants with HI results from the lack of stratum corneum desquamation and, recently, the basic defect in HI was suggested to be abnormality of lamellar granules, which play an important role in desquamation [7]. Infants affected with this severe type of congenital ichthyosis rarely survive the perinatal period due to respiratory and feeding difficulties and severe infection of the skin fissures [1], although a few cases of long-term survival have been reported [8–12]. Thus, once the parents have an HI infant, they are often reluctant to risk another pregnancy unless a reliable prenatal diagnostic test is available. Fetal skin biopsy has been shown to be valuable in identifying fetuses affected with a variety of genodermatoses (reviewed in [13]), including disorders of cornification. Fetal skin biopsy was performed in the twenty-first or twenty-second week of gestation in a few reported cases of positive prenatal diagnosis of HI [14,15]. In those cases, the observations of the skin samples revealed hyperkeratosis of the follicular and interfollicular epidermis and abnormal vacuoles within keratinized cells, and upon autopsy the fetus was found to have the harlequin phenotype.

We have examined by light microscopy (LM) and electron microscopy three fetal skin biopsy samples and cells from two aliquots of amniotic fluid obtained at 17 and 21 weeks estimated gestational age (EGA) from a fetus at risk of HI. The fetus was the second pregnancy in the family. The first offspring, affected with HI (to be reported elsewhere), provided information about the type of HI and upon autopsy the fetus was found to have the harlequin phenotype. We have examined cells from amniotic fluid obtained at 17 and 21 weeks' gestation and fetal skin biopsy samples from a fetus at risk of harlequin ichthyosis by light and electron microscopy. Clumps of abnormally keratinized cells that had a large number of lipid droplets in the cytoplasm were seen within both the 17- and 21-week amniotic fluid cell pellets. The cells in these clumps were similar to the thick layers of keratinized cells observed in the skin biopsy and autopsy samples. Morphologic examination of the fetal skin biopsy samples obtained at 21 weeks gestation revealed the characteristic changes of harlequin ichthyosis. The intraepidermal portions of hair canals had an excessive number of layers of keratinized cells. Normal lamellar granules were absent but abundant membrane-bound vesicles of a similar size and a number of dense bodies were observed in the cells of the upper intermediate layers of the epidermis. Autopsy skin samples of the terminated fetus at the twenty-third week of gestation showed structural changes that corresponded to those of the amniotic fluid cells and the fetal skin biopsy samples, although the periderm was gone in all the regions. Our findings of amniotic fluid indicate that the characteristic epidermal abnormality of harlequin ichthyosis has been expressed at 17 weeks gestation in some parts of the body or structures of fetal skin (e.g., hair canals) that keratinize before interfollicular epidermis. Moreover, the results suggest that harlequin ichthyosis can be detected in utero by morphologic analysis of amniotic fluid cells obtained by amniocentesis, Key words: prenatal diagnosis/lamellar granules/electron microscopy. J Invest Dermatol 102:210–213, 1994

 MATERIALS AND METHODS

Case History The patient was a 37-year-old Chinese woman in her second pregnancy. Her first child, born at 35 weeks of pregnancy, was affected with HI and died at 2 d of age. There was no family history of genodermatoses or consanguinity. Three months after the death of the first child, the...
patient became pregnant again and experienced no apparent complications of pregnancy. Initial ultrasound evaluation was performed at 17 weeks gestation using an Acuson 128XP10 (Acuson Corp., Mountain View, CA). There were no sonographic findings to suggest an affected fetus. The patient and her husband continued the pregnancy in part owing to the availability of prenatal skin biopsy to diagnose the disorder at 20–22 weeks of gestation. Because of advanced maternal age, they elected to have an amniocentesis at 17 weeks of gestation to determine fetal karyotype and to allow evaluation of fetal skin-derived amniotic fluid cells on a research basis.

Preliminary Study of the Proband Skin samples from the affected newborn infant, born at 35 weeks, were processed for LM and EM to confirm the diagnosis of the disorder and to characterize precisely the morphologic abnormalities expressed in an affected member of this family.

Amniotic Fluid Samples, Fetal Skin Biopsy Samples, and Skin Samples Obtained at Autopsy Amniocentesis of the fetus at risk was performed at 17 weeks gestation. Five milliliters of fluid were sent to Seattle for morphologic analysis of amniotic fluid cells and the remainder of the fluid was used for routine cytogenetic evaluation. Fetal skin biopsies were undertaken at 21 weeks gestation following the procedure published elsewhere [16]. A 14-gauge 3-inch angiocatheter was inserted above the biopsy site under ultrasound guidance. There were no sonographic findings to suggest an affected fetus. Additional 12-ml aliquots of amniotic fluid were withdrawn prior to fetal skin sampling. Three small samples of fetal skin were obtained from the back. There were no complications from the procedure.

Eight samples of skin from various regions of the body, including scalp and sole, were obtained at the time of autopsy after termination of the pregnancy at the twenty-third week of gestation.

Light and Electron Microscopy Amniotic fluid samples were mixed with an equal volume of one-half strength Karnovsky's fixative [Karnovsky MJ: J Cell Biol 27:137A–138A, 1965 (abstr)]. The fluid was then centrifuged and the cell pellets were post-fixed in 2% OsO₄. After dehydration in graded alcohols into propylene oxide, the cells were embedded in Epon 812 [17]. Skim samples were also pre-fixed in one-half strength Karnovsky's fixative post-fixed in 2% OsO₄, dehydrated, and embedded in Epon 812. All the amniotic fluid pellets (two pellets of 17 weeks gestation and five pellets of 21 weeks gestation) and skin samples were serially sectioned, sampled every 10–15 µm for LM (1 µm thick), and thin sectioned for EM (70 nm thick). The histologic sections were stained by the method of Richardson et al [18]. The thin sections were stained with uranyl acetate and lead citrate [19] and examined in JEOL 1200 EXII and Philips 420 transmission electron microscopes in the transmission mode at 60 or 80 kV.

RESULTS

Structure of Skin from the Affected Newborn (Proband) The granular and cornified layer cells showed the typical features of type I HI: lipid droplets in thick, compact stratum corneum, multivesicular bodies and autolysosomes in spinous and granular layer cells, and abnormal or absent lamellar granules. The transition between the granular and cornified cell was gradual and indistinct, in contrast to the abrupt transition between these two layers in normal epidermis. Lamellar granules were absent in spinous and granular layers. The cytoplasm contained empty vacuoles of various sizes. Cells of the stratum corneum contained the same type of vacuoles evident in the granular layer cells.

Amniotic Fluid at 17 Weeks EGA Amniotic fluid obtained by amniocentesis appeared clear and was normally colored to the naked eye.

Two different amniotic fluid cell pellets were examined. At the LM level, masses of aggregated keratinized cells were seen (Fig 1A), although these were observed infrequently; both of the cell pellets were cut through their entire thickness and five aggregations of keratinized cells were found.

Electron microscopic observation revealed that these masses contained fully keratinized stratum corneum cells joined by modified desmosomes (Fig 1B). The cytoplasm contained strikingly electron-dense amorphous material, filaments, and empty vacuoles, which probably had been occupied by lipids before chemical fixation (Fig 1C). These masses of keratinized cells were probably desquamated from the hair canals or from regions of the body that may keratinize early (e.g., palms and soles); the similar, thick layers of dense keratinized cells were observed in the stratum corneum of the autopsy sample from the sole.

Amniotic Fluid at 21 Weeks EGA Seventy-four sections from five different amniotic fluid cell pellets were examined. Two types of clumps of keratinized epidermal cells were found. One type

Figure 1. Amniotic fluid cells at 17 weeks estimated gestational age. Masses of aggregated keratinized cells are seen at the light microscopic level (A). Electron micrographs revealing that the clumped cells are fully keratinized, joined by modified desmosomes (B), and contain empty vacuoles (presumed lipid droplets) (C). Bars: A, 20 µm; B, 1 µm; C, 0.5 µm.
Figure 2. Skin biopsy samples of the fetus. An electron micrograph (A) showing abnormal lamellar granules and vacuolated cytoplasm in the intermediate layer cells. The skin surface is covered with the periderm (p). At higher magnification (B), vacuoles containing dense bodies, parallel membranes (arrowheads), and discontinuous keratin filaments (asterisks) are evident in the intermediate layer cell. The intraepidermal portion of a hair canal (C) has an excessive number of layers of abnormal keratinized cells containing multiple vacuoles. Bars: A and C, 1 µm; B, 6.3 µm.

Fetal Skin Biopsy Samples Three fetal skin biopsy samples were examined. In each, most of the interfollicular epidermis was still covered with periderm that was normal in structure although the hair canals showed hyperkeratinization. Basal cells of the samples from the fetus at risk were similar to basal cells of normal age-matched fetal epidermis except that there appeared to be a greater density of cytoplasmic ribosomes and a more mottled pattern of nuclear heterochromatin. Lower intermediate layer cells demonstrated the same features as basal cells and, in addition, the cytoplasm was vacuolated and appeared to have less glycogen compared with similar cells in normal fetal epidermis. Abnormal cytoplasmic characteristics were more pronounced in the cells of the upper intermediate layers, where the cytoplasm was highly vacuolated and disorganized (Fig 2A,B). Normal lamellar granules were absent but membrane-bound vesicles of a similar size were evident. Vacuoles in the size range of mitochondria were abundant and contained a number of inclusions such as parallel membranes, dense bodies, and glycogen. Keratin filament bundles appeared to be smaller than usual, short, and discontinuous. Even though most of the epidermis was not keratinized, its overall structure was clearly abnormal and showed the same characteristics as the keratinocytes of the affected proband and of postnatal individuals affected with HI.

The intraepidermal portions of hair canals had an excessive number of layers of keratinized cells and structurally abnormal cells of the granular and cornified layers (Fig 2C). Granular layer cells of the follicular epidermis contained small keratohyalin granules surrounded by ribosomes. In most cells, the keratohyalin granules showed little association with keratin filaments. Mitochondria were large, swollen, and contained few cristae. There was no evidence of lamellar granules in the cytoplasm of either spinous or granular layer cells. Large vacuoles containing myelin figure-like membranes were sequestered in pockets between both proximal and distal layers of aberrantly cornified cells. All cornified cells contained multiple vacuoles and cytoplasmic remnants.

Autopsy Skin Samples of the Terminated Fetus Skin samples from eight regions of the body including the scalp and the sole were examined at autopsy. The structure corresponded to that of the fetal skin biopsy samples although the periderm was gone in all the regions. There was some regional variation in the morphologic characteristics of the skin. Thick layers of keratinized cells with electron-dense cytoplasm joined by modified desmosomes were most prominent in skin of the palms and soles. The epidermis of the other areas, including scalp, showed a range of characteristics that reflected variation in the keratinization defects, i.e., abnormal or absent la-
mellar granules, the thick compact stratum corneum, and low density of keratin filaments in granular and upper spinous layer cells. The lipid vacuoles and dense granules were apparent in all autopsy samples.

The interfollicular epidermis from some regions showed a thickened epidermis with multiple layers of flattened, incompletely keratinized cells. The morphology was comparable to that observed in fetuses of the same age affected with lamellar ichthyosis [17]. However, the vacuoles, small, abnormal membrane-bound organelles, variously shaped dense bodies, and large vesiculated mitochondria evident in the incompletely keratinized layers, granular, and upper spinous cell layers were characteristic of HI.

**DISCUSSION**

Previously reported cases of successful prenatal diagnosis of HI were based on examination of fetal skin biopsy samples obtained at 20–22 weeks gestation [14,15]. There have been no reports of the condition of the skin of fetuses affected with HI before the twentieth week of EGA. In the present study, we found the debris of the hyperkeratic epidermis in the amniotic fluid of the fetus at 17 weeks EGA by LM and the cornified cells had many presumed lipid droplets that were characteristic for HI. From our findings of amniotic fluid, characteristic epidermal changes of HI are thought to have been expressed at 17 weeks EGA in some parts of the body or structures of fetal skin that keratinize before interfollicular epidermis on the body (e.g., hair canals). We cannot exclude the variability in expression of HI depending on the individual and the family.

To date, ultrastructural abnormalities of skin-derived amniotic fluid cells have been reported only in cases of bullous congenital lamellar ichthyosiform erythroderma (epidermolytic hyperkeratosis) [20,21]. The results of our observation of amniotic fluid from the seventeenth week of gestation suggest that it may be possible to use this material for prenatal diagnosis of HI. The putative diagnosis was upheld by the ultrastructural examination of the amniotic fluid cells from the twenty-first week of gestation and confirmed by the structure of the fetal skin biopsy samples at 21 weeks EGA and autopsy specimens of 23 weeks gestation. Clumps of epidermal cells with characteristic changes of HI have never been seen in amniotic fluid from normal 17–21-week pregnancies, although, on extremely rare occasions, clumps of keratinized epithelial cells have been found in a few amniotic fluid samples (Akiyama and Holbrook, unpublished observations). These results strongly indicate that HI might be detected in utero by morphologic analysis of amniotic fluid cells obtained by amniocentesis.

Prenatal sonographic findings of fetuses with HI at 28 and 31 weeks gestation were reported [22,23]. The sonographic features were specifically sought at 17 and 21 weeks gestation in this pregnancy, but were not observed. Thus, sonography alone may not be diagnostic before the third trimester. On the other hand, careful examination of the fetus with the fetoscope might reveal the affected phenotype before the third trimester because the terminated fetuses in the reports of positive prenatal diagnosis of HI [14,15] clearly show macroscopic changes of the HI phenotype.

Cases of HI were classified into three distinct types and a consistent defect among all the types was the abnormality or absence of lamellar granules [5]. Recently, a defect in lamellar granules and their function have been identified in HI and the possibility that the defect could account for the lack of desquamation and massive accumulation of scale was suggested [7]. In the present study, abnormal findings that reflected a defect in lamellar granules, i.e., presence of multivesicular bodies and absence of lamellar granules in the epidermal cells and lipid droplets in stratum corneum, were obtained in the fetal skin and amniotic fluid cells. These results may support the role of the defect of lamellar granules in the pathogenesis of HI.

**REFERENCES**


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