Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 22 No. 2 Ser. No. 75 2021

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Computed tomography (CT) scan findings in calves with hydranencephaly

Raoofi, A.[1]; Gorjidooz, M.[2*]; Dehghan Rahimabadi, P.[1]; Masoudifard, M.[1]; Mardjanmehr, S. H.[3] and Esmailinejad, M. R.[1]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Garmsar, Iran; [3]Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

[*]***Correspondence:*** *M. Gorjidooz, Department of Clinical Sciences, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Garmsar, Iran. E-mail: gorjidoozm@yahoo.com*

10.22099/ijvr.2021.37031.5403

(Received 21 Apr 2020; revised version 18 Dec 2020; accepted 16 Jan 2021)

Abstract

Background: Hydranencephaly is the unilateral or bilateral loss of cerebral hemispheres and their replacement by a cerebrospinal fluid-filled sac that is caused by some teratogenic viruses. This disorder can be recognized before death occurs, using computed tomography (CT) scan. Pathological findings are also useful for the confirmation of the diagnosis. **Aims:** The aim of this study was to determine features of hydranencephaly in calves by CT. **Methods:** In this study, CT images were obtained from the skulls of 7 same-age calves with neurological signs, affected by Akabane virus, after slaughter. To compare the Hounsfield unit (HU) obtained from each brain region with the normal one, a normal calf's (with the same age) CT images were considered. Then, HU was measured in different parts of the affected calves' brain and compared with the normal calf's brain. **Results:** Computed tomography images of affected calves indicated a hypoattenuated density of cerebral hemispheres, and the mean value of HU significantly decreased in these parts of the brain compared to normal calves (P=0.0001). There was no statistically significant difference between the mean value of the brain stem HU of the affected calves and the normal ones (P=0.066). In the gross pathology, asymmetric fluid-filled sacs that replaced cerebral hemispheres were observed, but the brain stems were intact in all cases. **Conclusion:** The results showed a strong correlation between CT scan and pathology findings in calves with hydranencephaly. This is the first CT scan-mediated study of calves with hydranencephaly.

Key words: Calf, CT scan, Hounsfield unit, Hydranencephaly

Introduction

Central nervous system disorders, such as hydranencephaly, are common in cattle (El-khodery *et al*., 2008). Hydranencephaly is described as the unilateral or bilateral loss of cerebral hemispheres, while they are replaced by a cerebrospinal fluid (CSF)-filled sac. The pathogenesis is not clear, but the destruction of germinal cells of the subventricular zone of the telencephalon probably affects this (Baiker *et al*., 2010; Nietfeld, 2012). To date, the occurrence of hydranencephaly has been reported in lambs (Cordy and Shultz, 1961; Osburn *et al*., 1971), calves (Barnard and Pienaar, 1976), human infants (Dixon, 1988), kittens (Greene *et al*., 1982; Sharp *et al*., 1999), and foals (Baiker *et al*., 2010). Hydranencephaly in human is considered to be a primary ischemic etiology with vascular involvement that occurs during gestation, while in animals, it is most commonly the result of viral infections in the first or beginning of the second trimester of pregnancy. Viral causes include Akabane virus, Aino virus, bovine viral diarrhea virus, Bluetongue virus, Schmallenberg virus, Chuzan virus, Cache Valley virus, Rift Valley fever virus, and Wesselsbron virus (Baiker *et al*., 2010; Nietfeld, 2012; Agerholm, 2015).

Many brain defects are diagnosed after necropsy through gross pathology and histopathology, and sometimes they can be diagnosed before death (Washburn and Streeter, 2004). One method of diagnosing brain defects before death is the use of computed tomography (CT) scan providing noninvasive and high-resolution images with a high power of differentiation between soft and bone tissues (Cabrera *et al*., 2015). Computed tomography was first utilized in human medicine in 1971 in England (Cierniak, 2011). Today, the use of CT scan in veterinary medicine is increasing as a result of increased access to advanced diagnostic and therapeutic tools. Although expensive, it can help improve the diagnosis (Tharwat *et al*., 2014). There are reports on the usefulness of CT in the diagnosis of many disorders in small animal such as musculoskeletal and lung diseases, abdominal illnesses and disorders of the nasal cavity, but there are few published reports of using CT in cattle (Baiker *et al*., 2010). This method has been used for the antemortem diagnosis of some conditions in large animal medicine, including porencephaly, cerebral abscess, pituitary adenoma, cervical stenotic myelopathy, spinal cord rupture, meningoencephalocele, and otitis interna/media (Constable *et al*., 2017). Computed tomography scan has also been used for the diagnosis of hydranencephaly in human neonates. An ever-increasing number of reports on brain disorders diagnosed by CT in cattle is appearing in previous literatures (Allen *et al*., 1987; Gerro *et al*., 1998; El-khodery *et al*., 2008; Lee *et al*., 2009; Baiker *et al*., 2010; Lee *et al*., 2011; Nietfeld, 2012; Tharwat *et al*., 2014). The aim of this study was to determine features of hydranencephaly in calves by CT.

Materials and Methods

Animals

By the end of winter and early spring in 2016, twenty calves with neurological signs were born in a dairy farm in Varamin (a county in Tehran province, longitude 51.39°E, latitude 35.19°N). Seven calves with neurological signs (3 male and 4 female calves) with the same age (under 3 month) were slaughtered (due to difficulty in nursing) and their skulls were transferred to the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Tehran, Iran, for gross and CT scan evaluation of their cerebral lesions.

Blood samples were collected in ethylene diamine tetra acetic acid (EDTA) coated tubes from the jugular vein of each calf prior to slaughter, for hematological examination. Cerebrospinal fluid was collected for cytologic examination.

CT scan protocol

Computed tomography images from the olfactory bulb to occipital condyles were obtained in transverse and dorsal planes from 7 skulls using a multidetector CT scanner (Somatom spirit 2, Siemens, Germany), with the follow characteristics: X-ray tube potential: 130 kV, tube current: 120 mA, slice thickness: 1 mm, pitch: 1, rotation time: 1 s, and reconstruction interval: 0.5-1 mm. The CT-images were reconstructed in transverse and dorsal planes using the program Image (Syngo image 5.5). The attenuation values of the cerebral hemispheres and brain stem were determined by measuring the hounsfield unit (HU) in the transverse plane. Therefore, HU was measured by manual placement of a region of interest (ROI) in each cerebral hemisphere and brain stem in 7 regions (for each hemisphere, rostral part: primary section of frontal sinus, medial part: near the temporomandibular joint and medial section of the temporal region, and caudal part: around the brain stem (Fig. 1)). The ROI's size in all regions were uniform (2 cm). To compare the HU obtained at each region with the normal brain, CT images of a normal calf (with the same age) were obtained and HU was measured at the same regions. In this study, the windows of the brain and bones were used for the evaluation of cranium details.

Necropsy

The 6 skulls were necropsied following the CT scan. After removing the cranium and dura mater, the remaining brain tissue was examined for formation of brain, brain stem, falx cerebri, and bone deformities, and was compared with the results of the CT. Then, 10 g of the remaining brain tissue was collected to detect the causative agent.

Statistical analysis

Statistical analysis was performed using a one-sample t-test method (SPSS-22 software, IBM Co., New York, USA) to analyze the differences between the mean value of the HU of calves with hydranencephaly, and that of a normal brain. The differences were considered statistically significant at $P<0.05$.

Results

The main clinical signs in affected calves included blindness, abnormal behavior such as head pressing, depression or dullness, unawareness of the environment,

Fig. 1: Transverse CT scan of the skull in 3 levels. Manual placement of a region of interest (ROI) in three regions: rostral part (**L**), middle part (**M**), and caudal part (**R**) of the brain in calves with hydranencephaly

wandering aimlessly, partial failure of suckling, rigidity and fixation in flexion (arthrogryposis) in one or more joints and associated muscles' atrophy. Hematological examination showed anemia (mean of hematocrit and erythrocytes: 15.9% and 3.55 × 10^6/µL, respectively), leukocytosis (mean of white blood cell (WBC): 14235/µL) and neutrophilia (mean: 6200/µL) .Cytologic examinations of the cerebrospinal fluid indicated normal values. The number of cells in this fluid varied from 5 to 18 cells/µL.

CT image analysis

In the transverse plane of the CT images, the presence of hypoattenuation of the hemisphers in cranial cavity was clear in various sizes, so much so that in some cases it completely replaced the cerebral hemispheres. In 5 cases, the size of left sac was larger than the right and in 2 cases, the right sac was larger than the left one (asymmetric fluid-filled sacs). Moreover, the calvarium was divided into two asymmetric halves, which was also confirmed in necropsy. In all cases, the cerebellum and brainstem were completely visible (Fig. 2). In addition, Falx cerebri was clearly seen in both transverse and dorsal planes as the hyper attenuation line (Figs. 2-4). In one case, the left lateral side of the frontal sinus was not detectable and was asymmetrically filled with bony structure (Fig. 3). The dorsal plane of the CT images also showed asymmetric hypoattenuated hemispheres in all cases.

Density values of cerebral hemispheres and brain stem

HU density values were measured in every transverse slice in all predefined regions in calves with

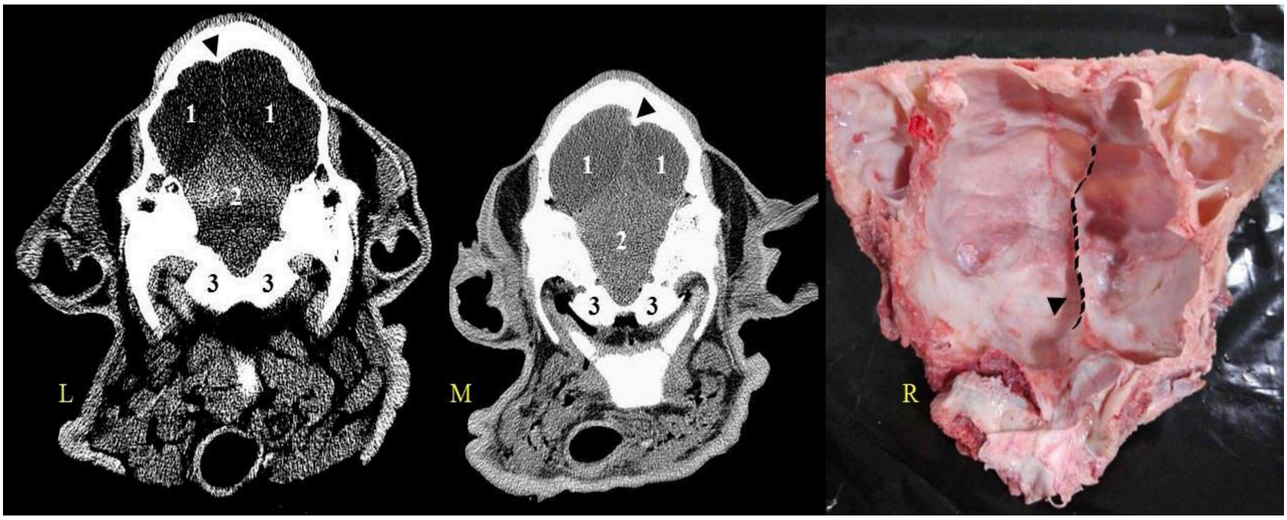

Fig. 2: CT scan transverse images of the skull in two calves with hydranencephaly in brain window (**L**: Calf No. 2 and **M**: Calf No. 3). 1: Asymmetric hypoattenuatied sacs, 2: Brain stem, 3: Occipital condyle. Arrowhead: Asymmetric calvarium (**R**: Necropsy of calf No. 3)

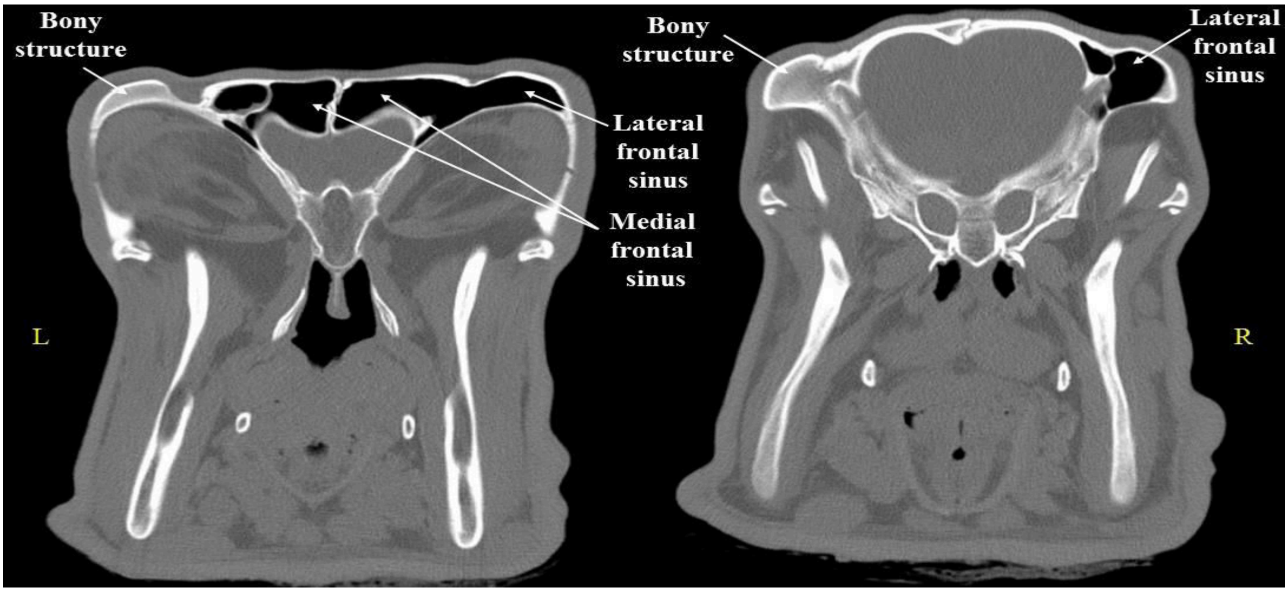

Fig. 3: Transverse CT scan images of the skull in calf No. 1 with hydranencephaly in bone window. Asymmetrically bony structure in left lateral side of the frontal sinus, rostral part of frontal sinus (left, **L**) and medial part of frontal sinus (right, **R**) is seen

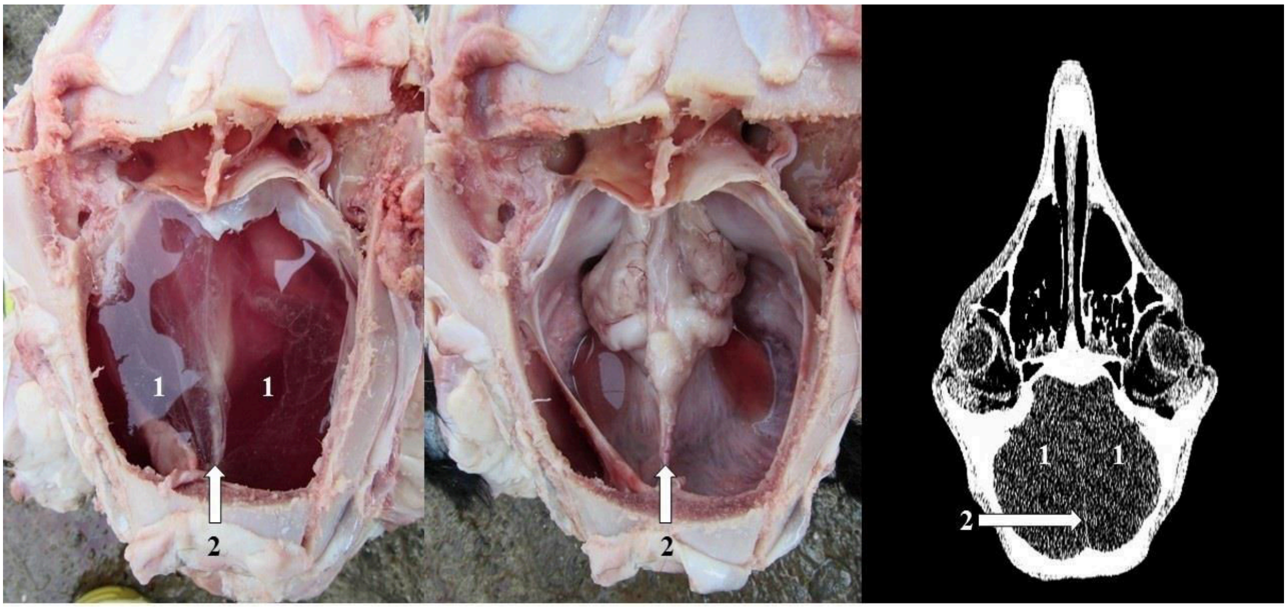

Fig. 4: Cranium necropsy in calf with hydranencephaly and its correlation with CT image (calf No. 4). 1: Presence of asymmetric fluid-filled sacs after opening of cranial cavity (left), the same case after fluid removal (middle) and CT image in dorsal plane (right), and 2: Falx cerebri

Table 1: The HU value of cerebral hemispheres and brain stem in calves with hydranencephaly and normal calf evaluated by quantitative CT

No.	Portion of brain	Side of hemisphere				
		Cerebral				Brain stem
		Rostral	Medial	Caudal	Mean±SD	
		HU	HU	HU	HU	HU
1	Left	10.8	16.7	7.5	10.71 ± 5.95	42.2
	Right	19	5.7	4.6		
2	Left	10.5	8.8	8.1	11.45 ± 6.79	57.7
	Right	16.5	2.8	22		
3	Left	16.6	7.7	7.5	10.03 ± 3.92	63.6
	Right	12.6	9.7	6.1		
4	Left	16.7	10.9	6.7	11.25 ± 3.41	53.1
	Right	13.3	10.3	9.6		
5	Left	21.6	8.4	12.3	13.86 ± 4.4	44.2
	Right	15.4	12.8	12.7		
6	Left	12	10.2	5.6	10.43 ± 3.91	50.4
	Right	16.1	12.2	6.5		
7	Left	18.8	9.7	13.2	14.51 ± 6.33	49
	Right	24.9	12.8	7.7		
8*	Left	44.7	33.9	33.1	38.1 ± 4.9	57.8
	Right	42.2	34.5	40.2		

* Calf with normal brain, CT: Computed tomography, and HU: Hounsfield unit

hydranencephaly and the normal calf (Table 1). The HU was measured in the right and left cerebral hemispheres and brain stem of all calves. X-ray attenuation of both hemispheres in calves with hydranencephaly showed remarkable cerebral hypodensity. Comparing the mean value of HU between calves with hydranencephaly and the normal calf showed a statistically significant difference among all regions of cerebral hemispheres (P=0.0001). There was no statistically significant difference between the mean value of HU of brain stem in calves with hydranencephaly and the normal calf (P=0.066).

The results obtained from CT correlated well with pathologic lesions. After removing the calvarium and dura matter, fluid-filled sacs seemed to be present in the cranial cavity. These sacs were surrounded by a thin membrane. The calvarium was divided into two different size halves (Fig. 4). Gross lesions of the remaining brain tissue were evaluated by examining the telencephalon. Only 2 cases showed incomplete fontal and occipital lobes, while in other cases, these parts were not formed. The parietal lobe was found to be incomplete in one case, while the temporal lobe was not seen at all. In all cases, the presence of cerebral peduncles were complete. Pyriform lobes were complete only in 3 cases but formed incompletely in the other three. Thalamus, rostral and caudal colliculi, cerebellum, pons, medulla oblongata and falx cerebri were completely formed and no major changes were observed.

Reverse transcription-polymerase chain reaction (RT-PCR) of the remaining brain tissue confirmed the presence of residual RNA of the Akabane virus, and so this virus could be considered as the causative agent of hydranencephaly in calves.

Discussion

Hydranencephaly is a result of impaired growth and necrosis of the cerebral tissue (Maxie, 2016) due to the disappearance or lack of development of the neopallial cells of the telencephalon and neocortex. The remaining neopallium is a thin and transparent membrane (Mayhew, 2008; Maxie, 2016). The brain tissue is replaced by fluid-filled sacs that are often seen in different sizes (Maxie, 2016). Hydranencephaly occurs in all species, but it is more common in calves and occurs either sporadically or as minor epizootics (Mayhew, 2008; Maxie, 2016). The most common cause of hydranencephaly in calves is the fetal infection of the teratogenic viruses during a specific stage of pregnancy. These viruses, attack the brain tissue and lead to tissue destruction and cell death (Maxie, 2016).

Computed tomography is a new method in medical sciences and is widely used for diagnosing bone and soft tissue disorders by providing high resolution images from between the two tissues (Cabrera *et al*., 2015). Computed tomography was originally developed for imaging the human brain; therefore, it can be a useful diagnostic tool in evaluating cattle cerebral lesions (El-khodery *et al*., 2008; Mohamed *et al*., 2011). In large animal practices, radiography is commonly used for diagnostic purposes, but the use of CT is limited in case reports. Computed tomography is an appropriate diagnostic procedure for the evaluation of both bone and soft tissue structures of the head (Tharwat *et al*., 2014). Although the diagnostic value of CT in cattle is similar to other candidates, there are few published reports on the use of CT in cattle. Porencephaly, intracranial arachnoid cysts and hydrocephalus have been diagnosed by means of CT scan in calves (Baiker *et al*., 2010). Post-mortem changes, reduce the quality of the resulting images (Allen *et al*., 1987), therefore, efforts should be made to obtain CT images as soon as possible after slaughtering infected calves.

The brain window allows the evaluation of brain parenchyma, hemorrhage, and other soft tissue details in the cranium. The presence of asymmetric hypoattenuated sacs that replaced the normal tissue of the brain was observed in CT images. According to the results, there is a highly significant difference between HU obtained from cerebral hemispheres in calves with hydranencephaly compared to normal cerebral hemispheres, indicating a marked tissue change. The HU of tissue density is based on air value (-1000 HU) and water value (0 HU), and the density of tissues is in the range of -1000 to + 1000, and higher (for bone density) HU (Thomas, 1999; Ohlerth and Scharf, 2007). The HU mean value of cerebral parenchyma of the affected calves was almost close to the HU value of water (but not zero), revealing the fluid nature of the structure (the lowest HU recorded in cases with hydranencephaly was 2.8 for the middle part of the right hemisphere of calf No. 2). The contents of the cerebrospinal fluid such as vascular structures, cells, ions, fat, proteins, oxygen, and carbon dioxide in CSF can change HU value (Boris *et al*., 1987). Due to the presence of cells in the CSF along with the other factors mentioned, the fluid density in the sacs slightly increased. In some cases, HU was slightly higher, which may be due to patches of brain tissue or partially formed lobes (as seen in the necropsy). There was no statistically significant difference between the brain stem's HU value of the affected calves and a normal calf, which is suggestive of normal tissue in the brain stem. Pathological lesions of the Akabane virus (as the cause of the hydranencephaly in calves in this study) involved cerebral hemispheres, and were intact in the brain stem of all cases (Kirkland, 2015). In all cases, CT findings were confirmed by necropsy, as the presence of fluid-filled sacs and an intact brain stem were clearly seen after removing the calvarium.

The bone window in the CT method allows for the examination of bone details such as fractures and other bone malformations. Computed tomography is highly sensitive to subtle bone changes, particularly in the head (Mohamed *et al*., 2011). Therefore, using CT scan to evaluate paranasal sinuses would be very useful (Morrow *et al*., 2000). In this study, one case showed bone deformity in the frontal sinus. In this case, the left lateral side of the frontal sinus was filled with bony structure, while the right frontal sinus was formed normally. However, this sinus change was not seen in the other affected calves.

In all calves with hydranencephaly, the inner surface of the calvarium (contacting with the brain) appeared asymmetrical in necropsy, resulting from the asymmetric growth of the fluid-filled sacs, which caused crest enlargement between the sacs (known as the groove for superior sagittal sinus in the human skull). This crest was clearly detectable in CT images. Normally, the interaction between the growth of the calvarium and the brain is mediated by a biomechanical mechanism. This mechanism is controlled by two hormones, leptin (secreted by the brain) and osteocalcin (secreted by the osteoblasts). Leptin receptors are found in osteoblasts and chondrocytes. This hormone can contribute directly to bone growth and metabolism. On the other hand, osteocalcin affects fetal brain development by crossing the blood-brain barrier and regulating energy metabolism (Khalid *et al*., 2012; Upadhyay *et al*., 2015; Obri *et al*., 2018). It is also hypothesized that in the embryonic period, the hydranencephaly-inducing viruses may affect the neural crest and subsequently the process of intermembranous bone formation that can lead to disorders in bone structure and osteogenesis (Fletcher and Weber, 2009). This hypothesis may justify a defect in formation of the frontal sinus. It is assumed that brain disorders caused by Akabane virus infections during gestation have led to these bone deformities.

Acknowledgement

The authors would like to thank Mr. M. Nowrouzi for his assistance with sample collection.

Conflict of interest

The authors declare that they have no conflict of interest.

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