Histological Study of Cerebrum, Cerebellum in Anencephalic & Non-Anencephalic Foetuses

Shilpa Karkera[1], Sumathi Shekar[2], Priya Ranganath[3]

[1]Department of Anatomy and Neuroscience, Trinity School of Medicine, Trinity medical Science University, St Vincent & Grendines, West indies. [2]Department of Anatomy, Bangalore Medical College and Research Institute, Bangalore, Karnataka, India. [3]Department of Anatomy, Bangalore Medical College and Research Institute, Bangalore, Karnataka, India.

ABSTRACT

BACKGROUND

Anencephaly is one of the most common birth defects. This was a hospital-based case finding study that covered 60 patients with anencephaly & their respective mothers, conducted in hospitals in Bangalore Medical College & Research Institute from 2014 to 2017.

METHODS

The study included 60 anencephalic foetuses (23 males & 37 females) of 20-30 weeks & 20 non-anencephalic foetuses (9 males & 11 females). Maternal history was analysed using a questionnaire which includes age factor, environmental factors, medications, family history, consanguineous marriage and febrile illness during pregnancy. The foetuses were examined for external abnormalities & dissected. Dissected foetal cerebrum & cerebellum tissues were processed & stained with H&E using tissue processor.

RESULTS

In 100% of cases, all layers of cerebrum & cerebellum of non-anencephalic foetuses were normal. In anencephalic foetuses, cerebrum in 75% cases showed primitive brain cells, astrocytes & glial cells, instead of 5 layers which was described in checklist & in 25 % cases angiomatous masses were seen but primitive brain cells & astrocytes were absent. 91.7% cases of anencephalic foetal cerebellum had 5 cell stages which were the same as non-anencephalic foetuses & 8.3% cases had all the layers but ill formed granular layer. Most of the organs were normally developed. Associated anomalies were also noticed in 70% of cases.

CONCLUSIONS

The study emphasizes the complexity of the aetiology behind anencephaly, variability of its presentation & yet unsatisfactory awareness among mothers about folic acid & its beneficial role in preventing anencephaly.

KEY WORDS

Anencephaly, Maternal Awareness, Angiomatous Masses

Corresponding Author:
Shilpa Karkera,
Trinity School of Medicine,
Trinity Medical Science University,
St. Vincent & Grendines, West Indies.
E-mail:
shilpa.karkera@trinityschoolofmedicine.org

DOI: 10.14260/jemds/2020/16

Financial or Other Competing Interests: None.

How to Cite This Article:
Karkera S, Shekar S, Ranganath P. Histological study of cerebrum, cerebellum in anencephalic & non anencephalic foetuses. J. Evolution Med. Dent. Sci. 2020;9(02):71-75, DOI: 10.14260/jemds/2020/16

Submission 29-11-2019,
Peer Review 21-12-2019,
Acceptance 02-01-2020,
Published 13-01-2020.

BACKGROUND

Anencephaly is a congenital anomaly characterized by absence of major portion of the brain, skull and scalp that occurs during the embryonic development. The nervous tissue subsequently undergoes degeneration and brain remains as a spongy vascular mass with some hind brain structures. Acrania is a condition in which the calvaria is absent (a roofless skull) & extensive defects of vertebral column are present. There is severe arrest of brain development & even degenerative regression causing anencephaly. The central hemisphere & other regions of brain usually suffer such damages. Occasionally the neural groove fails to close because of faulty induction of surrounding mesodermal structures & the neural tube then remains exposed to the surface. Such a defect is localised in the cephalic region. Moreover, the vault of the skull may be absent, giving the head the characteristic appearance, the eyes bulge forward, the neck is absent & the surface of the face & chest form a continuous plane. Anencephaly is a congenital anomaly characterised by gross defects of the head & often inability to swallow. Hydramnios (Amniotic fluid > 2 litres) is frequently associated with it. Acrania is a condition in which the calvaria is absent (a roofless skull) & extensive defects of vertebral column are present. There is severe arrest of brain development & even degenerative regression causing anencephaly. The central hemisphere & other regions of brain usually suffer such damages.[1] Acrania associated with mero anencephaly or anencephaly (partial absence of brain) occurs in about one in 1000 births & is incompatible with life. Meroanencephaly results from the failure of cranial end of neural tube to close during the fourth week & causes subsequent failure of formation of calvaria.[2]

It was first noted in 1971 that anencephaly and open spina bifida were associated with high amniotic fluid levels of alpha foetoprotein (AFP). AFP was also higher in maternal serum in patients with open neural tube defect. AFP is produced by foetal liver and yolk sack. It enters the amniotic fluid by diffusion across the foetal skin and by foetal urination. AFP enters the maternal circulation by diffusion across the placenta. In foetuses with open defects, larger than normal amount of AFP enter the amniotic fluid and maternal circulation. This forms the basis of use of maternal serum AFP for detection of open spinal defects.[2] Few other maternal factors like hyperglycaemia and obesity is also one cause of anencephaly. Glucose levels can be detected in the maternal serum. Hyperthermia, folate, zinc and copper deficiency may also lead into anencephaly. Folic acid supplementation 0.4 mg/day in the diet of women of childbearing age significantly reduces the incidence of neural tube defects such as anencephaly Elevated levels of acetyl cholinesterase levels on electrophoresis occur in virtually all cases of anencephaly.[3,4,5,6,7,8] Hyperthermia, folate, zinc and copper deficiency may also lead into anencephaly. Folic acid supplementation 0.4 mg/day in the diet of women of childbearing age significantly reduces the incidence of neural tube defects such as anencephaly.[9]

METHODS

The is a prospective, observational, hospital-based study conducted in Victoria Hospital attached to Bangalore Medical College & Research Institute (BMCRI), Bangalore; during the period of Aug 2014 to June 2017. The study included all the patients born with anencephaly, 60 anencephalic foetuses (23 males and 37 females) of 20-30 weeks and 20 non anencephalic foetuses of age 20-30 weeks; which were delivered in the Department of Obstetrics & Gynaecology, Victoria hospital, Bangalore.

Inclusion Criteria

The diagnosis was confirmed by an experienced observer following delivery of an abortus or still born infant. The diagnosis of anencephaly can be made in utero. The appearance of infant with anencephaly was unique. Diagnosis was made with virtual certainty when all the following criteria were met-

- A large portion of skull was absent.
- The scalp which extends to the margin of the bone was absent over the skull defect.
- Haemorrhagic, fibrotic tissue was exposed because of defects in the skull & scalp.
- Recognizable cerebral hemispheres were absent.

Exclusion criteria

- Errors in diagnosis have been described in literature.
- Scalp-covered lesions such as those seen in cases of microcephaly, encephalocele, atelencephaly, holoprosencephaly, hydranencephaly, skin lesions with intact bone as in cutis aplasia defects that do not extend to anterior skull (iniencephaly) were not included in the study.

A structural sociodemographic questionnaire was designed to capture basic data about gender, age, maternal history like socioeconomic data, environmental history, educational qualification and family history. A detailed proforma was developed for collecting the required details of the mother and the foetus. The patients who met the above criteria were selected. Approval was obtained from the Institutional Ethics Committee of Bangalore Medical College & Research Institute, Bangalore prior to data collection. Informed consent as per 'The Indian Council of Medical Research' guidelines was obtained from the participants before data collection. The fluid strength was determined by the condition of the body. As infant body tissue contains higher percentage of water than adult body tissue, adult 10% formalin was used but volume of fluid was reduced than adults; it depended upon size and weight of the body. Foetuses were cavity embalmed with 10% formalin and then dissected.

Incision Points

A curved incision was made bilaterally from the acromion process through the medial border of shoulder joint to mid-axillary line laterally, this continued to the iliac crest over the inguinal ligament to meet pubic symphysis. The skin with the

superficial tissue flap was reflected up the root of the neck, then to the inferior margin of mandible bilaterally, taking care not to injure the neck structures and rectus sheath. This way, whole of the front of the neck, chest and abdomen was exposed. The tissues are sliced by sharp forceps into 4 mm thickness. Dissected tissues of cerebrum & cerebellum of anencephalic foetuses and non-anencephalic foetuses were fixed for 24 hours and then tissue processing was performed. The staining was done by H& E technique. Slides were checked under low power (10 X) and then under high power (20x, 40x, 100x), identified, analysed, photographed and then reported using Olympus BX51 polarized microscope with inbuilt camera.

Cerebrum	Cerebellum (5 Layer Stage)
External marginal layer	Molecular layer
Cortical plate	Laminal dissecans
Subcortical layer	External granular layer
Intermediate zone	Internal granular layer
Ventricular zone	Purkinje layer
	Medulla
	Ganglionic cells

Table 1. Cell Layers of Cerebrum & Cerebellum

RESULTS

In this prospective study 60 anencephalic foetuses (23 males and 37 females) of 20-30 weeks and 20 non-anencephalic foetuses of age 20 - 30 weeks; which were delivered in the Department of Obstetrics & Gynaecology, Vanivilas Hospital, Bangalore during the period of Aug 2014 to June 2017. The study showed that 83.4% of the mothers were at 21-35 years & paternal age in 78.4% were at 21-35 years. Maternal age <20 years was in 1.6% & none in father. Maternal and paternal age >40 years was seen in 1.6%. Clinical history of 8.3% of mothers had multiple abortions, 1.6% had abortion with asthma, diabetes with obesity & hypertension, 5% were diabetic & 81.6 % were normal. Out of 60 cases only 9 (15%) mothers got consanguineous marriage, all were 1st cousins & remaining 51 (85%) cases were unrelated Morphological study of brain, brain stem, pituitary gland and cerebellum in 100% of non-anencephalic foetuses were well developed. Anencephalic foetuses had Acrania in 100% of cases & 45.3% foetuses had spina bifida along with acrania. Histopathological study of cerebrum & cerebellum: The study showed that in 100% of cases cerebrum of non-anencephalic foetuses had all five layers (checklist). In anencephalic foetuses, cerebrum in 75% cases showed primitive brain cells, astrocytes & glial cells, instead of 5 layers which is described in checklist & in 25% cases angiomatous masses were seen but primitive brain cells & astrocytes were absent. In 100% of cases cerebellum of non-anencephalic foetuses had the 5-cell stage. 91.7% cases of anencephalic foetal cerebellum had 5 cell stages & 8.3% cases had all the layers but ill-formed granular layer.

DISCUSSION

The study was conducted in main hospitals of Bangalore with main objective of histological variation of cerebrum & cerebellum in anencephalic & non anencephalic foetuses. Incidence of anencephaly is reported to be 1:1000 to 1:20000. The causes giving rise to this condition may be chemical, seasonality of birth, dietary or genetic. The different systems involved are the skeletal system where the vertebrae are involved; the cardiovascular system where the volume of the heart chambers may be affected; the nervous system where skull bones, basal ganglia, cerebrum and cerebellum are involved. Prenatal diagnosis has to be performed to detect this condition at an earlier stage. Folic acid supplements have to be given both pre and postnatally to avoid neural tube defects. Genetic counselling has to be given for concerned parents. In the present study the incidence of anencephaly in Victoria and Vanivilas hospital was 1.04 in 1000 births. Age of mother in 1.6% cases were <20, 83.4% were 21-35, 13.4% cases 36-40 & in 1.6%, >40.

Tables 2 A, B, C represent maternal risks as comparedwith some studies[10,11,12]

Socioeconomic Status	Singh[11] et al	Present Study
Low	13	86.6
Medium low	49.2	13.4
Medium	33	0
High	4.3	0

Table 2 A. Frequency of Anencephaly in Relation to Mothers' Education & Socioeconomic Status (Kuppuswamy Socioeconomic Status Scale)

Febrile Illness during Pregnancy	Kheir[12] et al	Present Study (%)
Flu-like illness	37	5
Malaria	18.5	6.7
Fever & skin rashes	11.1	1.6
Gastroenteritis	3.7	0
Others	29.6	0

Table 2B. Table 2 B. Febrile Illness during Pregnancy

Maternal Risk Factors	Kar[13] et al	Present Study
Abortion & Asthma	NRD	1.6
Abortion	4	8.3
Diabetes	8	5
Diabetes & Obesity	10	1.6
Hypertension	NRD	1.6
Folic acid intake	35	23.3

Table 2C. Maternal Risk Factors

The study showed that 100% of cases cerebrum& cerebellum of non-anencephalic foetuses had all the normal layers. In anencephalic foetuses, cerebrum in 75% cases showed primitive brain cells, astrocytes & glial cells, instead of 5 layers which was described in checklist & in 25 % cases angiomatous masses were seen but primitive brain cells & astrocytes were absent. In 91.7% cases of anencephalic foetal cerebellum had 5 layers of cells which was same as in non-anencephalic foetuses & 8.3% cases had all the layers but ill formed granular layer. Most of the organs were normally developed. Associated anomalies were also noticed in 70% of cases. A study on development of brain & spinal cord in anencephalic foetuses of gestational age of foetuses of 18 to 40 weeks. Majority were female (83.7%) & males were 16.3%.

72% had only anencephaly while 21% had spina bifida & 7% had meningomyelocele. The brain was observed as dark brown undifferentiated mass with complete absence of cerebellum, pons, medulla & midbrain. Histological examination of brain showed venous vessels with connective tissue, angioma along with islets of nervous tissue which mainly comprised of scattered nerve cells, astroglial cells & cavities lined by ependyma which was similar in this study.[13] Anencephalic foetal organs can be transplanted.[14]

A study on 43 cases of anencephaly with gestational age of 18 to 40 weeks, the majority being female foetuses 31 (72%) foetuses had only anencephaly while 9(21%) foetuses had additional spina bifida & 3 (7%) had meningomyelocele. Histological examination of brain showed venous vessels of varying caliber with connective tissue, similar to angioma along with islets of nervous tissue which mainly comprised of scattered nerve cells and cavities lined ependyma, which was found to be similar in present study. The cerebellum, pons, medulla, midbrain were absent. Spinal cord was normal in all foetuses with anencephaly.[15] Literature showed that anencephalic foetuses were successful donors of hearts & kidneys for transplantation. The intake of 4 mg per day of folic acid intake is recommended in mothers with history of neural tube defect.[3,16]

A study was conducted on NTDs occur when mutations accumulate in neuroepithelial cells, neural stem cells that eventually transform themselves into the brain & CNS, the problem occur after foetus is exposed to too much glucose, which can cause widespread cell death. They focused on a gene called Prkca, which plays a key role in regulating autophagy, the process by which cells dispose material they no longer need; often this material is broken or flawed in some way. In diabetes, the Prkca becomes overactive & as a result autophagy is suppressed. As a result, the flawed cellular material leads to major birth defects. They conducted a series of experiment on pregnant diabetic mice & came to a conclusion that to prevent NTDs in humans by using medicines that inhibit PK Calpha or mi-129-2 or activate PGC-1 alpha.[17,18]

CONCLUSIONS

Anencephaly is one of the most common birth defects. Experiencing any febrile illness, taking medications during pregnancy will increase the risk of birth of a foetus with anencephaly. The study emphasizes that maternal factors like age, alcohol consumption, smoking, febrile illness, consumption of certain drugs and chemicals, family history, season of birth, environmental factors, are risk factors of conceiving an anencephalic child and its associated anomalies; hence, these factors should be avoided. Consanguineous marriages should be avoided, and they should be told about the risks of the same in successive generations too. All pregnant mothers have to go in for triple marker tests; that is, beta HCG, alpha foetoprotein and estradiol. Amniocentesis could be made compulsory for mothers with a history of an anencephalic child. The mother has to be counselled regarding the risks of another such foetus. The family has to be told about the pedigree charts, incidence and occurrence of anencephaly in the population. They should be told about the importance of consuming folic acid before and during pregnancy. Majority of organs were normal anatomically & histologically, cerebrum in anencephalic foetuses has primitive brain cells, glial cells & astrocytes. Cerebellum was normal in majority of cases. Therefore, anencephalic foetal organs can be transplanted.

ACKNOWLEDGEMENT

This article is an extract of the research project titled "Anencephaly and its associated anomalies along with maternal serum testing".

REFERENCES

[1] Arey LB. In: Development anatomy - a textbook & laboratory manual of embryology. 7th edn. Philadelphia & London: W.B. Saunders Co, 1965.
[2] Moore KL, Persaud TVN. In: The developing brain-clinically oriented embryology. 6th edn. Philadelphia: W.B. Saunders Co, 1999;49:463.
[3] Cincore V, Ninios AP, Pavlik J, et al. Prenatal diagnosis of acrania associated with amniotic band syndrome. Obstet Gynecol 2003;102(5 Pt 2):1176-8.
[4] Moore L. Anencephaly. Journal of Diagnostic Medical Sonography 2010;26(6):286-9. Originally published online 27 Oct 2010. DOI: 10:1177/8756479310386486.
[5] Macri JN, Weiss RR. Prenatal serum alpha-foetoprotein screening for neural tube defects. Obstet Gynecol 1982;59(5):633-9.
[6] Amniotic-fluid alpha foeto protein measurement in antenatal diagnosis pf anencephaly and anencephaly and open spina bifida in early pregnancy. Second report of U.K. Collaborative Study on Alpha-foetoprotein in Relation to Neural-tube Defects. Lancet 1979;314(8144):651-62.
[7] Report of Collaborative Acetylcholinesterase Study. Amniotic fluid acetylcholinesterase electrophoresis as a secondary test in the diagnosis of anencephaly and open spinabifida in early pregnancy. Lancet 1981;318(8242):321-4.
[8] Ultrasound in pregnancy. ACOG technical bulletin No. 116. Washington, DC: American College of Obstetricians and Gynaecologists 1988.
[9] Carlson BM. In: Human embrylology and development biology. 2nd edn. St. Louis, Mosby 1999: p. 109-30.
[10] Singh J, Kapoor K, Kochhar S. Incidence of anencephaly in a tertiary care hospital in North West India. International Journal of Scientific & Research Publications 2015;5(9):2230-315.
[11] Kheir AEM, Eisa WMH. Neural tube defects: clinical patterns, associated risk factors & maternal awareness in Khartoum State, Sudan. Journal of Medicine & Medical Research 2015;3(1):1-6.
[12] Dudala SR. Updated Kuppuswamy's socioeconomic scale for 2012. J NTR Univ Health Sciences 2013;2(3):201-2.
[13] Kar A, Kar T, Kanungo S, et al. Risk factors, organ weight deviation & associated anomalies in neural tube defects: a prospective foetal & perinatal autopsy series. Indian Journal of Pathology & Microbiology 2015;58(3):285-91.

[14] Anand MK, Javia MD, Laxmi CJ. Development of brain & spinal cord in anencephalic human foetuses. International Journal of Experimental & Clinical Anatomy 2015;9(2):60-5.
[15] Bioethics Committee, Canadian Paediatric Society. Use of anencephalic newborns as organ donors. Pediatric Child Health 2005;10(6):335-7.
[16] Gilbert-Barness E, Kapur R, Oligny LL, et al. Potter's pathology of the foetus, infant & child. Vol. 1 & 2. 2nd edn. Mosby Publication 27 April 2007.
[17] Gupta P, Nain P, Singh J. Anencephaly: a neural tube defects – a review. American Journal of Pharmtech Research 2016;2(3).
[18] Yang P. Gene controls birth defect common in diabetes. University of Maryland School of Medicine, Science Daily 5 May 2017.