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Human Hypoglossal Neuronal Nucleus in Late Gestational Period: A Morphometric Study

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ABSTRACT

Introduction: Hypoglossal nerve (XII cranial nerve) innervates the tongue muscles, thus assists the motor activities like chewing, swallowing and vocalization. In addition, it is responsible for the modulation of the respiration and drinking behaviour. Clinically, injury to this nerve will lead to weakness and deviation of the tongue to one side. Development of the cranial motor system in central nervous system is a complex process involving events like neurogenesis, neuronal migration, cell death, and establishment of afferent and efferent connections. Since most studies regarding the nerve and its nucleus were conducted in animals, in depth study on the neuronal development of the hypoglossal nerve nucleus in humans is necessary.

Methodology: After obtaining the institutional ethical approval, explanation about the research was given to the mothers and their written consent was obtained. A total of 12 fetuses (Gestational age 19-40 weeks) were included in the study. They were divided into 4 groups based on their gestational age and Crown-Rump Length (CRL) measurements. As hypoglossal nucleus extends throughout the length of medulla oblongata in the para-median plane, tissues were collected from the section of medulla. The tissues were processed by routine histological procedure were stained with hematoxylin and eosin and also with Holmes' Silver nitrate to study the histological details. In this morphometric study, we measured the cell dimensions (length and breadth) and volumes of hypoglossal neurons and its nucleus. From these data, coefficients were drawn to identify the proportion of growth between cell and nuclear volume.

Results: Morphometric analysis of hypoglossal nerve neurons in human fetuses showed that the neurons are oval or pyramidal in shape beginning from 18 to 24 weeks gestational age. The neurons grow rapidly in the initial period of the gestational stage, but at the end it shrinks to maturity. In the late stage, this nerve nucleus has dendritic process.

Conclusions: The shape of the hypoglossal nerve nucleus was oval or pyramid. Though the neurons grew rapidly in the initial gestational weeks, it shrunk at the end to get matured. Dendritic process was found in the well matured nerve cells.

Key words: Hypoglossal nerve nucleus, Deglutition, Mastication, Morphometry, Histogenesis

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INTRODUCTION

Nervous system in the human being is very complex structure and this network is responsible for the transport of messages to and from the brain and spinal cord to different parts of the body [1]. Spinal nerves originate from the spinal cord whereas the cranial nerves arise directly from the brain stem except olfactory and optic [2,3]. There are 12 pairs of cranial nerves and its nuclei develop in the fourth week of gestation [2,4]. Motor neurons of the cranial nerves are within the brainstem, whereas the sensory ganglia are outside the brain. Unlike spinal nerves, cranial nerves are organized homologous. All cranial nerves do not have both motor and sensory fibers. Hypoglossal nerve, XII cranial nerve arises from the myelencephalon and belongs to the general somatic efferent nerve type. Nucleus of this nerve extends throughout the length of the medulla and as it is a motor neuron, it is located close to the midline. In the transected medulla it is visible as hypoglossal trigone, a raised area protruding towards the fourth ventricle. In the closed medulla, the gracile and cuneate nuclei are posterior to this. It is responsible for the motor innervation of the tongue muscles [4].

Hypoglossal nerve cells has both axons and dendrites like the other neurons, its axons leave the nucleus as a row of tiny fascicles and descends down the neck as the hypoglossal nerve near the hyoid bone; passes anteriorly and pierces through the body of the tongue to supply hypoglossus, styloglossus, genioglossus and intrinsic

muscles of tongue [5]. Motoneurons in the hypoglossal nucleus are the main source of motor innervation of the intrinsic and extrinsic muscles of tongue [6]. The somatotrophic organization of the hypoglossal nucleus is better understood as either individual representations or functional grouping of the extrinsic tongue muscles [7-9].

Though Hypoglossal nerve nucleus (HGN) controls certain phase of respiration, it is not explained as one of the important center in the nervous system. It is present in the dorsal part of the medulla oblongata [3]. HGN has 2 different types of neurons, namely motoneurons and inhibitory inter-neurons. Among these two, the predominant one is motoneurons which represent about 95% of the total neurons [10]. These neurons are large, multipolar and have more Nissl substances. On the other hand, the second type of neurons is formed by inhibitory inter-neurons, which are small, rounded or oval neurons with poor endoplasmic reticulum [11-13]. Both types of neuronal cells have significant functions in relation to tongue control [14-16].

Clinically, HGN is documented to be involved in the controlling the motor activities of the tongue like swallowing, chewing and vocalization, but later it is found to be responsible for breathing also [9]. Muscles of the tongue especially genioglossus is responsible for maintaining the patent airway during respiration [17-19]. Apart from this respiratory regulation, HGN is also responsible for modulating drinking behavior [20]. Most of the experimental studies on different animals gives us these current knowledge about HGN, however, research about HGN in human is limited [21- 23]. Thus to obtain a clear picture about the cell dynamics and its development, morphometric study of the histogenesis of hypoglossal nerve nucleus is essential.

RESEARCH METHODOLOGY

Ethical clearance and consent form

Before starting the research, ethical clearance were obtained from the Ethical Committee, MIMSR Medical College, Hospital and Research center, Latur and Dr. DY Patil Medical College, Pune, Maharashtra. Written consent was obtained from the mother of the fetuses.

Specimen collection

Fetuses from the 19th weeks of gestation to 24th week of gestation were obtained from the cases coming for medical termination of pregnancy and above 24 week of gestation were collected from the patients with spontaneous abortion. 12 fetuses between the gestational age of 19 to 38 weeks were collected after obtaining the necessary permission from the respective parents. Complete clinical history, pregnancy details especially from the mother were collected and recorded. Fetuses with anomalies were excluded from the study. These were well preserved fresh abortuses and were fixed in the formalin solution. The gestational age of the fetuses were determined by the CRL measurement, the measurement of the length of the fetuses from the top of the head (crown) to the bottom of the buttocks (rump) (Table 1). Fetuses below 10 weeks were not considered for this study as they were at a primitive stage of development. For convenience, fetuses were grouped into 4 based on their gestational age such as group-I from 19-22 weeks, group-II from 23-28 weeks, group-III from 29-35 weeks and group-IV from 36-40 weeks. The collected fetuses were preserved in 10% of formalin solution for further research.

Since hypoglossal nucleus extends throughout the length of medulla oblongata in the paramedian plane, complete transverse section of medulla was conducted. The tissues were processed by routine histological procedure. A total of 24 blocks [12 right and 12 left] were used for this study. 5 serial sections were taken from each block. The slides were numbered serially with 5 sections in each slide with a gap of 5 for each section. Sections were stained with hematoxylin and eosin and also with Holmes' Silver nitrate to study the histological details. These histological procedures were adopted from the previous research works [24,25].

Table 1: Mean CRL measurements with their range

Group	Gestational Age	No. of Foetuses Studied	Mean CRL Measurement	Range
I	19-22 weeks	3	19.53	18.6, 19, 21
II	23-28 weeks	3	26.03	24.8, 26.3, 27
III	29-35 weeks	3	32.66	31.4, 33, 33.6
IV	36-40 weeks	3	35.37	34.8, 35.3, 36

Measurement-morphometric study

Serial sections of the nucleus were taken from each tissue to study their morphometric measurements. Transversely cut section of the neurons in medulla will be measured with stage micrometer (0.01 mm scale) and eye piece micrometer with 100 divisions (0.1 mm Scale). Length and breadth of the nucleus seen through the eyepiece reticule were measured with the stage and eye piece micrometer. The volume of each cell (nucleus) was calculated by using formula $ab2\pi/6$ ($\pi=22/7$), where 'a' is the length and 'b' is the breadth of the nucleus, a standard method to minimize the error. Average length, breadth and the volume of the nucleus in each group were calculated and documented for analysis and comparison.

Statistical analysis

Statistical analysis was done using the SPSS 16.0 version to find the mean value of the length, breadth and volume in both nerve cell and nucleus of hypoglossal nerve. We studied the dimensions (length and breadth) as well as the volume of the hypoglossal nerve cell and its nucleus. The coefficient between the volume of the nerve cell and its nucleus in each group was done to find its proportion.

RESULTS

The mean lengths of the cell in hypoglossal nerve were 6.5, 20.8, 21.5 and 19.4 whereas its breaths were 4.8, 15.2, 16 and 14.25 respectively for the stage I, II, III and IV (Table 2). Similarly the mean lengths of the nucleus in hypoglossal nerve cell were 4.5, 13.1, 14.2 and 11.6 whereas its breaths were 4.5, 10, 11.4 and 8.7 respectively for the stage I, II, III and IV (Table 3). The calculated cell volumes were 78.444, 2517.19, 2882.99 and 2063.46 against the mean nucleus volumes of 47.73, 686.178, 966.64 and 459.9 gave the coefficient of proportion of 1.64, 3.67, 2.98 and 4.5 in the four different stages (Table 4).

Table 2: Cell dimensions

Name	Dimensions	Group-I	Group-II	Group-III	Group-IV
		19-22 weeks	23-28 weeks	29-35 weeks	36-40 weeks
Hypoglossal Nerve nucleus	Mean Length (μ)	6.5	20.8	21.5	19.4
	Mean Breadth (μ)	4.8	15.2	16	14.25
	Mean Volume (μ^3)	78.444	2517.19	2882.99	2063.46

Table 3: Nucleus dimensions

Name	Dimensions	Group-I	Group-II	Group-III	Group-IV
		19-22 weeks	23-28 weeks	29-35 weeks	36-40 weeks
Hypoglossal Nerve nucleus	Mean Length (μ)	4.5	13.1	14.2	11.6
	Mean Breadth (μ)	4.5	10	11.4	8.7
	Mean Volume (μ^3)	47.73	686.178	966.64	459.9

Table 4: Average volume of cell, nucleus and its coefficient

Name	Dimensions	Group-I	Group-II	Group-III	Group-IV
		19-22 weeks	23-28 weeks	29-35 weeks	36-40 weeks
Hypoglossal Nerve nucleus	Mean cell Volume (μ^3)	78.444	2517.19	2882.99	2063.46
	Mean Nucleus Volume (μ^3)	47.73	686.178	966.64	459.9
	Coefficient	1.64	3.67	2.98	4.5

DISCUSSION

This research has shown a clear picture about the morphological development of the neurons in hypoglossal nerve nucleus during the late gestational period (Figures 1-4). In this study, we investigated the length, breadth and volume of the hypoglossal nucleus and its nuclei from the 19th week of gestation to the full term (40 weeks). Many authors studied the morphometric characteristics of the hypoglossal nerve in order to support the anastomosis of hypoglossal and facial nerve in the treatment of peripheral facial palsy [26-28]. Tongue is a muscular organ which helps in the mastication, deglutition and vocalization. Highly organized and controlled movements of the tongue during these activities are done by the uniquely arranged lingual muscles and the reflex mechanism associated with it [29]. Experiments using retrograde tracers technique [7] and cytoarchitectonic method [30] explained the topographical representation of the hypoglossal nerve and lingual muscles inside the motor neurons of cranial nerve. Later, research reports found the most important and vital function of this nerve, namely modulation of the respiration [31] and drinking behavior [20].

In this research about the development of the hypoglossal nerve nucleus, we found that the neuronal cells grow dramatically from the 19th to 35th gestational week. But during the full term, it shrinks a bit when compared to the previous group. These results are in line with the previous research work of Kiran in the human sympathetic ganglion neuron [32] and that of Narasinga Rao B et al. study about the histogenesis of facial nerve neuron [25]. The cell grow at a higher rate in the group II (23-28 weeks) of about 32 times bigger than group I whereas in the group III it slightly increased to reach its peak volume of 2882.99 μ^3. Later in the group IV, the cell shrinks to 2063.46 μ^3 and the nucleus volume is half when compared to the previous group.

According to Shepard [33] there are 7 stages of development of the neurons in the central nervous system: (1) Proliferation stage-cells usually generates and clones (2) Commitment to a specific type of cell such as neurons or glia (3) Migration of the cell to its final destination (4) Differentiation of the cells depending on their morphological features like axon or dendritic extensions and cytoplasmic organelle (5) Maturation of the cells by accumulation and secretion of neurotransmitter substances (6) Myelination, means acquisition of myelin sheath and (7) Apoptosis of cells, which projects to abnormal target or not having enough neutrophic factor. Though these 7 stages are a guide for the neuronal histogenesis, it has been noted that the order of the development may vary in different structures of the nervous system [33].

Anatomical [34] and physiological [35] studies have shown that the multi-synaptic connections between the anterior solitary nucleus and the hypoglossal nucleus are involved in the gustatory-hypoglossal reflex. Motor control of muscles of the upper airway, particularly the genioglossus muscle of the tongue, is closely related to the modulation of the respiration [36]. Besides this vital function, protrusion of the tongue plays an integral part of the consummatory phase of drinking behaviour [37]. Anna Maria Lavezzi et al. studied the developmental pattern of the HGN from 17th gestational week to 10th month of life in sudden infant death and observed ssignificantly higher incidence of morpho-pathological features of this nucleus [38]. J Altman reported that the neurogenesis of the hypoglossal motoneurons is completed slightly earlier than that for the facial motoneurons [39].

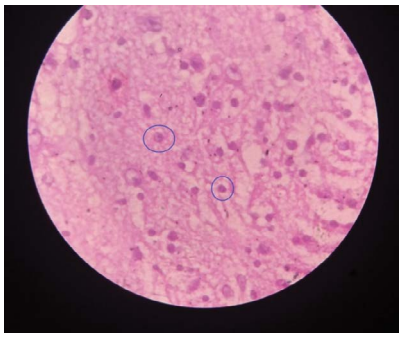

Figure 1: Cell bodies in the hypoglossal nerve nucleus at 20th week of gestation under 400 magnification [H&E Stain]

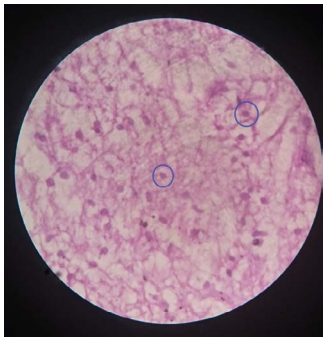

Figure 2: Cell bodies in the hypoglossal nerve nucleus at 26th week of gestation under 400 magnification [H&E Stain]

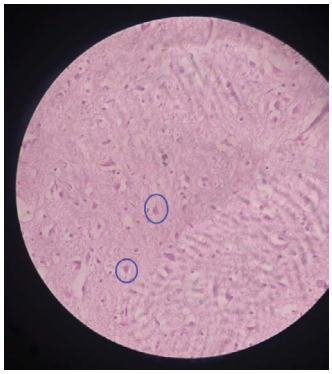

Figure 3: Cell bodies in the hypoglossal nerve nucleus at 32th week of gestation under 400 magnification [H&E Stain]

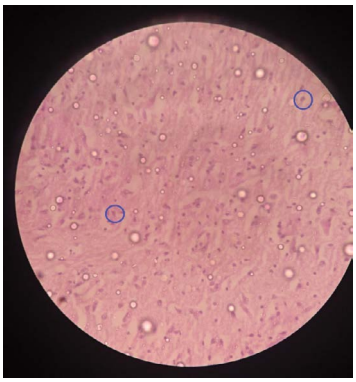

Figure 4: Cell bodies in the hypoglossal nerve nucleus at 36th week of gestation under 400 magnification [H&E Stain]

CONCLUSION

This current study about the morphometry of the neurons in human hypoglossal nerve nucleus from the 19th gestational week to the end of pregnancy found that the shape of the neurons were oval or pyramidal shape (from 18-24 weeks). The neurons grow rapidly in the initial period of the gestation, but at the end it shrinks to maturity. In the late stage, these nerve cells have dendritic process.

CONFLICT OF INTEREST

The authors' declares that they have no conflict of interest.

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