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MORPHOMETRICAL STUDY OF RETINA AT DIFFERENT GESTATIONAL AGE IN NORMAL AND FETUS WITH NEURAL TUBE DEFECT

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ABSTRACT

Therefore, retinopathy of prematurity, strabismus, refractive error, retinopathy of prematurity linked with myopia, and microphthalmos are the most usually related disorders with premature delivery. Microphthalmos is a condition of the eye that is often present at birth and is caused by a stop in the growth of the ocular tissues. When the eyeball is obviously underdeveloped, the diagnosis is straightforward; however, in cases that are on the diagnostic fence, distinguishing the normal size of the eyeball from the pathologically tiny size of the eyeball needs in-depth knowledge of the normal anatomy. Therefore, the normal ranges of foetal ocular measures taken during gestation may be useful in the diagnosis of syndromes linked with variations in the pace of ocular growth as well as other related foetal anomalies. Following the formation of the optic cup, the structure differentiates into the pigmented epithelium, the neuronal retina, and the optic stalk. The variable expression of transcription factors that are required for the specification is what distinguishes these unique areas from one another. Neural Tube Defects (also known as NTDs) are birth defects that affect the neural tube. They are caused by faulty development of the embryo's central nervous system.

keywords: *Morphometrical, retina, gestational age*

INTROUDUCTION

Our eyes are marvellous sense organs that enable us to take in all the splendour of the world in which we live, to read and educate ourselves, and to convey to one another our ideas and aspirations through the mediums of visual expression and the visual arts. When we lose the ability to see, which is the most fundamental of our senses, it is maybe the most tragic loss of all. Blindness deprives us of this modality. The retina is the layer that is most critical for vision, despite the fact that all portions of the eye are necessary for seeing an accurate image. The retina is essentially a portion of brain tissue that receives direct input from the lights and pictures that are present in the surrounding environment. There are between eight and twelve percent of all live births that are affected by premature birth, and the prevalence is growing as a result of advancements in neonatal care. Preterm birth can inflict a host of challenges on the developing ocular system, resulting in the visual manifestations of varied significance and pathological scope. However, many preterm infants survive, even those born as early as weeks of gestation. This is despite the fact that preterm birth can inflict these challenges. Therefore, retinopathy of prematurity, strabismus, refractive error, retinopathy of prematurity

linked with myopia, and microphthalmos are the most usually related disorders with premature delivery. Microphthalmos is a condition of the eye that is often present at birth and is caused by a stop in the growth of the ocular tissues. When the eyeball is obviously underdeveloped, the diagnosis is straightforward; however, in cases that are on the diagnostic fence, distinguishing the normal size of the eyeball from the pathologically tiny size of the eyeball needs in-depth knowledge of the normal anatomy. Therefore, the normal ranges of foetal ocular measures taken during gestation may be useful in the diagnosis of syndromes linked with variations in the pace of ocular growth as well as other related foetal anomalies. The morphological development of the eyeball throughout foetal life has only been the subject of a relatively limited number of research. The majority of the morphological studies of the eyeball have been conducted on children and human adults in vivo with the assistance of MRI or ultrasound, and they have required correction for optical or sonic distortion. As a result, the evaluating of ocular dimensions and shape in intrauterine period provides information on the baseline shape of the eyeball. The dimensions of the eyeball as well as the diameter of the lens have only ever been measured in vitro up to this point. If they were to be performed in vitro, many of the measurements of other ocular dimensions would be more simpler and more precise. On the other hand, relatively few data have been gathered on the human foetal eyeball since there is a restricted availability of human tissues that have been grown ex vivo. The purpose of this particular study was to investigate the development of the human eyeball from the time of conception till delivery. The human retina is formed over a period of several months, during which time it develops both in utero and after birth (postnatally). The careful examination of the retina throughout its development while the embryo is still within the mother's womb has the potential to yield valuable information that may be used to improve diagnostic criteria and contribute to the creation of treatment plans that are curative. 4 There aren't many in-depth reports that cover the timeline and the specifics of foetal development in all of the retinal layers that can be found in the literature. In addition, establishing exact timelines of human retinal development at known retinal loci is vital for both the understanding of what areas and layers of the retina may be damaged by events that occur while the foetus is still in the uterus as well as for the resolution of medicolegal concerns. Studies on the simian retina's anatomy have played a significant role in the accumulation of knowledge concerning the proper development of the foveal structure and the central retina. 6,7 There have been fewer observations conducted of the human fovea, and the number of eyes included in these investigations ranges anywhere from two eyes.

Histology and spectral domain optical coherence tomography are the two methods that are used in the few investigations that have been done on the retinas of preterm newborns and normal infants (SDOCT). The pictures produced by SDOCT have a lesser resolution than those produced by histology. Furthermore, the quality of the image might be negatively impacted by ocular media, movement, shadowing, and the inability to differentiate between neighbouring tissues with comparable relative reflectivity.

A wide variety of ophthalmological conditions can cause changes in the thickness of the retina; for example, diabetic macular lesions can lead to an increase in the thickness of the macular retina. Retinal atrophy can also be caused by glaucoma and other degenerative disorders, which ultimately results in a reduction in the thickness of the retina. The correct measurement of the thickness of the retina is one of the pieces of evidence that is regarded necessary for making a diagnosis of certain ophthalmological conditions. In the macula of adults as well as the areas surrounding the optic nerve head, a normative database for the thickness of the retina has been constructed.

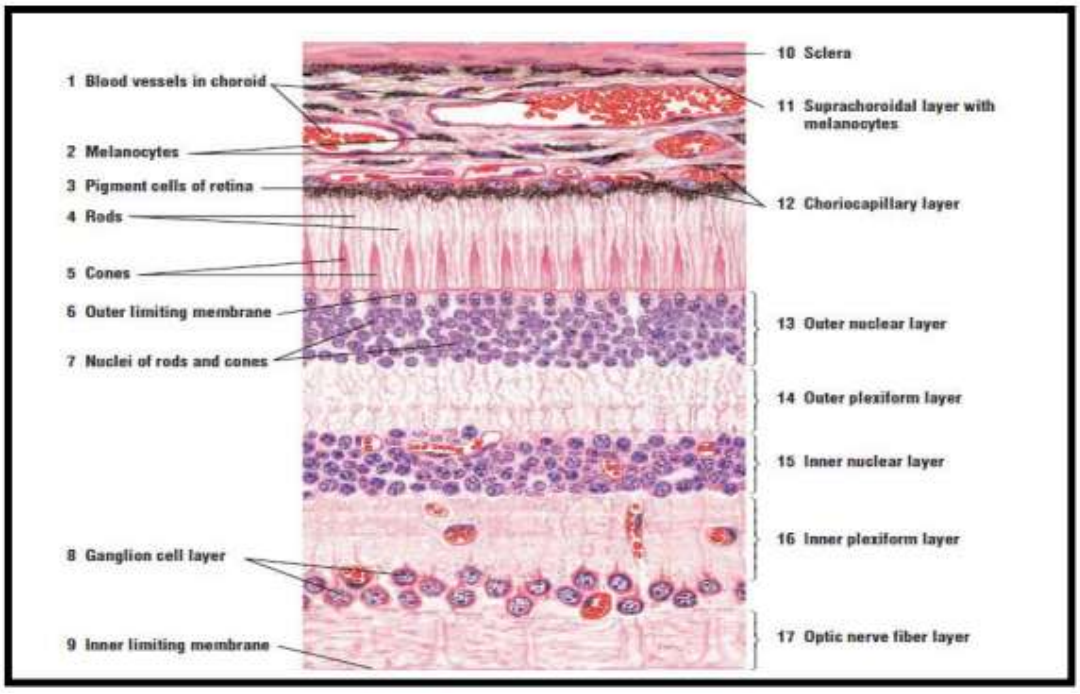
OBJECTIVES:

1. To study the morphometry of eyeball at different gestational age in normal and fetus with neural tube defect.
2. To study the vascular growth of retina at different gestational age by immunohistochemistry
3. To study the proliferative capacity of retinal progenitor cells by immunohistochemistry in normal and fetus with neural tube defect.

MICROSCOPIC ANATOMY

The retina is made up of millions of cells that are densely packed into a network that covers the whole surface of the back of the eye. These cells are capable of being broken down into three primary subtypes: photoreceptor cells, neuronal cells, and glial cells. The organisation of the retina into 10 layers, which can be seen with a light microscope, is the consequence of a very particular arrangement and connection of the nuclei and processes of the cells that make up the retina. There are many tiny layers in the retina, and each one has its own name and is composed of a unique set of components. These layers are as follows, beginning with the deepest layer (which is closest to the vitreous) and going outwards towards the choroid and sclera:

1. The membrane that serves as the internal boundary
2. The layer of the nerve fibres
3. The layer consisting of ganglion cells
4. The innermost layer of plexiform tissue 5. The closest layer to the nucleus
5. The outermost layer of plexiform cells 7. The most outermost layer of nuclear cells
1. The outer limiting membrane comes in at number 8.
2. The layer composed of rods and cones
3. The epithelium that contains the pigment (Figure-1)



Figure–1Layers of retina

Molecular regulation of eye development

In order for the eye to develop normally, there must be a pretty intricate interaction between its many tissues, which entails a number of distinct types of reciprocal inductive processes (Figure-2). Following the formation of the optic cup, the structure differentiates into the pigmented epithelium, the neuronal retina, and the optic stalk. The variable expression of transcription factors that are required for the specification is what distinguishes these unique areas from one another. Ch10, Pax6, Six6, and Rx are expressed in the neural retina, Mitf and Otx2 are expressed in the pigmented epithelium, and Pax2 is expressed in the optic stalk. (fig.2A,B,C,D) One of the most important participants in the process is a transcription factor that is encoded by the PAX6 gene. Before the process of neurulation ever begins, the neural plate already has a single eye field, which serves as the starting point for the development of the eye. The secretion of sonic hedgehog (shh) from the prechordal plate is necessary for the division of this single eyefield into two eyefields. It has been hypothesised that the sonic hedgehog protein in the anterior neural ridge downregulates the expression of the PAX6 gene while simultaneously upregulating the expression of the PAX2 gene, which results in the field being divided in half (Figure-14). Cyclopia is caused when there is a malfunction in either the production of the sonic hedgehog protein or its expression. When the optic vesicle buds off from the neuroectoderm in the third week of development, it triggers the formation of the lens placode in the overlying surface ectoderm by secreting the growth factor BMP4. The level of expression of the PAX6 gene in the surface ectoderm is what determines whether or not the surface ectoderm will be able to respond to BMP4. At this point, the lens placode assumes the role of the inducer and begins to release growth factors, one of which is fibroblast growth factor (FGF), which cause the optic vesicle to differentiate into the optic cup. The lens vesicle then grows from the lens placode, and as it does so, it secretes substances that promote the creation of the neural retina in the wall of the optic cup. This happens when the lens vesicle develops from the lens placode. In addition to this, the lens vesicle prompts the ectoderm that lies above it to initiate the formation of the cornea. Now that this stage has been reached, the neural retina takes on the role of the inducer and begins to release substances that cause the cells that line the inside of the lens vesicle to extend and differentiate into lens fibres. It is the secretion of transforming growth factor (TGF) by the mesenchyme that surrounds the optic cup that causes the creation of the pigmented retinal layer, as well as the choroid and the sclera. This occurs at the same time as the formation of the inner neuronal retinal layer.

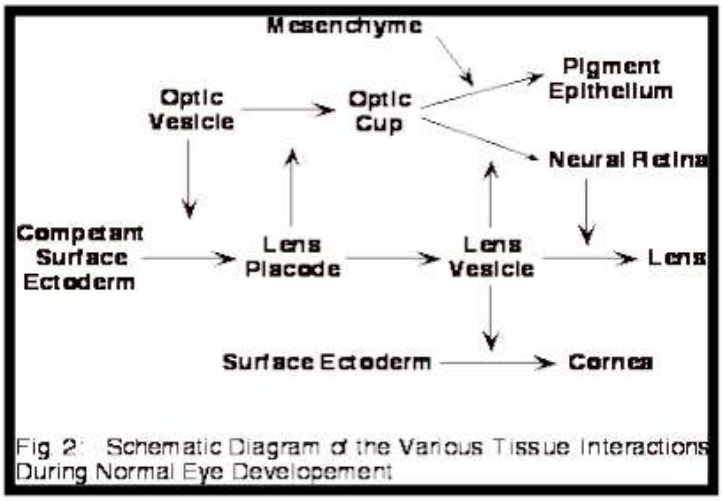
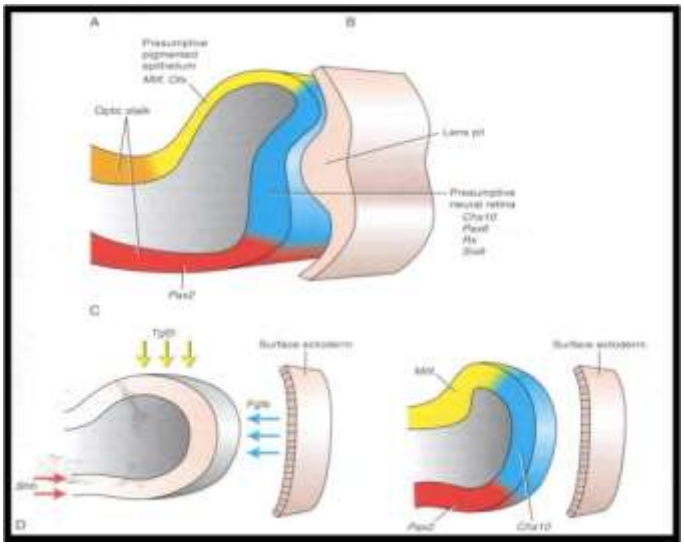


Figure – 2 various events during development of eye.



Figure–3 The expression of different transcription factor during development of eye

Development of Neural Tube Defects

Neural Tube Defects (also known as NTDs) are birth defects that affect the neural tube. They are caused by faulty development of the embryo's central nervous system. Spina bifida and anencephaly are the two most frequent forms of neural tube defects (NTDs). Both of these conditions affect distinct levels of the brain and spine, and they often reflect abnormalities in the embryonic processes that produce these structures. Birth defects such as neural tube defects (NTDs) are relatively uncommon, with a global prevalence among live births in the United States of one in one two hundred, and a worldwide prevalence ranging from one in one thousand (in Europe and the Middle East) to three to five in one thousand (in northern China as of 2014 with folate supplementation campaigns, bringing the prevalence down from ten per one thousand for years 2000–2004) Surprisingly little is known about the causes of NTDs in people despite the fact that these diseases have a significant impact on public health. A process in vertebrates known as neurulation is responsible for the

development of the central nervous system, which consists of the brain and spinal cord. This procedure takes place in human embryos anywhere between day 17 and day 28 after they have been fertilised. During the period of development that came before (gastrulation), the ectoderm was generated. This ectoderm will eventually thicken in response to certain chemical signals emitted by the notochord that lies underneath it, which will result in the formation of the neural plate. Elevation, juxtaposition, and fusion along the major neurulation of the body axis will result in the formation of the neural tube from this plate of ectodermal cells. In order to complete the process of neurulation (secondary), the cells in the caudal region must first condense and then undergo a transition from the mesenchymal to the epithelial layer. Primary neurulation is a multi-site process in animals, and recent research suggests that in humans there are two closure sites that are recognisable. This is an important discovery (one at the prospective cervical region and one over the mesencephalon-rombencephalic boundary) The creation of an anterior (ANP) and posterior neuropore is required for mammalian neurulation, which is a process that is very demanding on the organism's energy resources due to its stringent regulation (PNP). These openings, known as neuropores, will gradually get smaller until the ultimate fusion occurs, which will conclude the process of neural tube closure, also known as NTC (Figure 4).

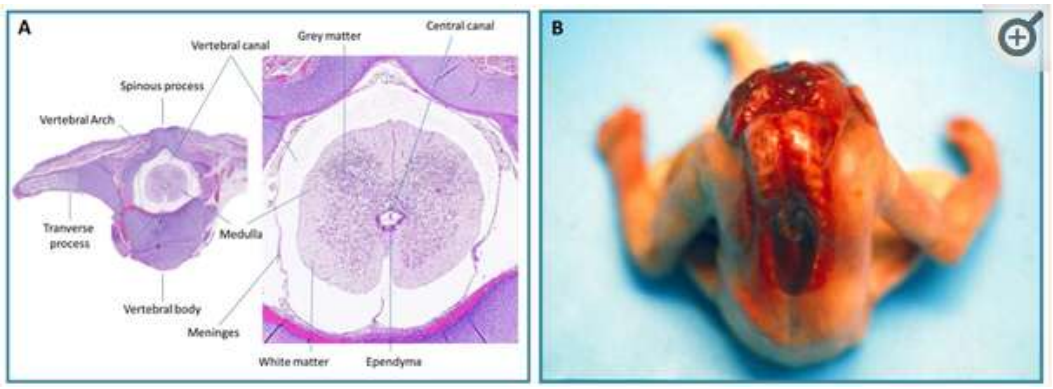


Figure 4. A: Transverse section of normal fetal vertebra with typical spinal cord section. B. Craniorachischisis. Note the absence of the cranial vault, the defect extends to vertebral arch into upper dorsal area, resulting in anencephaly and spina bifida

Different types of NTDs reflect the site of the interrupted neurulation. For example, craniorachischisis, which affects the brain and spinal cord, results from a failure of the initial closure site resulting in an open brain and spine, while anencephaly arises from abnormalities in the cranial neurulation process, and spina bifida results from incomplete caudal neurulation.

MATERIALS AND METHODS:

Numbers of specimen: 100 eyeballs of 55 normal fetuses and 6 eyeballs from NTD fetuses.

sex of fetuses: Dissection was done in both male and female fetus

Age of fetuses: between 13 to 40 gestational weeks.

Inclusion criteria:

1. Spontaneous aborted Fetuses
2. Still birth fetuses
3. From MTP

Exclusion criteria:

1. Decomposed fetuses
2. Developmental anomalies fetuses.
3. Macerated fetuses
4. Fetuses with history of different diseases in mother like TB, Hyperthyroidism and autoimmune diseases etc.

Determination of the age of fetuses:

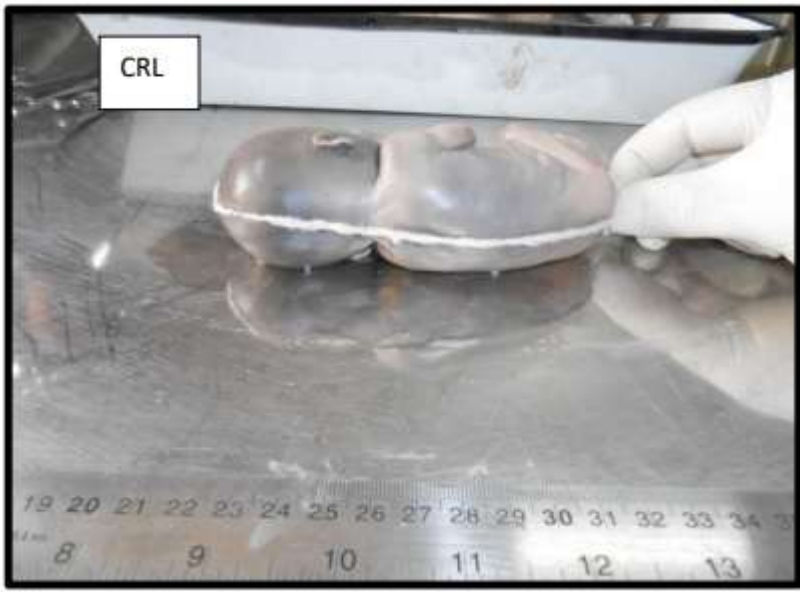
The obstetrical history, crown rump length (CRL), crown heel length (CHL), foot length (FL) with thread and scale in centimetres, and crown heel length (CHL) were used to establish the age of the foetuses, which was then followed by the weight of the foetuses in grammes.

The length of foetuses was measured from the top of the head (crown) to the bottom of the buttocks (rump) in order to determine their crown rump length.

The length of the crown to heel was determined by measuring the distance from the top of the head to the bottom of the heel of the foetuses.

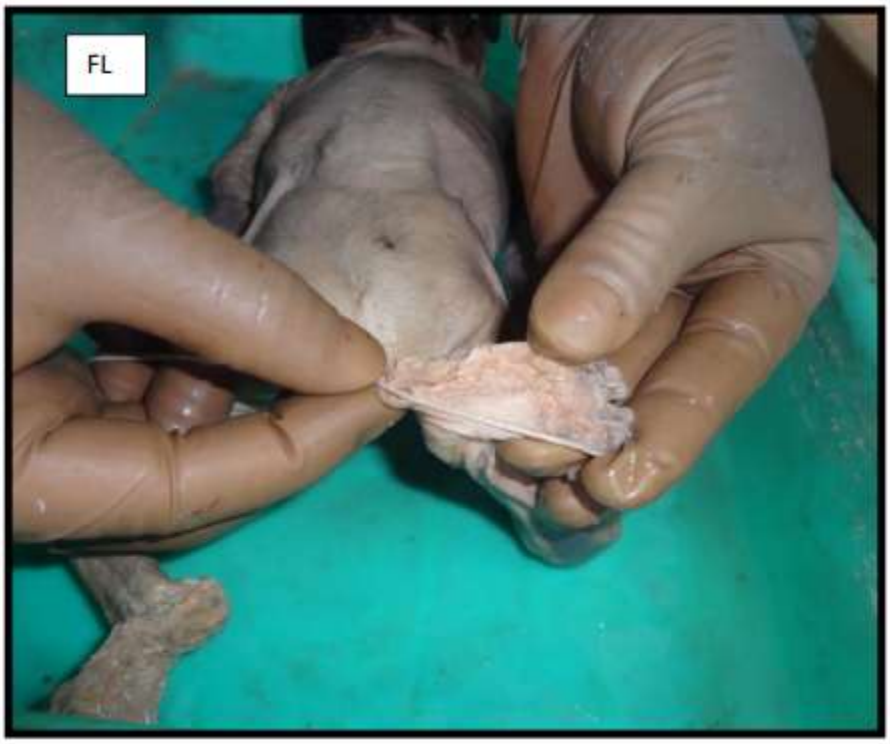
The length of the foot was measured along the medial border of the foot, starting at the heel and ending at the very tip of the big toe.

The foetal weight was determined using a computerised weighting equipment and expressed in grammes.



Figure–5.The measurement Crown rump length for determination of gestational age

Figure–6.The measurement Crown heel length for determination of gestational age



Figure–7.The measurement Foot length for determination of gestational age

Figure–8.Calibrating the weight of fetuses at different weeks of gestational age

Morphometrical Parameters

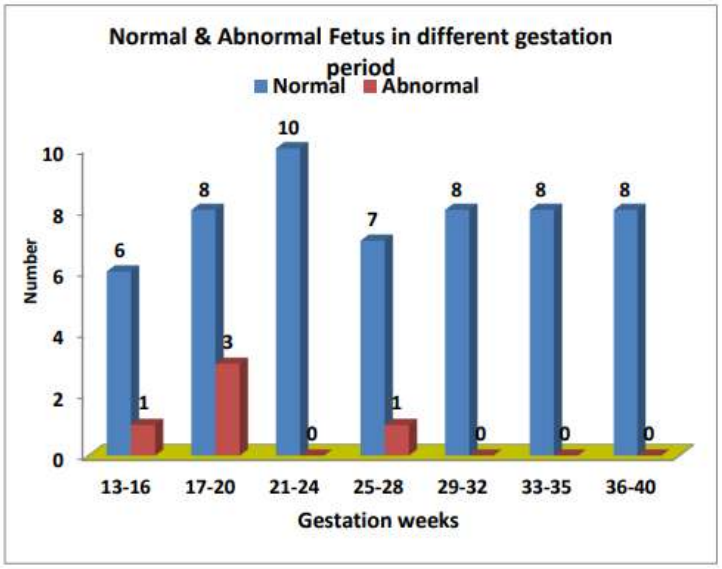
- The antero-posterior diameter of eyeballs in foetuses of normal mothers at different gestational ages, measured with a vernier calliper in millimetres
- The transverse diameter of eyeballs in normal mothers' foetuses at different gestational ages, measured with a vernier calliper in millimetres
- The vertical diameter of eyeballs in normal mothers' foetuses at different gestational ages,

RESULTS

The department of Anatomy at MGM Medical College in Navi Mumbai was responsible for carrying out the research for the current paper. In the current research, a total of 100 eyes from 55 foetuses were analysed between the ages of 13 and 40 weeks. The data for each group's normal and abnormal foetuses, including those affected by anencephaly, spina bifida, and menigocele, are presented in the table below.

Table 1: Number of fetuses for each group

Gestation weeks	Normal Fetuses		Abnormal Fetuses (NTD)		Total	
	N	%	N	%	N	%
13-16	6	10.9	1	20.0	7	11.7
17-20	8	14.5	3	60.0	11	18.3
21-24	10	18.2	0	0.0	10	16.7
25-28	7	12.7	1	20.0	8	13.3
29-32	8	14.5	0	0.0	8	13.3
33-35	8	14.5	0	0.0	8	13.3
36-40	8	14.5	0	0.0	8	13.3
Total	55	100.0	5	100.0	60	100.0



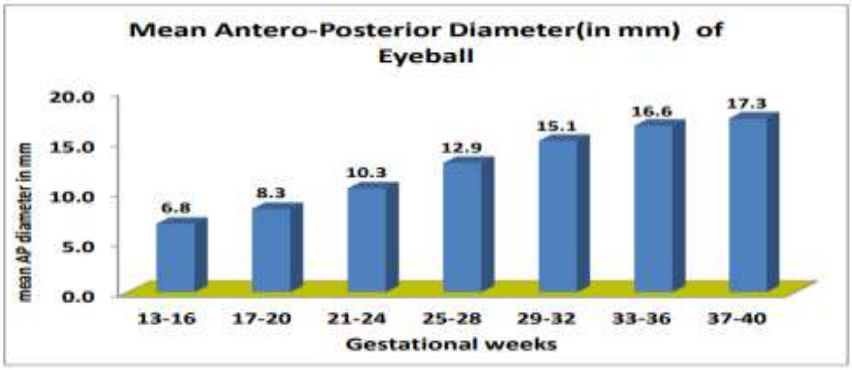
Graph -9.The number of fetuses for each group

Histogram illustrating the greatest number of normal foetuses present in the third group (21-24), as well as the smallest number of normal foetuses present in the first group (13-16) The following parameters were investigated in the current study, which encompassed all facets of morphometric research:

Table-2 Descriptive statistics for antero-posterior diameter of eyeball

	Antero-posterior Diameter(in mm) Mean								
Gestational weeks	No. of Fetuses	Min	Max	Mean	SD	SEM	95% CI of Mean		P-value
							Lower	Upper	
13-16	6	6.15	7.46	6.83	0.54	0.2220	6.26	7.40	
17-20	8	7.82	9.10	8.30	0.42	0.1491	7.95	8.66	
21-24	10	9.58	11.42	10.34	0.58	0.1824	9.93	10.75	
25-28	7	11.95	13.75	12.86	0.64	0.2427	12.27	13.46	<0.001
29-32	8	14.08	15.95	15.05	0.68	0.2416	14.48	15.62	
33-36	8	16.10	16.95	16.55	0.32	0.1129	16.28	16.82	
37-40	8	16.95	17.56	17.27	0.19	0.0688	17.11	17.44	

At the 13th – 16th weeks, the mean antero-posterior diameter was 6.83 mm (n = 6, SD = 0.54) and at the 37th – 40th weeks, the mean antero-posterior diameter was 59 17.27 mm (n = 8, SD 0.19). These findings are presented in the table below. The antero-posterior diameter, measured in millimetres, was subjected to a one-way analysis of variance. The findings suggest that there is a high significant rise in the antero-posterior diameter with increasing gestational age. (P- value < 0.001)



Graph–10.The mean antero-posterior diameter of the eyeball increases as gestational age increases

DISCUSSION

The estimation of the foetus' gestational age (GA) is of enormous significance in medical and legal contexts1. In obstetric and paediatric clinical practise, having an accurate calculation of the age of the foetus is of the utmost importance. The GA estimation may be accomplished using a variety of approaches; however, the approaches that are utilised most frequently include measures of foetal parameters such as foetal weight, FL,

HC, CRL, and CHL. The current study's calculation of gestational age by crown-to-heel length agrees very well with that of Sachin S. Patil et al. (2013) 60. Archie J.G. et al. (2006)78, Aryal DR et al. (2012)79, Davidson S. et al. (2008)80, Ghosh S. et al. (1971)81, and Mukherjee J.B. (2007)82 are some of the studies that have been conducted on this topic. Similar to what was found in Sachin S. Patil et al. (2013)60, the foetal age estimate utilising crown-rump length performed very well in the current investigation. Archie J.G et al (2006)78. A few of the findings were off owing to variations in population, sample size, genetic and environmental variables, all of which influence the development of the foetus and interfere with the ability to accurately estimate an individual's age in a given region. There was a strong connection between the GA determined by the obstetric technique and the GA that was computed using FL, HC, CRL, CHL, and AC. First place went to FL, then CRL, then CHL, and finally AC for precision. The findings of our observation are consistent with the findings of Panduranga Chikkannaiah et al. (2016)61. According to the findings of our research, the foot length provides the most accurate measurement of GA compared to the other characteristics. The foot length values observed in the 15th, 25th, 32nd, and 37th gestational weeks were 2 centimetres, 4.8 centimetres, 6.7 centimetres, and 7.6 centimetres respectively in the present study. These findings are nearly the same as the findings of Panduranga Chikkannaiah et al (2016)61, which were in the 15th, 25th, 32nd, and 37th gestational weeks 2.3 centimetres, 4.7.

CONCLUSION

In the current study, there were a total of 55 normal and 3 abnormal foetuses, and they were divided up according to their gestational ages. In this particular investigation, the anterior-posterior, transverse, and vertical diameters of the eyeball were measured. The biggest diameter was found in the antero-posterior direction. As gestational age progresses, there is an increase in the overall thickness of the retina. The thickness of each layer of the retina increases as the pregnancy progresses, with one exception: the thickness of the ganglion cell layer initially increases until the 19th week of gestation, after which point it decreases until the 35th week, after which point it remains stationary until the 40th week. The layers of the neuroblastic layer, the retinal pigment epithelium layer, the inner plexiform layer, the ganglion cell layer, and the nerve fibre layer are all visible in the retina up to 20 weeks. At the 20th week, the neuroblastic layer began to differentiate into the outer nuclear layer and the inner nuclear layer as the outer plaxiform layer began to develop. As gestational age grows, there is a corresponding increase in the horizontal expansion of the retina from the optic nerve to the nasal side and temporal side. Greater development occurred in the temporal region of the retina compared to the nasal region. When compared to normal babies of the same gestational age, anencephaly foetuses in the 16th week had shorter crown rump lengths, shorter crown heel lengths, and lower body weights. At 20 weeks gestation, the length of the crown rump, the length of the crown heel, the length of the foot, and the body weight of meningocele foetuses were almost identical to those of normal foetuses. At 16 and 17 weeks of gestation, the diameter of the eyeball was somewhat larger in foetuses with anencephaly and spina bifida than it was in normal pregnancies. At the 16th and 17th weeks of development, the thickness of the retina in foetuses with anencephaly and spina bifida was significantly smaller than that of normal foetuses. At 20 weeks of gestation, the thickness of the retina in meningocele foetuses was almost identical to the thickness of the retina in normal foetuses. At 16 and 17 weeks of gestation, the number of progenitor cells in foetuses with anencephaly and spina bifida was lower when compared to the number of progenitor cells in

normal foetuses. At 20 weeks of gestation, the number of progenitor cells in a foetus with meningocele was almost identical to the number of progenitor cells in normal foetuses.

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