ISSN 2230-9365

International Journal of Anatomical Sciences

Volume 2 No.1 March 2011

Official Journal of the
Association of Anatomists, Tamil Nadu, India

International Journal of

Anatomical Sciences

(Official Journal of Association of Anatomists, Tamil Nadu, India)
Volume 2 No. 1 March 2011

www.ijas.in

Editorial Board

Advisory Board

Published By

Association of Anatomists, Tamil Nadu, India
Regd. Under Societies Act, Government of Tamil Nadu – Reg. No:144/1988
Editorial Office, Department of Anatomy, University of Madras
Taramani Campus, Chennai 600 113, India
Phone: 91-44-24547020 Email: editor.ijas@gmail.com

International Journal of Anatomical Sciences
(Official Journal of Association of Anatomists, Tamilnadu)
March 2011; Volume 2 Issue 1

Contents **Page**

International Journal of Anatomical Sciences 2011,2(1):1-6

Case Report

Complex Congenital Heart disease with Sacral Agenesis – a Case Report

Srimathi, T.

Department of Anatomy, Sri Ramachandra University, Porur, Chennai 600 116, Tamil Nadu, India.

Key Words: congenital heart disease, sacral agenesis

Abstract: Complex congenital heart anomalies are less common. A thirty years old third gravida treated for secondary infertility, gestational diabetes mellitus and hypertension was admitted in Sri Ramachandra Medical College Hospital near term with a breech presentation of the fetus. Labour was induced due to Gestational diabetes and fetal bradycardia. She delivered by vaginal delivery / assisted breech delivery with episiotomy. The child had neonatal respiratory distress, did not cry at birth and was not breast feeding well. The child was treated in the neonatal Intensive care unit for the same. The foetal echocardiogram revealed small left ventricle, dilated right ventricle, single outflow from Right ventricle and probable Truncus arteriosus. Infantogram revealed cardiomegaly and Sacral Agenesis. The case is unique for its rarity and its embryological significance.

Complex congenital heart diseases are less common. A congenital heart defect (CHD) is a defect in the structure of the heart and great vessels that are present at birth either obstruct blood flow in the heart or vessels near it or cause blood to flow through the heart in an abnormal pattern. Congenital heart diseases constitute 1% of all congenital malformations. The cause is genetic in 8% and environmental in 2% of the cases. Mostly it is multifactorial. (Saddler, 2006)

Case Report

Thirty years old female patient was admitted near term .She was married six years ago. She had an induced conception. She was the third gravida with only Live born child in this conception. She had Gestational diabetes mellitus and was on insulin. She also had Pregnancy induced hypertension and was on treatment. Patient was taking antenatal care elsewhere before admission and said to have been taking Folic acid supplements.

Her Blood group was Rh negative. Her TORCH PANEL was positive for Herpes simplex virus. There was no positive family history of live births with congenital cardiac anomalies or other birth defects.

Antenatal ultra sonogram of the mother was done and following were the findings:

- At 36 weeks revealed no intrauterine growth retardation, Single live fetus with 36 wks gestation with breech presentation.
- Fetal bradycardia, small left ventricle (Fig.1)

Correspondence to: T. Srimathi, Department of Anatomy, Sri Ramachandra University, Porur, Chennai – 600 116, Tamilnadu, India.
Email: arima_tamil.yahoo.co.in

Accepted :15-March-2011

- Single outflow from right ventricle (Fig.2) and Single umbilical artery (Fig.3)

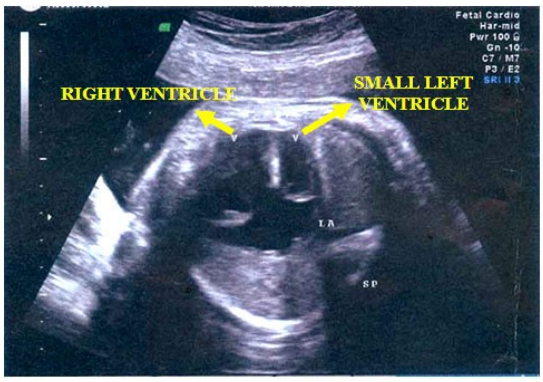
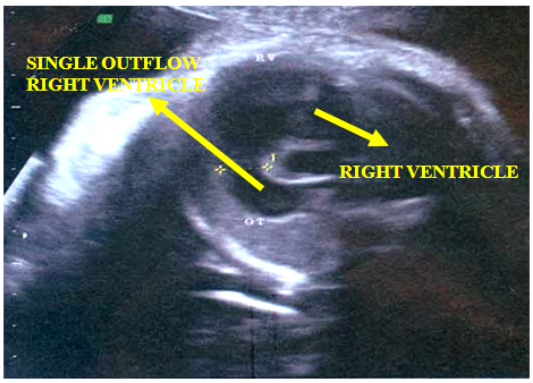
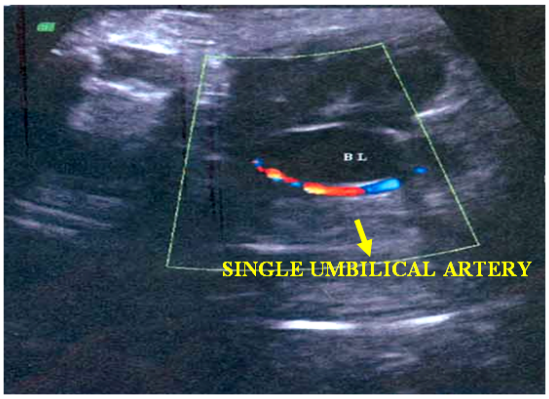


Fig 1.Ventricles, small left ventricle



(Fig: 2) Single outflow From right ventricle



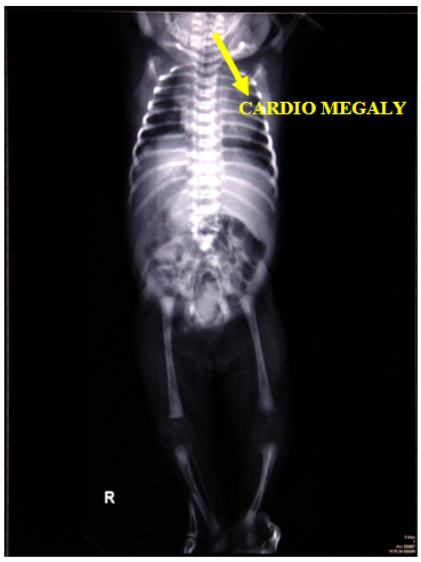
(Fig 3) Single umbilical artery

Patient delivered by assisted breech delivery after induction. Induction was done due to fetal bradycardia and gestational diabetes. A female child was born at term. The cry was weak and the baby did not feed well and had respiratory distress. Physical examination of the neonate revealed the following findings:

Birth weight was normal with reduced Apgar score (6/10) and normal head circumference and length. There was no cyanosis. The Respiratory rate was increased (76/mt). Oxygen saturation was reduced in arterial blood. Cardiovascular system examination revealed both heart sounds with a pansystolic murmur. Abdomen was soft. Both lower limbs were hypoplastic extended at knees and adducted. As the baby did not cry immediately after birth, assisted ventilation was provided and was treated with intravenous antibiotics after septic workup. The child picked up with normal respiratory effort.

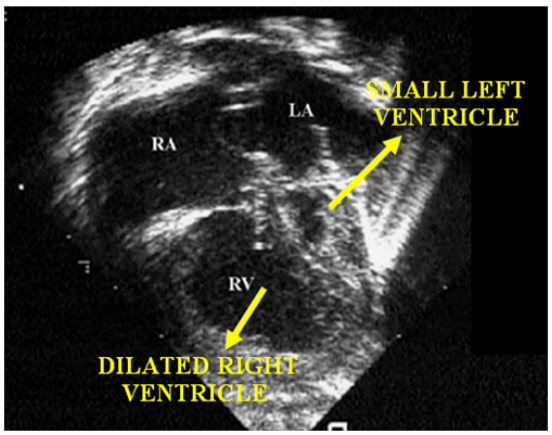
Investigations of the child revealed the following:

1. Infantogram - Revealed cardiomegaly (Fig.4).
2. Echocardiogram showed Complex congenital heart disease - Hypoplastic mitral ring, Small left ventricle, Dilated right ventricle, Single outflow from Right ventricle (Probable *Truncus arteriosus*) (Fig.5).
3. X ray LS Spine showed Sacral Agenesis (Fig.6)

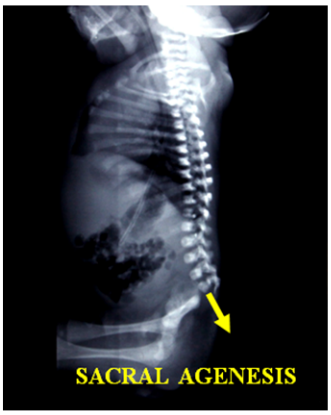


(Fig4) Infantogram showing cardiomegaly

IJAS 2011, 2(1): 1-6



(Fig 5) Small left ventricle, Dilated right ventricle



(Fig 6) Sacral agenesis in X ray Lumbosacral Spine lateral view

Discussion

The clinical features of this case being suggestive of a syndrome called Hypoplastic Left Heart syndrome. This was first described in 1952 by Lev as "hypoplasia of aortic tract" including cases of hypoplasia of aorta, Venticular septal defect, aortic stenosis or atresia, with or without mitral atresia. In 1958 Noonan and Nadas described these lesions as Hypoplastic Left Heart syndrome (Rao, 2009). The prevalence of hypoplastic Left Heart syndrome is 0.016-0.27 per 1000 live births. It constitutes 1.4-3.8% of congenital heart disease. The incidence of congenital heart disease as such in India is 8 per 1000 live births.

Out of these 180,000 children born each year nearly 60,000 have critical congenital heart disease that requires early intervention (Saxena, 2005). The incidence of severe congenital heart disease that will require expert cardiologic care is about 2.5 to 3/1,000 live births. The moderately severe forms of CHD probably account for another 3 per 1,000 live births. (Hoffman, and Kaplan, 2002)

Combination of this syndrome with sacral agenesis is a rare occurrence. Sacral agenesis is rare affecting 1 in 25,000 (Hoffman 1980). While hypoplastic Left Heart syndrome is more common in males but sacral agenesis has equal incidence in both sexes.

The probable causes of the Hypoplastic Left Heart syndrome in this case could be due to abnormal partitioning of the *Truncus arteriosus* resulting in small aortic outflow tract and a hypoplastic valve annulus. This impedes the blood flow through the aorta from the left ventricle. Since the stimulus for normal development of the aorta is thus interrupted aorta becomes hypoplastic. Normal development of the left ventricle and the mitral valve could have been secondarily affected resulting in hypoplasia of these structures. (Bardo *et al.*, 2001).

The probable causes of sacral agenesis could be due to disorders of primary or secondary neurulation which commonly occurs during the thid to seventh week of pregnancy. (Fellous, *et al.*), Folic acid deficiency is a probale cause but the patient was taking antenatal care elsewhere before admission and said to have been taking Folic acid supplements. Though she was termed as having gestational diabetes mellitus probable pre gestational undetected diabetes is also a possible cause since the previous blood sugar values were not available. Sacral agenesis may also occur as a part of Curarino's syndrome .This syndrome is an autosomal dominant disorder with sacral agenesis,mass in the

presacral space, and anorectal mal-formations. This occurs due to mutation of HLXB9 gene most probably microdeletion involving HLXB9.There is a linkage to chromosome 7q36. Homeobox genes (Hox) are required for development of various organs and skeletal develop-ment. Altered patterns of expression or mutations of Hox genes have been shown to produce congenital abnormalities.

It is more likely that the combination of congenital heart disease with sacral agenesis and single umbilical artery, hypoplasia of the limbs in this case is a part of Caudal regression syndrome. The majority of Caudal regression syndrome is sporadic. The role for HLXB9 in caudal regression has not yet been demonstrated, although it is possible that a mutation or DNA sequence variation in the noncoding region of HLXB9 could be present in these individuals, it is more likely that these malformations are due to other factors or defects in other gene(s) (Belloni and Martucciello, 2000). Bernard Duhamel first described the syndrome as a spectrum of congenital malformations, which consist of anomalies of the rectum, the urinary and genital systems, the lumbosacral spine, and the lower limbs. The most severe end of the spectrum is the fusion of the lower limbs and the major organ malformations. This is known as sirenomelia or mermaid syndrome. (Zaw and Stone, 2002)

Caudal regression syndrome is associated with maternal diabetes which is also a possibility in this case. Hyperglyce-mia leads to release of free radicals which can be teratogenic.

Abnormal migration of the neural crest cells is also a probable cause for both the congenital heart disease and the sacral agenesis in this case. Identifying the High Risk mothers for the development of babies with congenital anomalies, proper prenatal screening, possible preventive measures and sometimes intrauterine corrective measures for the birth defect are the only tools to prevent and treat such congenital anomalies.

Identifying High Risk mothers :

Elderly mothers (age 35 and above), diabetic mothers,previous pregnancy with birth defects or genetic disorder, Exposure to teratogens like chemicals, drugs,radiation and TORCH infection,positive family history of genetic disorder, mothers with congenital heart disesases and syphilis are the High Risk mothers for congenital anomalies.(Ganesh Dangal, 2007).

Prenatal Tests:

1. Ultrasonogram
2. Chorion villus sampling
3. Amniocentesis
4. DNA analysis
5. Genetic testing for parents with previous child with birth defects or genetic disorder
6. Routine antenatal screening tests
7. Maternal serum screening for Triple marker and Quad marker (elevation of alfafetoprotein, unconjugated estriol,Human chorionic gonadotrophin and inhibinA) .This test is useful to detect neural tube defects and Down's syndrome.
8. Preimplantation genetic diagnosis though there are ethical issues .Detects potential defects in an embryo within first few days of conception. A blastomere is removed from embryo and tested for genetic abnormalities in Invitro fertilization.If there are are no such abnormalities then the embryo is implanated.
9. Fetoscopy, Fetal skin sampling, cordocentesis. (Alberman and Berry, 1979)

Possible preventive measures:

1. Proper vaccination during pregnacy
2. Folic acid supplements
3. Avoidance of unnecessary medications, alcohol and smoking
4. Consultation with a genetic counselor for the high risk mothers
5. Genetic screening for the high risk mothers
6. Appropriate treatment of maternal diabetes and associated maternal diseases

Intrauterine corrective measures:

Intrauterine corrective surgeries can be done for neural tube defects like spina bifida, congenital heart diseases, sacrococcygeal teratomas and congenital diaphragmatic hernias.,though there are ethical issues. Tworetzky (2004) performed a study in which 3 of 9 fetuses that had a technically successful valvotomy (intra uterine) for aortic stenosis and a continuing pregnancy went on to a 2-ventricle circulation at birth showing Hypoplastic Left Heart Syndrome is preventable in utero

Conclusion:

Once a congenital anomaly is detected in the newborn, this should be disclosed to the family. The nature of the illness and treatment options has to be explained. The family should be helped to accept that this anomaly is predetermined and not the result of birth injury. Counselling should be given. Periodic follow up is mandatory. The treatment options for the hypoplastic left heart syndrome include Norwood procedure, heart transplantation and Supportive expectant Care. (Norwood and Lang 1981) Identifying the High Risk mothers for the development of babies with congenital anomalies, proper prenatal screening, possible preventive measures and sometimes intrauterine corrective measures for the birth defect are the only tools to prevent and treat such congenital anomalies.

It is a collective term describing a group of cardiac malformations with various degrees of hypoplasia of the structures of the left side of the heart. Over 95% of patients with Hypoplastic Left Heart Syndrome will die if left untreated during the first month of life. Echocardiogram is the diagnostic procedure. As a result of advanced technology, refined surgical techniques (including intrauterine correction), and catheter based interventions, the mortality has reduced for children born with hypoplasia of the left heart. The syndrome is still considered as one of the most complex congenital cardiac malformations to manage. Treatment of patients with sacral agenesis is best provided by a team including an orthopaedic surgeon, urologist, neurosurgeon, pediatrician, physical therapist, and onthotist-prosthetist.

Genetic counseling and antenatal diagnosis of such diseases may be the tools for the prevention and management of this disorder. (Connor and Thiagarajan 2007).

The case history and investigations were collected after getting informed consent from the patient (mother).

Acknowledgements

I express my acknowledgements to Dr. Palaniappan, Dr. Buvana, Dr. Chitra, Department of Obsteterics and Gynaecology, Sri Ramachandra University, Chennai, Dr. Sumathylatha, Department of Anatomy, Sri Ramachandra University, Chennai for their guidance.

References

Alberman E, Berry C (1979) Prenatal diagnosis and the specialist in Community medicine. *Community medicine*, 1: 89-96.

Angelini P (1995) Embrylogy of congenital heart disease. *Tex Heart Inst J*, 22: 1-12.

Belloni E, Martucciello (2000) Involvement of the HLXB9 Homeobox Gene in Currarino Syndrome. *Am J Hum Genet*, 66: 1.

Bardo DME, David G. et al., (2001) Hypoplastic Left Heart Syndrome. *Radiographics, the journal of continuing medical education*, 21: 705-717.

Clark EB (1986) Cardiac embryology: its relevance to congenital heart disease. *Am J Dis Child*, 140: 41-44

Connor JA, Thiagarajan R (2007) Hypoplastic left heart syndrome. *Orphanet Journal of Rare Diseases*, 2: 23doi:10.1186/1750-1172-2-23.

Dangal G (2007) High-risk Pregnancy. *The Internet Journal of Gynecology and Obstetrics*, ™ ISSN: 1528-8439 7: 1.

Dutta AK (2000) In:Essentials of Human Embryology. 4th Eds. Calcutta: Current Books International. 183Saxena A (2005) Congenital heart disease –A status Report. *Ind J Paediatrics*, 72:595-598.

. Fellous M, Boue J et al., (1982) A five-generation family with sacral agenesis and spina bifida: possible similarities with the mouse T-locus. *Am J Med Genet*, 12: 465-487.

Hoffman JIE, Kaplan S (2002) The incidence of congenital heart Disease. *J Am Coll Cardiol,* 39: 1890-1900.

Norwood WI, Lang P et al., (1981) Experience with operations for hypoplastic left heart syndrome. *J Thorac Cardiovasc Surg*, 82: 511-519.

Pang D, Hoffman HJ (1980) Sacral agenesis with progressive neurological deficit. *J Neurosurg*, 7: 118-126.

Rao SP (2009) Hypoplastic Left Heart Syndrome. eMedicine Specialties > Pediatrics: Cardiac Disease and Critical Care Medicine Cardiology,: 1-64. (http://emedicine. medscape. com / article /890196)

Saddler TW (2006) In : Langman's Medical Embryology. 10th Edition, Philadelphia, USA: Lippincott Williams & Wilkins. 172.

Tworetzky W, Wilkins-Haug L, et al., (2004) Balloon dilation of severe aortic stenosis in the fetus: potential for prevention of hypoplastic left heart syndrome: candidate selection, technique, and results of successful intervention. *Circulation Journal of the American Heart Association*, 110: 2125-2131.

Williams DL, Gelijns AC (2000) Surgery for congenital heart disease. *J Thorac Cardiovasc Surg*, 119: 720-731Zaw W, Stone DG (2002) Caudal Regression Syndrome in twin pregnancy with Type II Diabetes. *J Perinatology*, 22: 171-174.

.

International Journal of Anatomical Sciences 2011,2(1):7-10

A Study on the Variations of Median Nerve formation in South Indian Population

Sarala Devi KV,[*] Udhaya K,[*] Sudha Seshayan[¥]

[*]*Department of Anatomy, Vinayaka Mission's Kirupanandha Variyar Medical College, Salem 636 308, Tamilnadu, India*
[¥]*Registrar, Tamilnadu Dr.M.G.R. Medical University, Chennai, India.*

Key Words: Median nerve, lateral cord, musculocutaneous nerve

Abstract: The median nerve is one of the commonest nerve showing variations from the level of its formation to its termination. This is an important region where anesthetist, plastic surgeon and oncologist land up with problems more frequently due to variations and the mismanagement will result if they are not aware of these variations. The median nerve was studied regarding its formation in 60 upper limb specimens by conventional dissection method. Two specimens showed variations in our study. In one specimen, low level formation of median nerve with innervation of arm muscles was found. In another specimen the median nerve was formed at a higher level by the fusion of medial root with lateral cord of brachial plexus. These findings will provide anatomical knowledge for the reliable clinical correlation and surgeries.

Median nerve is formed by the union of lateral root from lateral cord (C5, C6 and C7) and medial root from medial cord (C8 and T1) of brachial plexus lateral or antero-lateral to the 3rd part of axillary artery (Turner, 1864; Williams *et al.*, 1999; McMinn, 1990). Variations in the formation of median nerve are common, more frequent and have been observed by different authors in India (Satyanarayana *et al.*, 2010; Krishnamurthy *et al.,* 2007). Such study in south Indian population is comparatively sparse. Knowledge of these variations is essential for performing nerve repair, nerve transplant and reconstructive surgeries. Hence this study would be of great help to the plastic surgeons and orthopedic surgeons. Hence we undertook this study to find out the variations in formation of median nerve in South Indian population.

Correspondence to: K.V. Sarala Devi, Department of Anatomy, Vinayaka Mission's Kirupanandha Variyar Medical College, Salem, Tamilnadu. India
Email: sarala.shrihari@gmail.com

Accepted :15-April-2011

Materials and Methods

Sixty upper limb specimens from 30 embalmed human cadavers of South Indian population irrespective of sex were used for the present study. They were dissected by conventional dissection method (Romanes, 1986) and examined for anatomical variations in median nerve formation.

Observations

The median nerve showed two variations in two specimens (out of 60 specimens). No associated muscular and vascular anomaly was noticed.

IJAS 2011,2(1): 7-10

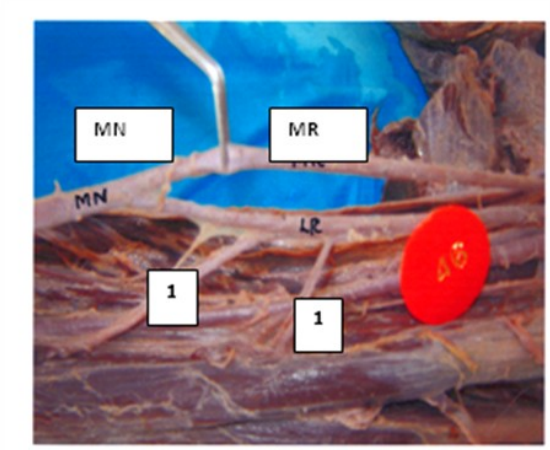


Fig 1: Low level formation of median nerve
MN – median nerve; MR – medial root;
1 – muscular branches to arm muscles

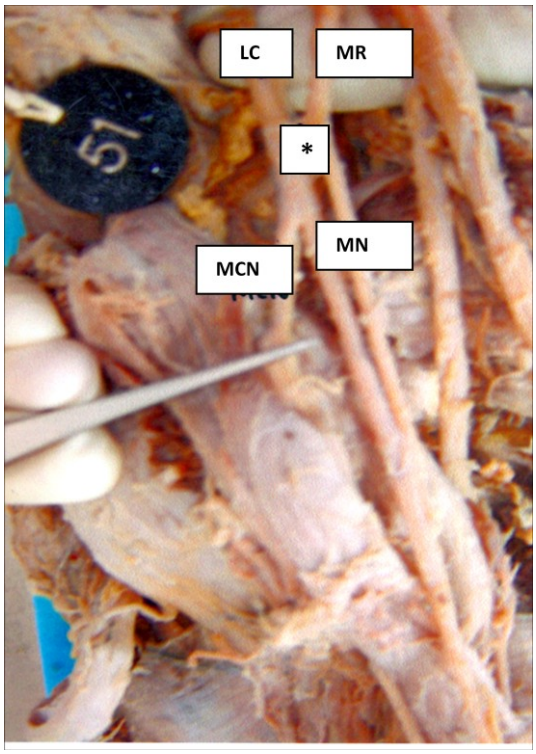


Fig 2: High level formation of median nerve.
LC – lateral cord; MR – medial root;
*MN–median nerve; MCN – musculocutaneous nerve; * - site of formation of median nerve (accidentally it was splitted when dissecting)*

One specimen (fig. 1) showed the formation of median nerve at the junction of middle and lower 1/3rd of arm. The medial root of median nerve was longer than usual (13.6 cm). The lateral cord of brachial plexus after giving the lateral pectoral branch, continued down in the arm. The medial root crossed in front of brachial artery from medial to lateral side and then united with the lateral cord to form the trunk of median nerve. The musculocutaneous nerve was absent. The lateral cord supplied branches to coraco-brachialis, biceps brachii, and brachialis. This anomaly was unilateral and the incidence was 1.66%.

One specimen (Fig. 2) was noticed with median nerve formation at a higher level than usual. After the lateral pectoral nerve was given off, lateral cord of brachial plexus was joined by medial root to form median nerve. About 0.7cm distal to the formation of median nerve, mus-culocutaneous nerve was given off. It was presenting unilaterally and the incidence was 1.66%

Discussion

Anatomical variations of median nerve are frequent and there are many studies reported. Chauhan and Roy (2002) reported the formation of median nerve by two lateral roots and one medial root. Satyanarayana *et al.* (2008) and Uzun and Seelig (2001) noted that the median nerve was formed by four roots, three lateral and one medial root. In other study, it was observed that musculocutaneous nerve was absent and so the median nerve supplied muscular branches to coracobrachialis, brachialis and biceps brachii (Guha and Palit, 2005). Satyanarayana *et al.* (2010) also reported that formation of median nerve by three roots, two from lateral cord and one from medial cord. In another study, the C7 fibers were traced from the median nerve to the musculocutaneous nerve (Walsh, 1877). In our study the median nerve was contributed by the lateral cord and root from medial cord but the musculocutaneous nerve was given off from median nerve; the evidence of this anomaly was found to be less in previous literature. The surgeon has to keep these

variations in mind especially during radical axillary dissection and shoulder arthroscopy wherever sparing of the nerve is mandatory

The incidence of distal formation of median nerve was more common (8.5%, Uysal *et al.*, 2003; 12%, Matejcik 2003; 2.1%, Mohammed and Badawoud, 2003) than that of high level formation. Due to these neural variations, sometimes the arm muscles are innervated by median nerve and in most of such cases, musculo-cutaneous nerve was found to be absent (Prasada Rao and Chaudhary, 2000; Song *et al.*, 2003, Satheesha nayak 2007, Beheiry 2004). In contrast to our study, Melani Rajendran and Nivedha, (2004) noted that the median nerve in addition to its formation in the middle of arm, gave off muscular branches to arm muscles and musculocutaneous nerve. The finding of our present study coincides with that of Satheesha nayak (2007). The knowledge of these variations of median nerve should be borne in mind during evaluation of unexplained nerve palsy after trauma, surgical intervention of particular area, neurotization of neural injuries and in reconstructive surgeries

In humans, the muscles of the upper limb develop from the mesenchyme of the paraxial mesoderm during the 5th week of embryonic life (Larsen, 1977). The neurons penetrate into the mesen-chyme in different direction (Brown *et al.*, 1991; Williams *et al.*, 1995). Immediately after the rearrangement of nerves, they enter the limb buds and establish an intimate contact with the differentiating mesoderm condensations. This early contact between the nerve and muscle cells is a prerequisite for their complete functional differentiation (Saddler, 2006). These variations of the nerves are the result of alterations in signaling between mesenchymal cells and neuronal growth cones during development which once formed could persist postnatally (Sannes *et al.,* 2000). Probably, the neurons of the nerve in our study would have also taken such anomaly.

Conclusion

These neural variations should be thought of while managing recurrent neuropathies with unusual clinical symptoms and signs. This is an important region where the anesthetists, plastic surgeons, oncologists and radiologists land up with problems frequently due to variations and mismanagement will result if they are not aware of these anatomical variations.

References

Beheiry EE (2004). Anatomical variations of the median nerve distribution and communication in the arm. *Folia Morphol (Warsz)* 63: 13-18.

Brown MC, Hopkins WG, Keynes, RJ (1991) Essentials of neural development. In: Axon guidance and target recognition. Cambridge University Press. Cambridge. pp 46 – 66.

Chauhan R, Roy TS (2002) Communication between the median and musculocutaneous nerve – a case report. *JASI* 52: 72–75.

Guha R, Palit S (2005) A rare variation of anomalous median nerve with absent musculocutaneous nerve and high up division of brachial artery. *J Interacad* 9: 398–403.

Krishnamurthy A, Nayak SR, Venkatraya Prabhu L, Hedge RP, Surendran S, Kumar M, Pai MM (2007) The branching pattern and communications of musculocutaneous nerve. *J Hand Surg Eur* 32: 560-562.

Larson WJ (1997) Human Embryology.In: Development of limbs. 2nd Edition, Churchill Livingston, Edinburgh. pp 311- 339.

Matejcik V (2003) Anatomic deviations in the origin of the median nerve. *Ro zhl chir* 82: 526-528.

McMinn RMH (1990) Upperlimb. In: Last's Anatomy. 8th Edition. Churchill Livingstone, Edinburgh. pp 82.

Melani Rajendran S, Nivedha R (2004) An anomalous median nerve. Anatomical Adjuncts. 4: 23-25.

Badawoud MHM (2003) A study on the anatomical variations of median nerve formation. *Bahrain Medical Bulletin* 25: 1-5.

Prasada Rao PV, Chaudhary SC (2000) Communication of the musculocutaneous nerve with median nerve. *East Afr Med J* 77: 498-503.

Romanes GJ (1986) Cunningham's Manual of Practical Anatomy. Vol I 15th Edition, Oxford University Press, London. pp 67-74.

Saddler TW (2006) Muscular system. In: Langman's Medical Embryology. 10th Edition. Lippincott Williams & Wilkins, Philadelphia. pp 146–147.

Sannes HD, Reh TA, Harris WA (2000) Development of the nervous system In: Axon growth and guidance. Academic Press, New York. pp 189-197.

Satyanarayana N, Guha R (2008) Formation of median nerve by four roots. *J College of Medical Sci-Nepal*, 5: 105–107.

Satyanarayana N, Reddy CK, Sunitha P, Jayasri N, Nitin V, Praveen G, Guha R, Datta AK, Shaik MM (2010) Formation of median nerve by three roots – a case report. *J College of Medical Sci-Nepal* 6: 47-50.

Satheesha Nayak (2007) Absence of musculo-cutaneous nerve associated with clinically important variations in the formation, course and distribution of the median nerve – a case report. *Neuroanatomy* 6: 40–50.

Song WC, Jung HS, Kim HT, Shin C, Lee BY, Koh KS (2003) A variation of the musculo-cutaneous nerve absent. *Yonsei Med J*, 44: 1110–1113.

Turner WM (1864) On some variations in the arrangements of the nerves of the human body. *Natural History Review* 4: 612–617.

Uysal II, Sekar M, Kasabulut AK, Buyukmumlu M, Ziylan T (2003) Brachial plexus variations in human fetuses. *Neurosurg,* 53: 676-684.

Uzun A, Seelig LL *Jr* (2001) A variation in the formation of the median nerve; communicating branch between the musculocutaneous and median nerves in man. *Folia Morphol (Warsz)* 80: 99–101.

Walsh JF (1877) The Anatomy of the brachial plexus, *Am J Med Sci*, 74: 387-397.

Williams PL, Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, Ferguson D (1995) Nervous system In: Gray's Anatomy. 38th Edition. Churchill Livingston, Edinburgh. pp 1266–1274.

Williams PL, Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, Ferguson D (1995) Embryology and development In: Gray's Anatomy. 38th Edition. Churchill Livingstone, Edinburgh. pp 231–232.

International Journal of Anatomical Sciences 2011, 2(1):11-17

Research Article

Long-Term Hyperglycemic effect on rat Epididymis and Sperm

Suresh S, Shanthi Santhosh Kumari S, Preethi U, Venkatalakshmi N, Karthik Ganesh M, Ganesh L, Prithiviraj E, Prakash S.

Department of Anatomy, Dr. Arcot Lakshmanasamy Mudaliar Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, INDIA.

Key Words: hyperglycemia, epididymis, sperm

Abstract: The epididymis, an important organ of male reproduction, imparts sperm maturation acquiring motility and fertilizing potential and removal of defective sperm and providing good microenvironment. In addition the epididymis plays a significant role in the transport, concentration, protection, and storage of spermatozoa. The objective of the present study was to analyse the patho-physiological changes in epididymis, sperm morphology, count, maturation and transport under long-term hyperglycemic condition using Wistar Albino rats as diabetic (hyperglycemic) model. Twenty male Wistar albino rats (*Rattus norvegicus*) were used for this study (body weight 225– 250 g) The rats were randomly selected for the following groups i.e. group I – control (received 0.1M Citrate buffer) and group II – Diabetes (single injection of streptozotocin 60 mg/kg b.w. in 0.1M citrate buffer). At the end of 120 days animals were sacrificed by over dose of anesthesia (i.p), and followed by transcardial perfusion using 4 % paraformaldehyde in 0.1 M phosphate buffered saline. Epididymides were dissected out and after gross measurements were taken, processed for paraffin technique. Sections were stained using hematoxylin (Harris's) and eosin and observed under light microscope and histomorphometry and stereological analyses were done. Spermatozoa were collected from caudal portion of epididymis to estimate sperm viability and motility, morphology and morphometry, cytoplasmic droplets and epididymal transit time. The present data revealed major damage in structure of epididymis and altered sperm quality and quantity under the influence of long-term diabetes. The alteration in epididymis might have influenced the sperm damage and poor sperm parameters leading to infertility. With the age limit to acquire diabetes is coming down rapidly and thus increasing the risk of infertility in younger generation. Consequently, more number of basic and clinical researches should be focused on the prevention and cure of diabetes.

Infertility in males is a topping list of male related sexual problems nowadays. It affects as many as one in six couples, the possible causes comes under the following Idiopathic, Varicocele, ageing, hormonal imbalance, Substance Abuse (alcohol, nicotine), Testicular factors, sedentary lifestyles, environmental factors, thyroid disease, hypertension, heart disease and diabetes mellitus (DM). DM may affect male reproductive function at multiple levels including spermatogenesis as a result of its endocrine impairment (Baccetti et al., 2002; Ballester et al., 2004). Animal studies using rodent models of streptozotocin-induced diabetes mellitus have demonstrated a

Correspondence to: Prakash S, Department of Anatomy, Dr. Arcot Lakshmanasamy Mudaliar Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, Tamil Nadu 600113, India.
E-mail: seppanprakash@yahoo.com

Accepted: 15-Mar-2011

reduction in sperm count and quality (Ballester et al., 2004; Amaral et al., 2006; Scarano et al., 2006). Human diabetic study showed reduction in all semen parameters (semen volume, sperm count, motility and morphology) (Garcia-Diez et al., 1991).

The epididymis, an important organ of male reproduction, imparts sperm maturation acquiring motility and fertilizing potential and removal of defective sperm and providing good microenvironment (Turner, 2007). In addition to sperm maturation, the epididymis plays a significant role in the transport, concentration, protection and storage of spermatozoa and all these process are androgen dependent (Meistrich et al., 1975).

The objective of the present study was to analyse the patho-physiological changes in epididymis and the sperm morphology, count, maturation and transport under long-term hyperglycemic condition using Wistar Albino rats as diabetic (hyperglycemic) model.

Materials and Methods

Animal Used

Twenty male Wistar albino rats (Rattus norvegicus) were used for this study. Male rats of body weight (b.w.) 225– 250 g were selected. They were housed individually in separate standard cages and maintained under standard laboratory conditions (temperature 24–28°C, relative humidity 60–70%, and 12-hour light–dark cycle) with free access to solid pellet diet and water *ad libitum* throughout the study. The study was approved by Institutional Ethical Committee. Quarantine procedures and animal maintenance was according to the recommendations of Canadian Council Guide to the Care and Use of Experimental Animals (1993) and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines for laboratory animal facility (2003). Experimentation on these animals carried out after subjecting to quarantine period of not less than 15 days each.

Experimental Design and Induction of Diabetes

In this present study, animals were grouped as, group I – control (received 0.1M Citrate buffer) and group II – Diabetes (single injection of streptozotocin (Sigma-Aldrich, USA) 60 mg/kg b.w. in 0.1M citrate buffer). At the 4th day after injection the animal blood glucose level were estimated. Animals which showed above 250 mg/dl were considered as diabetic (Suresh and Prakash, 2010).

Tissue Harvesting

At the end of 120 days after recording final body weights, animals were sacrificed by over dose of anesthesia (i.p), and followed by transcardial perfusion using 4 % paraformaldehyde in 0.1 M phosphate buffered saline. Epididymides were dissected out and gross measurements were taken. The tissues were fixed for histological studies in freshly prepared Bouin's fluid and processed for paraffin technique. Sections were stained using hematoxylin (Harris's) and eosin and observed under light microscope (Nikon. Japan)

Sperm Analysis

Sperm count:

Sperm count was done according to the procedure described previously (Suresh et al., 2010). Briefly spermatozoa were collect from caudal portion of epididymis, by mincing caudal epididymis with anatomical scissors in 5 ml of pre warmed (35º C) physiological saline, placed in a rocker for 10 min. and incubated at room temperature for 2 min. Supernatant fluid was diluted 1:100 with solution containing 5 g Sodium bicarbonate, 1 ml formalin (35 %) and 25 mg Eosin/ 100 ml H_2O. Total sperm count was determined using haemocytometer. Approximately 10µl of diluted sperm suspension was transferred to each counting chamber and was allowed to

stand for 5 min and then counted under a light microscope at 400 X magnification.

Sperm Viability and Motility:

About 20µl of sperm suspension was mixed with an equal volume of 0.05% eosin-Y and nigrosin. After 2 min incubation at room temperature, slides were viewed by bright-field microscope with 400 X magnification (Nikon, Japan). Dead sperms appeared pink and live sperms were not stained (Suresh et al., 2010). Percentage of motile sperm was assessed using graded semi-quantitative scale of 0 to 5 and the spermatozoa were evaluated for the rate of forward movement and graded accordingly, i.e., 0 = No movement, 1= Sluggish or tail movement alone, 2 = Intermittent sluggish movement, 3 – 4 = Fair & Good movement and 5 = Maximum movement in forward direction.

Morphology and Morphometry:

The fixed sperm were smeared on a glass slide and stained with phosphate buffered saline solution of Giemsa (Merck, Germany) (Hafez, 1977). Morphological alterations in sperm and morphometrical data acquired by measuring length of the head and flagellum were evaluated. A total of one hundred spermatozoa were analyzed per animal using ocular micrometer scale fitted to a light microscope under 40X magnification.

Cytoplasmic Droplet Containing Sperm

The presence of cytoplasmic droplet containing sperm was determined using the method of Syntin & Robaire (2001). Spermatozoa were assessed for the presence or absence of cytoplasmic droplet, for which minimum of 100-spermatozoa/ animal were evaluated.

Estimation of Epididymal Transit Time

The epididymal transit time were calculated according to the methods of Scarano et al., (2006). Briefly the epididymal sperm count were calculated in individual region (Caput, corpus and caudal). Then transit time of caput/corpus and caudal epididymis were calculated by dividing the number of sperm within each of these three regions by the daily sperm production.

Histomorphometry and stereological analyses

The conventional stereological principles and accepted morphometric procedures as outlined by Elias and Hyde, (1980) were used to obtain quantitative information, details of the procedure have been described previously (Prakash et al., 2008). Systematic unbiased random sampling (SURS) protocol was adopted. All the data's are expressed in relative value.

Statistical analysis

The significant difference between the mean value of control and experimental groups was determined by student't' test. P value <0.05 was considered as statistically significant (Zar, 1974). All the analysis was done by using Microsoft Excel 2003 and SPSS statistical package version 7.

Observations

Sperm analysis

Significant reduction in percentage of viable and increase of the dead sperm were observed in the long term hyperglycemic rats when compared with normoglycemic rats. There was significant decline in motility in hyperglycemic when compared with normoglycemic rats.

The sperm concentrations and motility were markedly decreased in the long term hyperglycemic rats. No significant changes were observed in the morphometry study in all the experimental groups. Morphological analysis shows wide degree of abnormality in long term diabetic rat sperm such as defects in the head (microcephalic, bicephalous, amorphous, and acephalic), neck and tail when compared to the group I.

Cytoplasmic droplet containing sperm

Cytoplasmic droplet study showed significant increase of the sperm containing cytoplasmic droplets in long term hyperglycemic rats (45 ± 1.12) when compared to normoglycemic rats (3 ± 0.39). These cytoplasmic droplets were located at various levels in the sperm; tail remnant was more in number. Observations are summarized in table 2.

Epididymal transit time

Significant reduction in the epididymal transit time was observed in the long term diabetic rat when compared with normal rats. Data's were shown in table 1.

Table 1 Sperm analysis data and epididymal transit time of control and long term diabetic animals. Each value indicates the mean ± SD of (n – 6) animals. a – control, *** - p<0.001.

PARAMETER	CONTROL	DIABETES
Sperm viability (%)		
Live	98 ± 1.23	60 ± 2.12 a***
Dead	2 ± 0.89	40 ± 1.65 a***
Motility (%)	98 ± 2.15	50 ± 1.86 a***
Count (10^6)	371 ± 12.02	128 ± 3.25 a***
Morphology (%)		
Normal	96 ± 2.12	67 ± 3.20 a***
Abnormal	4 ± 0.35	33 ± 1.32 a***
Epididymal sperm count ($X10^6$/organ)		
Caput/ Corpus	123.67 ± 4.68	45.23 ± 2.17 a***
Caudal	247.33 ± 2.63	82.77 ± 1.86 a***
Epididymal sperm transit time (days)		
Caput/ Corpus	6.110 ± 0.25	2.47 ± 0.86 a***
Caudal	12.22 ± 1.01	4.52 ± 1.06 a***

Epididymal Morphological Changes

There were morphological alteration such as decrease in the length, breadth, height, weight and volume in all the three regions of epididymis i.e. caput, corpus and cauda of long term hyperglycemic rats when compared to the normoglycemic rats. Data's were shown in table 2)

Table 2 Morphology of epididymis in control and long term diabetic animals. Each value indicates the mean ± SD of (n – 6) animals. a – control, * - p<0.05, ** - p<0.01 and *** - p<0.001

PARAMETER	Control	Diabetes
Length (cm)	5.33 ± 0.24	4.1 ± 0.15 a **
Breadth (cm)		
Caput	0.63 ± 0.12	0.25 ± 0.11 a**
Corpus	0.3 ± 0.10	0.13 ± 0.09 a*
Caudal	0.85 ± 0.08	0.39 ± 0.11 a**
Width (cm)		
Caput	0.53 ± 0.12	0.15 ± 0.09 a***
Corpus	0.15 ± 0.03	0.1 ± 1.59 a*
Caudal	0.54 ± 0.06	0.24 ± 0.08 a**
Volume (ml)	0.48 ± 0.11	0.15 ± 0.09 a**
Weight (gm)	0.643 ± 0.06	0.31 ± 0.03 a***

Histological observation

Epididymal histology shows the thickening of basement membrane, vacuolation of the epithelial cells and absence of mature spermatozoa and presence of immature germ cell in the lumen of tubules in the long term diabetic animals when compared to the control (Fig. 1).

Histomorphometric Changes

There was significant change in volume of connective tissue, tubule and epithelia. The number of tubule was found to be increased and the diameter found to be decreased in diabetic group (Figure 2 to 7).

Discussion

The present data's revealed that major changes in structure and functions of epididymis plays a significant role in altering sperm quality and quantity under the influence of long-term diabetes. This includes reduction in gross weight and volume of the epididymis concomitant with decrease in the volume of spermatozoa in the tubular lumen may be partly accounted for the reduction in epididymal weight and volume in the diabetic rats. This may be due to the impaired spermatogenesis in diabetic condition (result not shown).

IJAS 2011, 2(1):11-17

Fig. 1 Morphology and histology of the epididymis in control and long term diabetic rats. In diabetic animal (arrow) showing vacuole formation in the epithelium Arrow head indicates the immature germ cells in the lumen and the star indicates the occluded lumen.

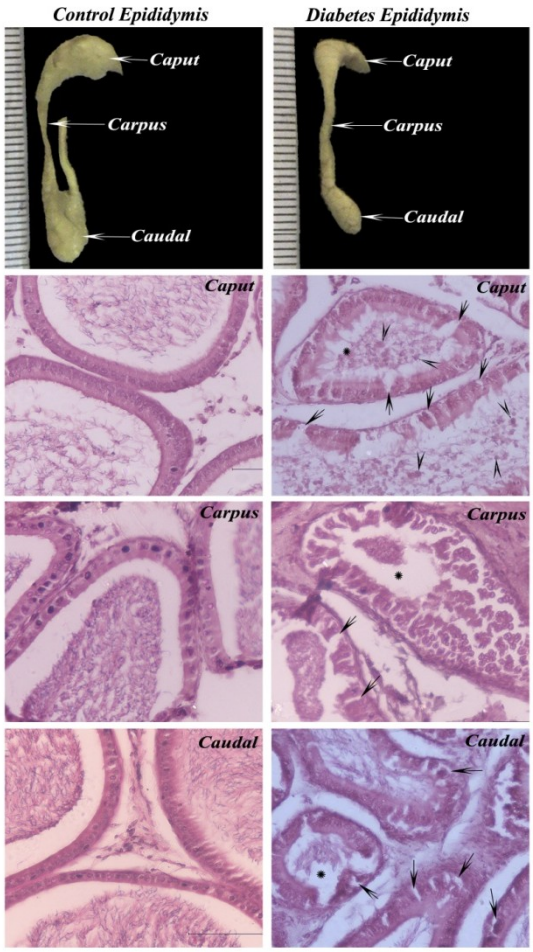


Fig. 2 Diameter of the epididymal tubules of control and long term diabetes animals. Each bar indicates mean ± SEM of (n-6) animals. a - control, *** - p< 0.001.

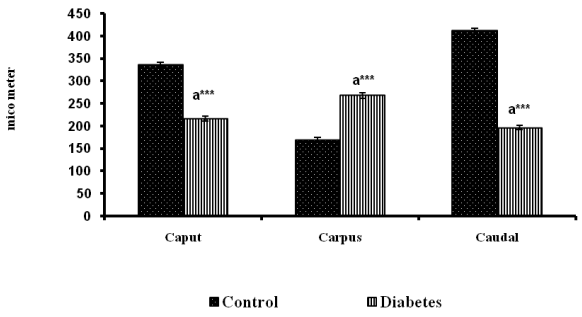


Fig. 3 Height of the epididymal epithelium of control and long term diabetes animals. Each bar indicates mean ± SEM of (n-6) animals. a - control, * - p< 0.05, ** - p<0.01 and NS - Not significant.

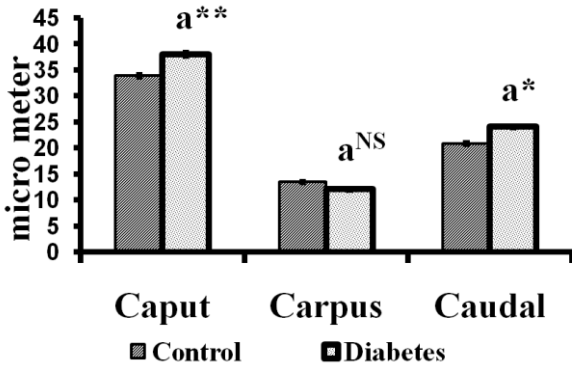


Fig. 4 Volume of connective tissues of control and long term diabetes animals. Each bar indicates mean ± SEM of (n-6) animals. a - control, *** - p< 0.001, ** - p<0.01.

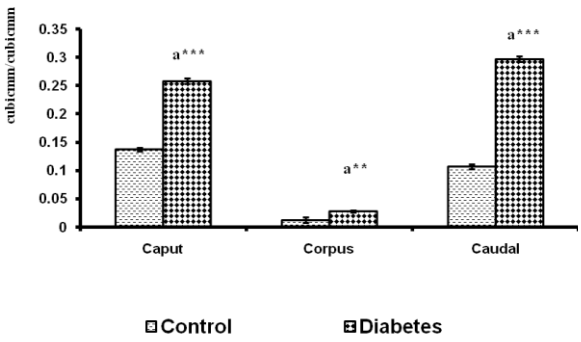


Fig. 5 Volume of epididymal epithelium of control and long term diabetes animals. Each bar indicates mean ± SEM of (n-6) animals. a - control, *** - p< 0.001.

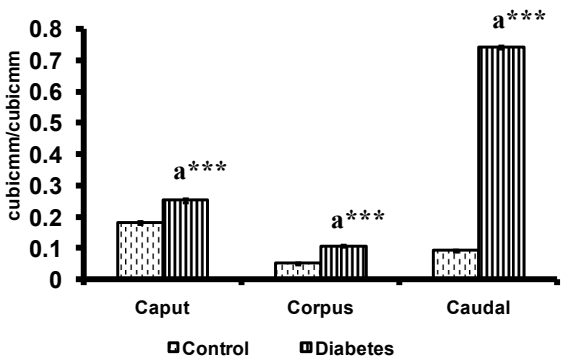


Fig. 6: Volume of epididymal tubules of control and long term diabetes animals. Each bar indicates mean ± SEM of (n-6) animals. a - control, ** - p<0.01 and *** - p< 0.001.

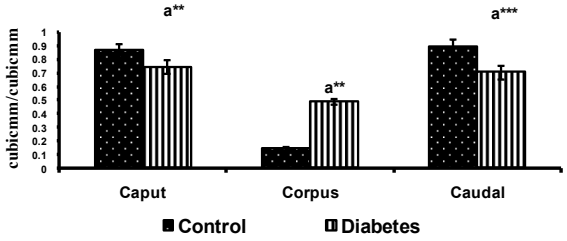
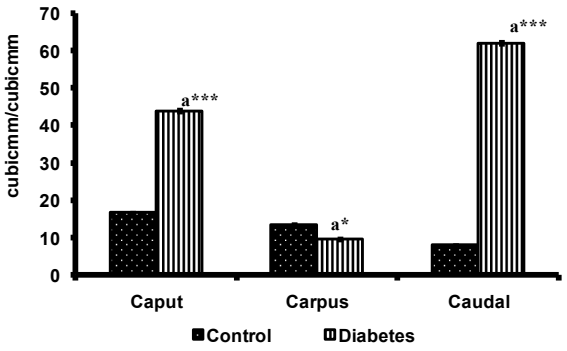


Fig 7 Number of the epididymal tubules of control and long term diabetes animals. Each bar indicates mean ± SEM of (n-6) animals. a - control, * - p<0.05 and *** - p< 0.001.



In general epididymis depends on androgens to maintain their structure and function (Amman et al., 1993). The morphological alteration seen in diabetic epididymis may be predominantly due to the androgen deficiency. The serum testosterone and luteinizing hormone were reduced in diabetic condition as observed in our previous study (Suresh and Prakash, 2010). Usually in normal rats deprived of testosterone, there will be increase in the rate of epididymal sperm transit time (Meistrich et al., 1975). However, in this study the diabetic rats deprived of testosterone showed significant decrease in epididymal transit time for sperm. There was increased amount of immature and abnormal sperm in long term diabetic rats when compared with control rats. Present observations clearly shows that epididymal dysfunction and disturbed homeostasis. The pathology could have been enhanced by the impaired spermatogenesis and immature spermiation which produce immature or sperm with cytoplasmic droplets (Suersh and Prakash, 2010).

The changes in epididymis had severely affected sperm parameters when compared to control. The sperm count and motility were decreased with increased number of abnormal sperm in the hyperglycemic epididymis. The sperm with cytoplasmic droplet was also more in hyperglycemic rat indicating patho-physiological state of the testis and epididymis. The presence of sperm with cytoplasmic droplet can increase free radical production and oxidative stress in the epididymis, a condition seen in our previous study on aged rat epididymis (Suresh et al., 2010).

The histological observation of diabetic epididymis showed vacuolation, epithelial fibrosis and lumen filled with immature and degenerative sperm cells, demonstrating the deleterious effect of long-term hyperglycemia. Histomorphometeric observations showed more number of tubules per unit area. This might be due to the shrinkage of tubular diameter. Similarly this shrinkage or concentration of more tubules might have caused increase in epithelial height and volume. There was increased amount of connective tissue proportion.

These structural changes suggested that the epididymal physiology could have severely altered and consequently, resulted in sperm damage which may lead to infertility. With the age limit to acquire this disease is coming down rapidly and thus increasing the risk of infertility in younger generation. Given the finite nature of the human existence, an individual man's most precious and lasting gift to the world is the legacy created by his offspring. The most

unfortunate thing is that India will be the diabetes capital of the world in 2030 according to a World Health Organization (WHO) report. Hence, more basic and clinical research is required for the prevention and cure of diabetes in India.

References

Ali ST, Shaikh RN, Siddiqi NA (1993) Semen analysis in insulin-dependent/non-insulin-dependent diabetic men with/without neuropathy. *Arch Androl*, 30: 47–54.

Amaral S, Moreno AJ, Santos MS, Seica R, Ramalho-Santas J (2006) Effects of hyperglycemia on sperm and testicular cells of Goto-Kakizaki and streptozotocin-treated rat models for diabetes. *Theriogenology*, 66: 2056–2067.

Amman RP, Hammerstedt RH, Veeramachaneni DNR (1993) The epididymis and sperm maturation: a perspective. *Reprod Fert Dev*, 5: 361–381.

Baccetti B, Marca Al, Piomboni P, Capitani S, Bruni E, Petraglia F, De Leo V (2002) Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. *Hum Reprod,* 17: 2673–2677.

Ballester J, Muñoz MC, Domínguez J, Rigau T, Guinovart JJ, Rodríguez-Gil JE (2004) Insulin-dependent diabetes affects testicular function by FSH- and LH-linked mechanisms. *J Androl*, 25: 706–719.

Elias H, Hyde H (1980) An elementary introduction to stereology. *Am J Anat*, 159: 411–446.

Cameron DF, Rountree J, Schultz RE, Repetta DF, Murray T (1990) Sustained hyperglycemia results in testicular dysfunction and reduced fertility potential in BBWOR diabetic rats. *Am J Physiol*, 259: E881–E889.

Committee for the Purpose of Control and Supervision on Experiments on Animals CPCSEA guidelines for laboratory animal facility (2003) Committee for the Purpose of Control and Supervision on Experiments on Animals. *Ind J Pharmacol,* 35: 257–274.

Olfert ED, Cross BM, McWilliam AA (1993) Guide to the care and use of experimental animals. Volume 1. 2nd ed. Canadian Council for Animal Care.

Frenkel GP, Homonnai ZT, Drasnin N, Sofer A, Kaplan R, Kraicer PF (1978) Fertility of the streptozotocin-diabetic male rat. *Andrologia*, 10: 127–136.

García-Díez LC, Corrales Hernandez JJ, Hernandez-Diaz J, Pedraz MJ, Miralles JM (1991) Semen characteristics and diabetes mellitus: significance of insulin in male infertility. *Arch Androl*, 26: 119–128.

Hafez ESS (1977) Techniques of human andrology. Published by Elsevier/North- Holland Biomedical press. 471. Amsterdam, Netherland.

Handelsman DJ, Conway AJ, Boylan LM, Yue DK, Turtle JR (1985) Testicular function and glycemic control in diabetic men. A controlled study. *Andrologia*, 17: 488–496.

Meistrich ME, Hughes TH, Bruce WR (1975) Alteration of epididymal sperm transport and maturation in mice by oestrogen and testosterone. *Nature*, 258: 145-147.

Mori H, Christensen AK (1980) Morphometric analysis of Leydig cells in the normal rat testis. *J Cell Biology*, 84: 340-354.

Phil-Ok Koh (2007) Streptozotocin-Induced Diabetes Increases Apoptotic Cell Death in Rat Epididymis. *Lab Anim Res*, 23: 381-384.

Prakash S, Prithiviraj E, Suresh S (2008) Development of seminiferous tubules in Prenatal, Postnatal and adult Testis of Bonnet Monkey (*Macaca radiata*). *Anat Hist Emb*, 37: 19 - 23.

Syntin P , Robaire B (2001) Sperm structural and motility changes during aging in the brown Norway rat. *J Androl*, 22 : 235–244.

Scarano WR, Messias AG, Oliva SU, Klinefelter GR, Kempinas WG (2006) Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats. *Int J Androl*, 29: 482–488.

Sexton WJ, Jarow JP (1997) Effect of diabetes mellitus upon male reproductive function. *Urology*, 49: 508–513.

Suresh S, Prithiviraj E, Prakash S (2010) Effect of *Mucuna pruriens* on oxidative stress mediated damage in aged rat sperm. *Int J Androl*, 33: 22–32.

Turner TT (2008) De Graaf's Thread: The Human Epididymis. *J Androl*, 29: 237-250.

Vignon F, Le Faou A, Montagnon D, Pradignac A, Cranz C, Winiszewsky P, Pinget M (1991) Comparative study of semen in diabetic and healthy men. *Diabete Metab*, 17: 350–354.

Zar JH (1974) Biostatistical analysis. Engle wood cliffs NJ; Prentice Hall Inc.

International Journal of Anatomical Sciences (IJAS)
Instructions to Contributors

1. IJAS welcomes original research findings / observations on all aspects of bio-medical field which has a relevance to structure and structural changes. Articles without any reference to structure are not in the scope of the journal
2. IJAS accepts articles from authors with an assumption that they were not previously published in any form and if accepted for publication in IJAS shall not be published in any other form.
3. Papers must be submitted only through its online portal www.ijas.in and submission in any other form will not be accepted.
4. A paper can be of maximum 20 pages in A4 paper size including illustrations, legends, acknowledgements, and references.
5. All the papers submitted to IJAS will be subjected to review by at least two referees before editorial takes a decision on the acceptance of the paper for publication. Review process of IJAS is an anonymous double-blind process.
6. IJAS is an open-access journal. Therefore, anyone can download published articles free of cost from its online portal. Authors of accepted articles have to pay Rs.4000/- (US $100 in the case of foreign authors) or the charges as fixed by the Association of Anatomists, Tamil Nadu from time to time. These charges are towards publication cost and online uploading charges.
7. No paper reprints will be supplied to authors of accepted articles since the journal itself is an open access type. However, if authors wish to purchase hard copy of the journal, they should contact, editorial office and pay the cost as fixed by the Association of Anatomists, Tamil Nadu from time to time. Currently the rate of one single hard copy of the journal is Rs.1000/-
8. Authors must prepare their papers as per the detailed instructions available in the website. Failure to follow the instructions in any manner will result in the rejection of the submitted paper.
9. Please visit www.ijas.in for details regarding preparing papers for submission to IJAS. In case of any difficulty in accessing the online portal, contact the journal office at Department of Anatomy, Dr.A.L.M. PGIBMS, University of Madras, Taramani Campus, Chennai 600 113, India. Phone: 91-044-24547020 Email: editor.IJAS@gmail.com

International Journal of Anatomical Sciences
(Journal of Association of Anatomists, Tamil Nadu, India)
Volume 2 No. 1 March 2011

Contents