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Original Article

STATUS OF ANTIOXIDANTS MOLECULES AND LIPID PEROXIDATION IN THE DIABETIC TESTES

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ABSTRACT

Introduction: According to the epidemiological studies, diabetes mellitus has become a potential cause of male infertility. Knowledge regarding how diabetes mellitus interferes with the process of spermatogenesis and results in infertility needs the molecular study in the testis in diabetic condition. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus. So, this study was established to investigate the activity of enzymatic antioxidants and oxidative stress in the testis of diabetic model rats.

Material & Methods: Diabetes mellitus was induced in the rat by intraperitoneal injection of Streptozotocin. The rats were sacrificed and the dissection was done to take out the testis. The testes were processed for the activity of enzymatic antioxidants.

Results: It was found that oxidative stress was increased in the testes of diabetic rats. The sperms were also affected by the chronic hyperglycemia.

Keywords: Antioxidants, oxidative stress, diabetes mellitus, testes.

INTRODUCTION

Diabetes mellitus (DM) is a major concern of the global health due to its high prevalence and its serious complications. Besides being a metabolic and endocrine disorder, diabetes mellitus has also been associated with reproductive impairment in men [1] and its impact on reproduction can be profound, as seen by diminution in fertility and increase in reproduction losses [2-5]. A very common pathology experienced by diabetic men is the consistent inability to achieve and maintain penile erection (erectile dysfunction) sufficient for adequate sexual relation [6,7]. Erectile dysfunction (ED) and retrograde ejaculation is well recognized and examined in diabetic men [8-10].

It has been proved that DM alters structural and functional changes in the testes [11-13] but how the DM alters the normal organization of testis is not clearly established. There could be some molecular changes in the testes of diabetic men which is responsible for the histopathological changes in the testis and results in the decline of fertility. Further, time factor also play an important role in the pathology of any disease. The complete evaluation of whole parts of gonads in diabetic men is a challenging factor. Therefore, this study was designed to evaluate the activity of antioxidants molecules and status of oxidative stress in the testis of diabetic rats.

The body constantly interacts with oxygen during various physiological processes in the cell. Most of the body's energy is produced by the enzymatically controlled reaction of oxygen with hydrogen during oxidative phosphorylation occurring within the mitochondria. Due to this enzymatic reduction of oxygen a highly reactive molecules are produced as free radicals which are known as oxidants. A free

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radical is an oxygen containing molecule that has one or more unpaired electrons [14] making it highly reactive with other molecules. Since they are highly active and derived from oxygen, they are also called reactive oxygen species (ROS). ROS includes superoxide anion (O_2-), hydroxyl (OH), hydrogen peroxide (H_2O_2), organic hydroperoxide (ROOH), hypochlorus acid (HOCL) etc. [15]. Aerobic environment is a constant source of ROS through in vivo mechanisms such as electron leakage during biologic oxidations and by physical activation of oxygen by external agents such as radiation e.g. UV sunlight.

Reactive oxygen substance can chemically interact with other cell components such as DNA, protein and lipid. Depending on their cellular concentration they can either exert beneficial physiologic effects (e.g. killing invading pathogens or microbes) or pathological damage to cellular components including gene, amino acids and lipids [16].

To counteract the destructive action of oxidants, the body produces other molecules known as antioxidant. These molecules present in the cells, prevent the oxidative damage done by oxidants. Antioxidants can be enzymatic such as catalase, superoxide dismutase, and glutathione peroxidase/ reductase or non- enzymatic antioxidants such as vitamin C, vitamin A, vitamin E, pyruvate and glutathione [17]. Superoxide Dismutase (SOD) is an enzymatic antioxidant that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. SOD catalyzes the reduction of superoxide anions (0_2-) to hydrogen peroxide and oxygen [18-20]. This is the first line of defense to protect cells from the injurious effects. Catalase is another important antioxidant molecule which is used by cells to decompose hydrogen peroxide a very reactive molecule, into oxygen and water molecules [21].

Oxidative stress is a pathological state that arises when free radicals chemically damage biological molecules and the decrease in the ability of the body to counteract their harmful effects through neutralization by antioxidants. It is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases [22-24] such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, myocardial infarction, cardiovascular diseases, chronic inflammation, aging and other degenerative diseases in human [25,26]. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus [27,28]. Lipid peroxidation is a well-defined mechanism of cellular damage in animals. Lipid peroxides are unstable indicators of oxidative stress in cells that decompose to form more complex and reactive compounds such as malondialdehyde (MDA). Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage [29-31].

Analysis of the sperm reflects the state of ongoing spermatogenesis in the testis and the sperm analysis is the initial standard test to evaluate the status of male fertility [32]. Semen analysis provides useful information concerning sperm count, motility and morphology as well as ejaculation and emission. Although the semen analysis reveals useful information for the initial evaluation of the infertile male, it is not a test of fertility [33]. However; it reflects some basic preliminary guidelines regarding fertility.

Most of the research in diabetes mellitus has been focused on the metabolic disorder rather than its effect on the reproductive system. The onset of diabetes mellitus (usually type 2 which accounts for 90% prevalence) is seen in later stages of life and by this time fertility of the individual is reduced naturally. However, in recent years, diabetes mellitus is occurring rampantly in the young age group due to stressful lifestyle [34] and also DM has become a potential cause of male infertility. With this view, this study has been designed to investigate the activity of enzymatic antioxidants and lipid per oxidation in the testis of diabetic model rats. Besides oxidative stress, this study also analyzed sperm of diabetic rats.

MATERIAL AND METHODS

Adult male Wistar rats weighing 200-250 grams were included in this study. Only the euglycemic rats were taken for the study. They were maintained in captivity in cages under natural light conditions, laboratory chow and water ad libitum were available. On the basis of duration of study; one week, one month and six months, the rats were divided into three groups. Each group contains control and diabetic rats. Diabetes mellitus was induced in the rat by intraperitoneal injection of Streptozotocin (STZ) at a dose of 50 mg/kg dissolved in freshly prepared citrate buffer (pH 4.5). The control group were given the same volume of citrate buffer. After 72 hrs of STZ injection, the blood sample of STZ group rats were

taken from the tail vein to measure the fasting blood glucose level by automated glucose analyzer. The fasting blood glucose level above 200 mg/dl was considered as diabetic rat. The fasting glucose level of control group was also measured to confirm the non diabetic. The fasting glucose level was monitored periodically to confirm the diabetic and non diabetic rats. The rats were sacrificed after one week, one month and six months according to the duration of study. The dissection was done to take out the testis and epididymis.

The testes were quickly excised, washed in sodium phosphate buffer (pH 7.2) and stored at -80˚C. The testes were decapsulated and the tissue was homogenised by the tissue homogenizer at the concentration of 50 mg/ml in 0.1M of ice cold phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 r/min at 4˚C for 5 minutes separately. Each supernatant was measured for the estimation of catalase, superoxide dismutase and MDA. Estimation of superoxide dismutase (SOD) was done by Marklund and Marklund method [35]. Estimation of catalase activity was done by Sinha method [36]. Estimation of malondialdehyde (MDA) was done by Sathoh's method [37]. The sperms taken out from the epididymis were examined for count, motility and morphology following standard methods [38].

OBSERVATIONS AND RESULTS

The SOD activity in the diabetic rats after one week increases significantly. Thereafter, SOD activity decreases markedly in the diabetic rats when compared to the non-diabetic rats (Table 1, Fig. 1).

Table 1: Descriptive statistics of superoxide dismutase activity

Study Group	Duration of study	Mean ± SD	SEM	t-stat	df	P value
Control	One week	2.98 ±0.11	0.04	-4.11	10	0.001*
Diabetic	One week	3.19± 0.05	0.02			
Control	One month	2.77 ±0.11	0.04	8.26	10	<0.001**
Diabetic	One month	1.56 ±0.34	0.13			
Control	Six months	2.47 ±0.17	0.07	5.91	10	<0.001**
Diabetic	Six months	1.34 ±0.43	0.17			

*The mean value is expressed in Units/mg; * Significant, ** highly significant.*

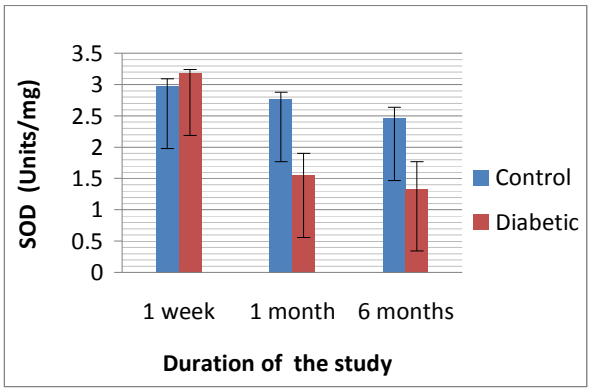

Fig. 1: Comparison of superoxide dismutase activity between diabetic and non-diabetic rats

The mean value of catalase activity in diabetic rats was less than the non-diabetic rats except in the first group where the catalase activity was increased but not statistically significant (Table 2, Fig. 2)

Table 2: Descriptive statistics of catalase activity

Study Group	Duration of study	Mean ± SD	SEM	t-stat	df	P value
Control	One week	2.9 ± 0.21	0.08	0.86	10	0.2
Diabetic	One week	3.01± 0.21	0.08			
Control	One month	3.32±0.17	0.07	18.73	10	<0.001**
Diabetic	One month	1.72±1.31	0.53			
Control	Six months	3.2±0.17	0.07	25.66	10	<0.001**
Diabetic	Six months	0.91±0.12	0.05			

*The mean value is expressed in Units/mg of protein; * Significant, ** highly significant.*

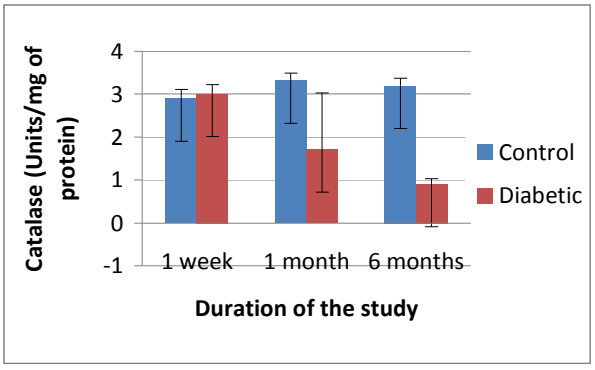

Fig. 2: Comparison of catalase activity between diabetic and non-diabetic rats

The MDA level was increased in the testis of diabetic rats when compared with the non-diabetic

rats. At the end of the one week, there was no significant change in MDA level between the diabetic and control rats. However, statistically significant change was there after one month and six months of diabetes (Table 3, Fig. 3).

Table 3: Descriptive statistics of MDA

Study Group	Duration of study	Mean ± SD	SEM	t-stat	df	P value
Control	One week	1.48±0.13	0.05	-0.9	10	0.19
Diabetic	One week	1.56± 0.15	0.06			
Control	One month	1.52±0.13	0.05	-5.82	10	0.001*
Diabetic	One month	2.26±0.28	0.11			
Control	Six months	1.76±0.14	0.06	-15	10	<0.001**
Diabetic	Six months	3.6±0.26	0.1			

*The mean value is expressed in nmol/ml; * Significant, ** highly significant.*

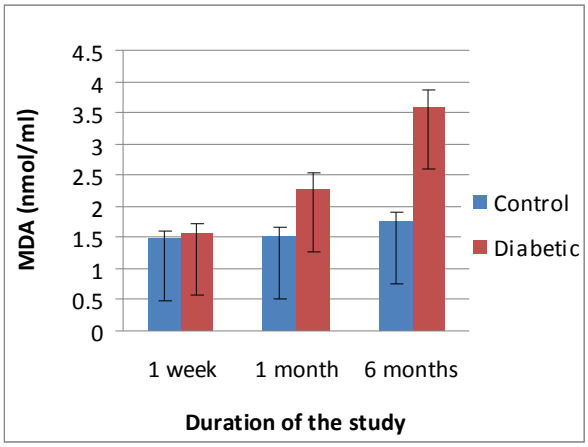

Fig. 3: Comparison of MDA level between diabetic and non-diabetic rats

The number of sperms in diabetic rats decreased significantly in comparison to control rats except in the group of first week study. A significant decrease in the count was seen after a long term of diabetic conditions (Table 4, Fig. 4). The motility of the sperms in the diabetic group decreased as compared to non-diabetic groups (Table 5, Fig. 5). The abnormal morphology of the sperm of diabetic rats increased significantly than the non-diabetic rats in one month and six months of study (Table 6, Fig. 6).

Table 4: Descriptive statistics of sperm count

Study Group	Duration of study	Mean ± SD	SEM	t-stat	df	P value
Control	One week	152.73 ± 14.1	5.75	1.71	10	0.05
Diabetic	One week	141.72± 6.92	2.82			
Control	One month	164±18.95	7.74	4.15	10	<0.001**
Diabetic	One month	77.18±47.52	19.4			
Control	Six months	138.82±28.66	11.7	6.54	10	<0.001**
Diabetic	Six months	35.1±26.22	10.7			

*The mean value is expressed in million/ml; ** denotes highly significant.*

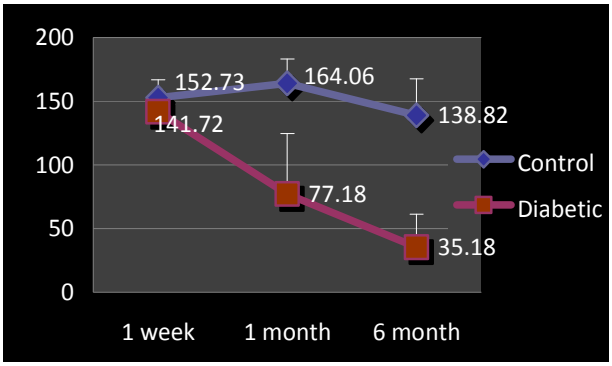

Fig. 4: Mean value of the sperm count in the diabetic and non-diabetic rats

Table 5: Descriptive statistics of motility of the sperms

Study Group	Duration of study	Mean ± SD	SEM	t-stat	df	P value
Control	One week	75.08 ± 7.5	3.06	-0.43	10	0.33
Diabetic	One week	77.16± 6.99	2.85			
Control	One month	75.33±9.82	4.01	2.74	10	<0.010
Diabetic	One month	57.16±12.86	5.25			
Control	Six months	73.58±8.36	3.41	6.54	10	<0.001**
Diabetic	Six months	38±9.13	3.73			

*The mean value is expressed in percentage (%); * Significant, ** highly significant.*

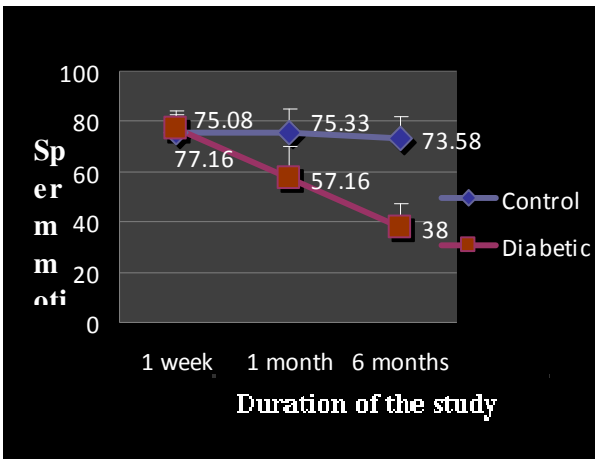

Fig. 5: Total motility of the sperms of diabetic and non-diabetic groups

Table 6: Descriptive statistics of abnormal morphology of the sperms of the rats

Study Group	Duration of study	Mean ± SD	SEM	t-stat	df	P value
Control	One week	9.91 ± 2.78	1.13	0.22	10	0.41
Diabetic	One week	9.5± 3.68	1.5			
Control	One month	9.5±3.31	1.35	-2.16	10	0.02
Diabetic	One month	15.16±5.5	2.24			
Control	Six months	11.83±2.65	1.08	-3.95	10	0.001
Diabetic	Six months	21.83±5.59	2.28			

*The mean value is expressed in percentage (%); * Significant, ** highly significant.*

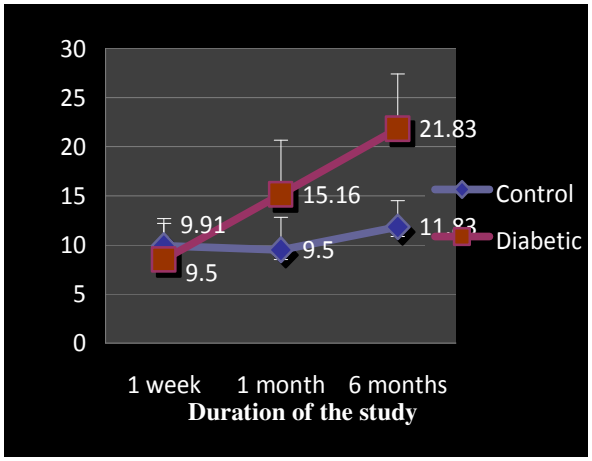

Fig. 6: Abnormal morphology of the sperms of diabetic and non-diabetic groups

DISCUSSION

Millions of sperms are produced per ejaculation, from the testis of male reproductive system. This suggests that there is a high cell division in the germinal epithelium of the seminiferous tubules of the testis. The high rate of cell division of the germ cells and the ability of the sperms to move forward implies high consumption of energy and oxygen by the mitochondria of germ cells. However, the vascularisation of the testes is very poor so that oxygen tension in the tissue of testes is low [39] and the competition for this oxygen within the testes is extremely intense. Both spermatogenesis [40] and Leydig cell steroidogenesis [41,42] are vulnerable to oxidative stress. However, the presence of low oxygen tension may be an important mechanism to protect the tissue from reactive oxygen species. In addition, the testes also contain a number of antioxidant enzymes by which the testis protects itself from free radical–mediated damage. These antioxidant defence mechanism are of major importance because peroxidative damage is currently regarded as the single most important cause of impaired testicular function in wide range of pathological conditions [43-50].

Reactive oxygen species (ROS) or free oxygen radical are normally generated by Sertoli cells that cause alteration in cellular structures and induces morphological changes in spermatids during spermiogenesis [51] and controlled amount of ROS is essential for capacitation and acrosome reaction [52]. But increased free radical induce lipid peroxidation and DNA fragmentation of the spermatozoon, disrupting both the motility of these cells and their ability to fertilize the ovum [53-59]. At the level of the testes, oxidative stress is capable of disrupting the steroidogenic capacity of Leydig cells [60] as well as the capacity of the germinal epithelium to differentiate normal spermatozoa [61].

Superoxide Dismutase

In this study, after one week of induction of diabetes SOD activity was increased significantly in diabetic group when compared to control group. Since SOD is the first line of defence against the free radical, its production is accelerated during the early phase of diabetes i.e. after one week. Thereafter, SOD activities are markedly decreased after one month and six months group. This decline in SOD activity was due to tremendous increase in free radical where SOD may not be able to counteract destructive action of radical. There are reports of decreased antioxidant enzyme

levels in streptozotocin induced diabetes mellitus [62-65].

Various observations indicate that spermatozoa may be more exposed and vulnerable to oxidative stress than germ cells. Spermatozoa found in the epididymis and vas deferens is not protected like germ cells in the seminiferous tubules by the microenvironment provided by the Sertoli cell barrier. Furthermore, the membranes of spermatozoa may be particularly susceptible to free radical attack because of their high level of polyunsaturated fatty acids [66]. Several studies have demonstrated that, in contrast to spermatogonia, primary spermatocyte and round spermatid, elongated spermatids and spermatozoa have a reduced capability or are even unable to repair DNA damage [67,68].

Catalase

We found the decrease in the activity of catalase in long term diabetic (six months) than one month diabetic rats. This shows negative correlation of catalase activity with the severity of diabetic. The increase activity of catalase in one week of diabetic rats is not significant ($p>0.05$). Other studies done by Singh et al., 2013 [69] and Adewole et al., 2007 [64] are also in accordance with our results in which catalase level were reduced in the testes of diabetic rats than the non-diabetic rats. When catalase activity is decreased, as in the present study, H_2O_2 is reduced to a very highly oxidizing OH radical in the presence of Fe^{2+} or other transition metals. The OH radical cannot be enzymatically removed from the cells but a free radical scavenger can detoxify it. The activity of catalase was lowered in diabetic rats, probably due to glycation of the enzyme due to hyperglycemia. Kaleem et al*.,* 2006 [70] proposed that decrease in activities of SOD and CAT in diabetic state may be due to over-production of reactive oxygen species in diabetic animals.

Catalase works closely with superoxide dismutase to prevent free radical damage to the body. SOD converts the dangerous superoxide radical to hydrogen peroxide, which catalase converts to harmless water and oxygen [71]. Hydrogen peroxide is a naturally occurring but destructive waste product of all oxygen-dependent organisms. It is produced in the human body when fatty acids are converted to energy, and when white blood cells attack and kill bacteria. Catalase, which is located in the cell's peroxisome, prevents this naturally occurring hydrogen peroxide from harming the cell during these processes. It also helps prevent the conversion of hydrogen peroxide to hydroxyl radicals, potentially dangerous molecules that can attack and even mutate DNA. Catalase also uses hydrogen peroxide to break down potentially harmful toxins in the body, including alcohol, phenol and formaldehyde. Catalases are some of the most efficient enzymes found in cells; each catalase molecule can convert millions of hydrogen peroxide molecules into water and oxygen every second.

Malondialdehyde

The result of the present study showed an increase in the level of MDA in all diabetic groups which are consistent with results of Shaikh et al., 2014; Rabbani et al., 2009; Adewole et al., 2007; Ozkaya et al., 2002 [62-65]. Increase in MDA resulted from hypoinsulinemia that increases the activity of fatty acyl coenzymes A oxidase, which initiates β-oxidation of fatty acids, resulting in lipid peroxidation. Also, protein glycation and glucose auto-oxidation can lead to the formation of free radicals, and this can equally induce lipid peroxidation. In this study, there is non-significant increase in the level of MDA after one week. This may be due to significant increase in the level of SOD (after one week) which prevent the lipid peroxidation by neutralizing the toxic effect of superoxide radical.

The spermatozoa are particularly susceptible to OS-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFAs) and their cytoplasm contains low concentrations of scavenging enzymes. OS–mediated damage to the sperm plasma membrane may account for defective sperm function observed in a high proportion of infertility patients [72]. Indeed, it has been shown that excess amounts of ROS and free radicals in spermatozoa and seminal plasma have adverse effects on sperm motility and fertility. It has also been reported that levels of plasma antioxidants in infertile men are significantly lower than those in plasma from controls [72]. Moreover, ROS-induced DNA damage may accelerate the process of germ cell apoptosis, leading to a decline in sperm counts associated with male infertility, and thus to the apparent deterioration of semen quality [73]. In fact, mitochondrial DNA mutations are associated with a decline of motility in human sperm, probably leading to male infertility. Additionally, mitochondrial respiration defects gave rise to meiotic arrest and abnormalities in sperm morphology, stressing the requirements of mitochondrial respiratory function in mammalian spermatogenesis [74].

Spermatogenesis is the integral part of the male reproductive system which cannot remain untouched

by the effects of diabetes mellitus. For every cell of the testis, insulin is required for uptake of glucose from the blood to the cell. But insulin is deficient or resistance in diabetes mellitus, which affects the homeostasis of glucose in the testis which ultimately affects the spermatogenesis.

The findings regarding semen quality in diabetes mellitus have not been consistent, showing variations from normal to altered sperm count, motility, morphology or a combination of both. In a study conducted by Bartak et al., 1975 [75] in a young generation (16–22 years old), involving 25 diabetic and 24 control individuals showed that juvenile diabetics presented lower sperm values and significant differences in sperm motility and morphology. A few years later, another study by the same group compared the ejaculated semen of 65 diabetic and 77 control men and it was reported as a negative effect of diabetes mellitus on the ejaculate. The parameters mostly affected were sperm motility, morphology, volume and count [76]. These findings are concordant with our result. In our study, the sperm count and motility were decreased in diabetic group when compared with the control group. The total percentage of abnormal morphology of sperms was increased in the diabetic group. The effects of diabetes on sperms parameters in long term diabetic conditions are more than the short term diabetic. Though there is a divergent in semen parameters in all diabetic groups from control group but the result of one week study is statistically not significant. The decrease in sperm count, motility and increase abnormal morphology of sperms in diabetic conditions were also reported similarly by Seethalakshmi et al., 1987 and Kim and Moley, 2008 [77,78]. Slightly different results were reported by Pardron et al., in 1984, where diabetic adolescents presented a minor, non-significant, decrease in sperm count relatively to control individuals. The semen from these juvenile diabetic patients had lower volume and motility, as well as altered morphology, and presented significantly higher fructose and glucose levels, evidencing that an ineffective metabolic control can be deleterious or responsible for the observed alterations in the semen [79].

CONCLUSION

The study was conducted in order to clarify the relationship between oxidative stress originated by a diabetic condition and parameters related to testicular function in the rat. We concluded that the induction of diabetes in rats causes increase in the oxidative stress in the testis. Diabetes increases in the level of MDA in the testis. Furthermore, significant reduction in the activity of the antioxidant enzyme was a common feature in all the samples, which is suggestive of the ongoing oxidative disturbances. Prolonged hyperglycemia leads to decrease in the level of SOD, catalase. Decreased activity of these antioxidants might be due to its inactivation caused by excessive ROS production. Initial stage of diabetes mellitus increases the production of SOD since it is the first line of defence against free radical. Since the production of free radical is highly accelerated as the diabetes becomes chronic with time, the level of SOD decreases leading to increase oxidative stress in testis.

The sperms are severely affected in long term diabetes than in short term diabetes. Diabetes mellitus affects significantly in all the parameters of the sperms: count, morphology and motility. Besides decrease in number of spermatozoon, diabetes mellitus also alters structural (morphology) and functional (motility) aspects of spermatozoon. This result shows that diabetes mellitus does not cause only erectile dysfunction and retrograde ejaculation but also affects directly on spermatogenesis leading to infertility.

The consequences of oxidative stress may be a factor for errors in spermatogenesis which leads to loss of motility, count and increased abnormal morphology of sperms in diabetes rats. These findings now support a role of oxidative stress as a significant cause of male infertility in diabetes mellitus.

REFERENCES

1. Baccetti B, La Marca A, Piomboni P, Capitani S, Bruni E, Petraglia F, De Leo V. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. Hum Reprod. 2002; 10:2673-7.
2. Lucas MJ, Leveno KJ, Williams ML, Raskin P, Whalley PJ. Early pregnancy glycosylated hemoglobin, severity of diabetes and fetal malformations. Am J Obstet Gynecol. 1989; 161:426-431.
3. Mills JL, Knopp RH, Simpson JL, Jovanovic-Peterson L, Metzger BE, Holmes LB, Aarons JH, Brown Z, Reed GF, Bieber FR. Lack of relation of increased malformation rates in infants of diabetic mothers to glycemic control during organogenesis. N Engl J Med. 1988; 318:671-676.
4. Miller E, Hare JW, Cloherty JP, Dunn PJ, Gleason RE, Soeldner JS, Kitzmiller JL.Elevated maternal hemoglobin A1c in early pregnancy and major congenital anomalies in

infants of diabetic mothers. N Engl J Med. 1981; 304:1331-1334.

5. Greene MF. Prevention and diagnosis of congenital anomalies in diabetic pregnancies. Clin Perinatol. 1993; 20:533-547.
6. Rehman K, Beshay E, Carrier S. Diabetes and male sexual function. J Sex Reprod Med. 2001; 1:29–33.
7. De Young L, Yu D, Bateman RM, Brock GB. Oxidative stress and antioxidant therapy: their impact in diabetes-associated erectile dysfunction. J Androl. 2004; 25(5):830-6.
8. Rodriguez-Rigau LJ. Diabetes and male reproductive function. J Androl.1980; 1:105-110.
9. Sun P, Cameron A, Seftel A, Shabsigh R, Niederberger C, Guay A. Erectile dysfunction—an observable marker of diabetes mellitus? A large national epidemiological study. J Urol. 2006; 176:1081–1085.
10. Greene LF, Kelalis PP. Retrograde ejaculation of semen due to diabetic neuropathy. J. Urol.1967; 696.
11. Cameron DF, Murray FT, Drylie DD. Interstitial compartment pathology and spermatogenic disruption in testes from impotent diabetic men. Anat Rec. 1985; 213 (1):53-62.
12. Orth JM, Murray FT, Bardin CW. Ultrastructural changes in Leydig cells of streptozotocin-induced diabetic rats. Anat Rec. 1979; 195(3):415-30.
13. Hassan G, Abdel Moneium T. Structural changes in the testes of streptozotocin-induced diabetic rats. Suez Canal Univ Med J.2001; 4(1): 17-25.
14. Pryor WA. Free Radicals.1966; McGraw-Hill, New York.
15. Das A, Chaudhuri D, Mandal N, Chatterjee A. Study of antioxidant and reactive oxygen species scavenging activity of the edible tuber of "Greater Yam" (Dioscorea Alata L.) from North-East India . Asian J Pharm Clin Res. 2012; 5(3): 74-84.
16. Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. Urology. 1996; 48:835-50.
17. Sikka SC. Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. J Androl. 2004; 25:5-18.
18. McCord JM, Fridovich I. "Superoxide Dismutase, An Enzymic Function for Erythrocuprein (Hemocuprein)". Journal of Biological Chemistry.1969; 244 (22): 6049–6055.
19. Fridovich I. "The trail to superoxide dismutase". Protein Science.1998; 7: 2688–2690.
20. Bannister WH, Bannister JV. "Isolation and characterization of superoxide dismutase: a personal history and tribute to Joe McCord and Irwin Fridovich". Free Radical Biology and Medicine.1988; 5: 371–6.
21. Gaetani GF, Ferraris AM, Rolfo M, Mangerini R, Arena S, Kirkman HN. "Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes". Blood.1996; 87 (4): 1595–9.
22. Halliwell B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging. 2001; 18:685-716.
23. Isabella DD, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of Oxidative Damage in Human Disease. Clinical Chemistry. 2006; 52(4):601-623.
24. Maritim AC, Sanders RA, Watkins JB: Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol. 2003; 17:24-38. 10.
25. Freidovich I. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? N.Y. Acad. Sci. 1999; 893:13.
26. Yun-Zhong F, Sheng Y, Guoyao Wu. Free radicals, antioxidants, and nutrition. Nutrition. 2002; 18:872–879.
27. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes.1999; 48: 1–9.
28. Wolff SP, Jiang ZY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic Biol Med.1991; 10: 339–52.
29. Moore K, Roberts LJ. Measurement of lipid peroxidation. Free Radic. Res. 1998; 28 (6): 659–71.
30. Del RD, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis. 2005; 15 (4): 316–28.
31. Durak D, Kalender S, Uzun FG, Demir F, Kalender Y. Mercury chloride-induced oxidative stress and the protective effect of vitamins C and E in human erythrocytes in vitro. Afr J Biotechnol. 2010; 9 (4): 488- 495.
32. Barratt CL. Semen analysis is the cornerstone of investigation for male infertility. Practitioner. 2007; 251:8.
33. Jequier AM. Semen analysis: a new manual and its application to the understanding of semen and its pathology. Asian J Androl. 2010; 12:11–3.
34. Pinhas-Hamiel O, Zeitler P. The global spread of type 2 diabetes mellitus in children and adolescents. J Pediatr. 2005; 146:693-700.
35. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47(3):469-74.
36. Sinha KA. Colorimetric assay of catalase. Anal Biochem. 1972; 47: 389-394.
37. Sathoh K "Serum lipid peroxide in cerebrovascular disorder dertermined by a new colorimetric method". Clin Chin Acta.1978; 90: 37-43.
38. Godkar PB, Godkar DP. Textbook of Medical Laboratory Technology 2nd ed. Mumbai: Bhalani Publishing house.2003; 957-970.
39. Free MJ, Schluntz GA, Jaffe RA. Respiratory gas tensions in tissues and fluids of the male rat reproductive tract. Biol Reprod.1976; 14:481-8.
40. Peltola V, Mantyla E, Huhtaniemi I, Ahotupa M. Lipid peroxidation and antioxidant enzyme activities in the rat testis after cigarette smoke inhalation or administration of polychlorinated biphenyls or polychlorinated naphthalenes. J Androl. 1994; 15:353-61.

41. Quinn PG, Payne AH. Oxygen-mediated damage of microsomal cytochrome P-450 enzymes in cultured leydig cells: Role in steroidogenic desensitization. J Biol Chem.1984; 259:4130-5.
42. Chen H, Liu J, Luo L, Baig MU, Kim JM, Zirkin BR. Vitamin E, aging and Leydig cell steroidogenesis. Exp Gerontol. 2005; 40:728-36.
43. Guimaraes SB, Aragao AA, Santos JM, Kimura Ode S, Barbosa PH, Vasconcelos PR. Oxidative stress induced by torsion of the spermatic cord in young rats. Acta Cir Bras. 2007; 22:30-3.
44. Anim JT, Kehinde EO, Prasad A, Varghese R. Morphological responses of the rabbit testis to ischemic/reperfusion injury due to torsion. Urol Int. 2005; 75:258-63.
45. Unsal A, Eroglu M, Avci A, Cimentepe E, Guven C, Derya Balbay M, Durak I . Protective role of natural antioxidant supplementation on testicular tissue after testicular torsion and detorsion. Scand J Urol Nephrol. 2006; 40:17-22.
46. Dokmeci D, Inan M, Basaran UN, Yalcin O, Aydogdu N, Turan FN, Uz YH. Protective effect of L-carnitine on testicular ischaemia-reperfusion injury in rats. Cell Biochem Funct. 2007; 25:611-8.
47. Hendin BN, Kolettis PN, Sharma RK, Thomas AJ Jr, Agarwal A. Varicocele is associated with elevated spermatozoa reactive oxygen species production and diminished seminal plasma antioxidant capacity. J Urol.1999; 161:1831-4.
48. Saleh RA, Agarwal A, Sharma RK, Said TM, Sikka SC, Thomas AJ Jr. Evaluation of nuclear DNA damage in spermatozoa from infertile men with varicocele. Fertil Steril. 2003; 80:1431-6.
49. Zamoner A, Barreto KP, Filho DW, Sell F, Woehl VM, Guma FC, Silva FR, Pessoa-Pureur R. Hyperthyroidism in the developing rat testis is associated with oxidative stress and hyperphosphorylated vimentin accumulation. Mol Cell Endocrinol. 2007; 267:116-26.
50. Choudhury S, Chainy GB, Mishro MM. Experimentally induced hypo- and hyper- thyroidism influence on the antioxidant defence system in adult rat testis. Andrologia. 2003; 35:131-40.
51. Hipler UC, Gorning M, Hipler B, Romer W, Schreiber G. Stimulation and scavestrogen-induced inhibition of reactive oxygen species generated by rat Sertoli cells. Arch Androl. 2001; 44:147–54.
52. Baumber J, Sabeur K, Vo A, Ball BA. Reactive oxygen species promotes tyrosine phosphorylation and capacitation in equine spermatozoa. Theriogenology. 2003; 60: 1239 – 47.
53. Aitken RJ, De Iuliis GN. Origins and consequences of DNA damage in male germ cells. Reprod Biomed Online. 2007; 14:727–733.
54. Aitken RJ, Baker MA. Oxidative stress, sperm survival and fertility control. Mol Cell Endocrinol. 2006; 250:66–69.
55. Aitken RJ. Founders' Lecture: Human spermatozoa: Fruits of creation, seeds of doubt. Reprod Fertil Dev. 2004; 16:655–664.
56. Aitken RJ. The Amoroso Lecture: The human spermatozoon—A cell in crisis? J Reprod Fertil.1999; 115:1–7.
57. Sikka SC. Relative impact of oxidative stress on male reproductive function. Curr Med Chem. 2001; 8:851–862.
58. Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. Curr Opin Obstet Gynecol. 2006; 18:325–332.
59. Kumar TR. Muralidhara Male-mediated dominant lethal mutations in mice following prooxidant treatment. Mutat Res. 1999; 444:145–149.
60. Hales DB, Allen JA, Shankara T, Janus P, Buck S, Diemer T, Hales KH . Mitochondrial function in Leydig cell steroidogenesis. Ann N Y Acad Sci. 2005; 1061:120–134.
61. Naughton CK, Nangia AK, Agarwal A. Pathophysiology of varicoceles in male infertility. Hum Reprod Update. 2001; 7:473–481.
62. Shaikh H, Shrivastava VK. Effect of streptozotocin induced diabetes mellitus type 1 on the rat brain antioxidant status and activity of acetyl-cholinesterase: A novel and potential treatment by Vitex Negundo.Int J Pharm Pharm Sci. 2014; 6(10):252-256.
63. Rabbani SI, Devi K, Khanam S. Inhibitory effect of Glimepiride on nicotinamide- streptozotocin induced nuclear damages and sperm abnormality in diabetic Wistar rats. Indian J Exp Biol. 2009; 47: 804-810.
64. Adewole SO, Caxton-Martins EA, Salako AA, Doherty OW, Naicker T. Effects of oxidative stress induced by streptozotocin on the morphology and trace minerals of the testes of diabetic wistar rats.Pharmacologyonline. 2007; 2:478-497.
65. Ozkaya YG, Agar A, Yargicoglu P, Hacioglu G, BilmenSarikcioglu S, Ozen I, Alicigüzel Y. The effect of exercise on brain antioxidant status of diabetic rats. Diabetes Metab. 2002; 28: 377–84.
66. Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol. Reprod. 1989; 40:183-197.
67. Ono T, Okada S.Radiation-induced DNA single-strand scission and its rejoining in spermatogonia and spermatozoa of mouse Mutat. Res.1977; 43:25–36.
68. Van Loon AA, Den Boer PJ, Van der Schans GP, Mackenbach P, Grootegoed JA, Baan RA, Lohman PH. Immunochemical detection of DNA damage induction and repair at different cellular stages of spermatogenesis of the hamster after in vitro or in vivo exposure to ionizing radiation. Exp. Cell Res.1991; 193:303–309.
69. Singh R, Bhardwaj P, Sharma P. Antioxidant and toxicological evaluation of Cassia sopherain streptozotocin-induced diabetic Wistar rats. Pharmacognosy Res. 2013; 5(4): 225–232.
70. Kaleem M, Asif M, Ahmed QU, Bano B. Antidiabetic and antioxidant activity of Annona squamosa extract in streptozotocin-induced diabetic rats. Singapore Med J. 2006; 47:670–5.
71. Searle AJ, Wilson RL. Glutathione peroxidase: Effect of superoxide, hydroxyl and bromine free radicals on enzyme activity. Int J Radiat Biol Relat Stud Phys Chem Med. 1980; 37:213.

72. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. 2003; 79:829-43.

73. Wei YH, Kao SH. Mitochondrial DNA Mutation and Depletion Are Associated with Decline of Fertility and Motility of Human Sperm Zoological Studies. 2000; 39:1-12.

74. Nakada K, Sato A, Yoshida K, Morita T, Tanaka H, Inoue S, Yonekawa H, Hayashi J. Mitochondria-related male infertility.Proc Natl Acad Sci. USA. 2006; 103:15148-53.

75. Barta´k V, Josı´fko M, Hora´ckova´ M. Juvenile diabetes and human sperm quality. Int J Fertil. 1975; 20:30–32.

76. Bartak V. Sperm quality in adult diabetic men, Int. J. Fertil. 1979; 24: 226–232.

77. Seethalakshmi L, Menon M, Diamond D. The effect of streptozotocininduced diabetes on the neuroendocrine-male reproductive tract axis of the adult rat. J Urol. 1987; 138:190–194.

78. Kim ST, Moley KH. Paternal effect on embryo quality in diabetic mice is related to poor sperm quality and associated with decreased glucose transporter expression. Reproduction. 2008; 136:313–322.

79. Hicks, Rojas L, Rosado A. Insulin regulation of spermatozoa metabolism, Endocrinology.1973; 92:833–839.

Original Article

MUTATIONAL ANALYSIS OF MYBPC3 GENE IN DILATED CARDIOMYOPATHY PATIENTS IN NORTH INDIAN POPULATION

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ABSTRACT

Introduction: Today, molecular cardiology is characterized by the integration of high-technology laboratory studies and clinical medicine. Molecular genetics has redefined the etiology and diagnostic criteria for numerous diseases and has led to the development of new, individualized treatment regimens for several cardiovascular diseases. Amongst all, dilated cardiomyopathy is the commonest cause of heart failure. This study was conducted to identify the possible genetic change in dilated cardiomyopathy in North Indian population.

Material & Methods: Blood samples of dilated cardiomyopathy patients were collected from Cardiology OPD, Sir Sunderlal Hospital, Banaras Hindu University. DNA was isolated using salting out method. PCR was done to amplify exons 32, 33 and 34 of MYBPC3 gene. The PCR product was sequenced to detect the mutational changes in Exons 32-34 of MYBPC 3 gene.

Results: There were 65 control samples and 65 DCM samples were collected. Total 76 intronic variations were reported. In three (BHU/15/425, BHU/15/450, BHU/16/89) patients, disease causing pathogenic variant c3624_3625insC (rs397516029, HMGD CD0910628) was reported in MYBPC3 gene. Insertion of G at 47354119_47354120 position was reported that lead to a frameshift mutation in three subjects. Several Missence variants were also reported 47354121G>C, 47353899C>T, 47353715C>A, 47353647A>C, 47353626G>T that are present in coding region and may lead to alteration in protein structure and function.

Conclusion: Evidence from previous study reported that MYBPC3 play important role in cardiac contraction and responsible for pathogenesis of dilated cardiomyopathy. Therefore, the identification of frequent genetic transmission of dilated cardiomyopathy provides an important tool for the study of pathogenesis of this disease, which is a frequent cause of admission to the hospital and of heart failure.

Keywords: Dilated cardiomyopathy, myosin binding protein C, sequencing, DNA.

INTRODUCTION

Disorders of the heart leading to heart failure are leading cause of morbidity and mortality. Since the term "cardiomyopathy" was coined 30 years ago to describe a group of myocardial diseases of unknown cause, which was termed as idiopathic cardio-myopathy [1].

Out of all types of cardiomyopathies, dilated cardiomyopathy (DCM) and hypertrophic cardio-myopathy (HCM) are the two major cardiomyopathies (Fig. 1). Other clinical cardiomyopathies include restrictive cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy [2].

DCM is defined by the presence of: a) fractional shortening (FS) less than 25% (> 2SD) and/or ejection fraction less than 45% (> 2SD); and b) left ventricular end diastolic diameter (LVEDD) greater than 117% (>2SD of the predicted value of 112% corrected for age and body surface area, BSA) [3] excluding any known cause of myocardial disease. In the context of a familial DCM, these criteria are used to diagnose the

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proband in a family.

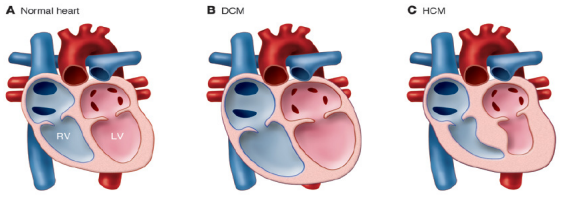


Fig. 1: Morphological changes to the heart in cardiomyopathy. (A) Normal heart. (B) In DCM, the heart enlarges with increased diameter and reduced function. (C) In HCM, the myocardium especially in the LV becomes thickened, leading to impaired filling and emptying [4]

Recent studies report that 20-35% cases of dilated cardiomyopathy are considered to be familial. Therefore, it is important to study the genetic factors responsible for DCM. Many genes are responsible for DCM out of which the most common are MYBPC3, MYH7, TNNT2, TTN, SGCD. In this study we screened familial DCM patients for the presence of mutation in MYBPC3 gene by DNA isolation and by DNA sequencing [5].

MYBPC 3 (Myosin Binding Protein C) Gene

The MYBPC3 gene provides instructions for making the cardiac myosin binding protein C (cardiac MYBP-C), which contains 35 exons and is found in heart (cardiac) muscle cells. In these cells, cardiac MYBP-C is associated with a structure called the sarcomere, which is the basic unit of muscle contraction. Sarcomeres are made up of thick and thin filaments. The overlapping thick and thin filaments attach to each other and release, which allows the filaments to move relative to one another so that muscles can contract. Regular contractions of cardiac muscle pump blood to the rest of the body [6].

In cardiac muscle sarcomeres, cardiac MYBP-C attaches to thick filaments and keeps them from being broken down. Cardiac MYBP-C has chemical groups called phosphate groups attached to it; when the phosphate groups are removed; cardiac MYBP-C is broken down, followed by the breakdown of the proteins of the thick filament. Cardiac MYBP-C also regulates the rate of muscle contraction, although the mechanism is not fully understood [7].

Chromosomal Location

Cytogenetic location: 11p11.2, which is the short (p) arm of chromosome 11 at position 11.2 (Fig. 2).

Molecular location: base pairs 47,331,406 to 47,352,702 on chromosome 11 (Homo-sapiens Annotation Release 108, GRCh38.p7) [7].

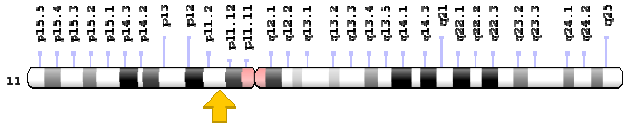


Fig. 2: Cytogenetic location of MYBPC3 gene

The main objective of our study was to evaluate mutational changes in genes MYBPC3 responsible or causing DCM as well as HCM in the population of North Indians from Eastern Uttar Pradesh region.

MATERIAL AND METHODS

Sample Collection

3 to 5 ml of peripheral blood was collected in EDTA coated vials after taking inform consent from patients of dilated cardiomyopathy, samples were collected from the Outpatient Department of Cardiology, Sir Sunderlal Hospital of Institute of Medical Sciences, Banaras Hindu University, Varanasi (from 2015-2017). Echocardiogram (ECG) and clinical history of patient were noted; ischemic dilated cardiomyopathy patients were excluded from this study. The study protocol was approved by Ethical Committee of SS hospital, BHU, Varanasi.

DNA Extraction and PCR Amplification

DNA isolation was done by “Salting out method” and dissolved in TE buffer. Patient samples can be readily checked for concentration and quality using the Nano Drop 1000 Spectrophotometer. After DNA extraction coding region of exons 32-34 were amplified by using following primers exon 32-34F 5’GGCTCAGCCACTGACTTGT 3’and 32-34R 5’ AGGGGCCTAGCTTTGTGTG 3’ after amplification 5µl PCR amplified products were loaded with DNA loading dye to check the amplification.

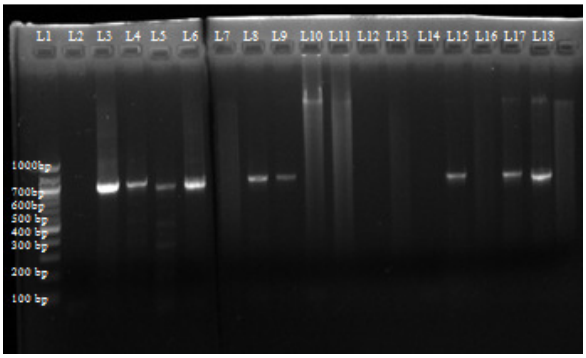
DNA Sequencing

When PCR amplification was complete, PCR products was purified with Exo Sap reagent. The purified PCR products were then sequenced using the sequencing kit and sequencer from Applied Biosystems, USA. Sequencing reactions were analysed using 3130xL Genetic Analyzer (Applied Biosystem R). Sequencing files were obtained from the 3130xL Genetic Analyzer (applied Biosystems R) were analysed using FinchTV

viewer. Further analysis was done using MEGA 6 software and Mutation Taster software.

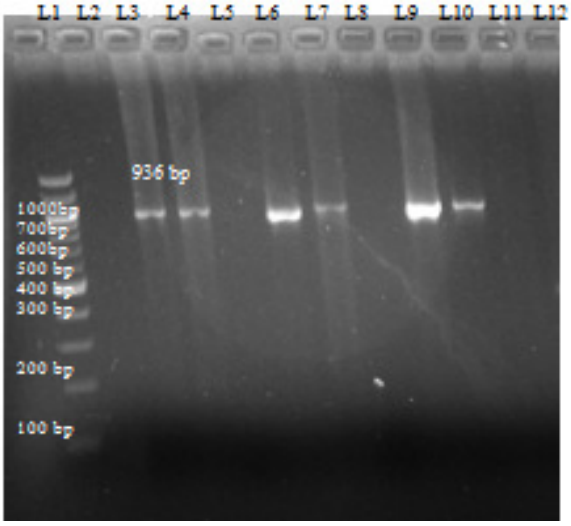
OBSERVATIONS AND RESULTS

Total of 65 patients samples were collected out of which 50 were male and 15 were female. Agrarose gel electrophoresis results (Fig. 3-5) and sequencing results (Fig. 6,7) exhibited various synonymous and non-synonymous mutations (Table 1). A disease causing pathogenic variation was found in one of the patient.



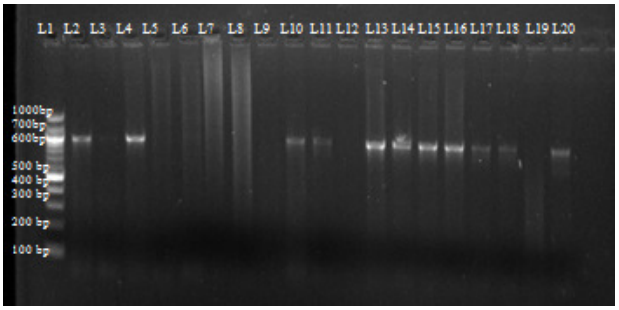
L1: Ladder
L2: NC
L3: **BHU/15/407**
L4: **BHU/15/408**
L5: **BHU/15/423**
L6: **BHU/15/424**
L7: EMPTY
L8: **BHU/15/425**
L9: **BHU/15/464**
L10: BHU/15/465
L11: BHU/15/511
L12: BHU/15/512
L13: BHU/15/513
L14: BHU/16/8
L15: **BHU/16/9**
L16: BHU/16/58
L17: **BHU/16/59**

Fig. 3: 2% Agarose Gel Electrophoresis for MYBPC3 having amplicon size 953 bp



L1: Ladder
L2: NC
L3: **BHU/15/464**
L4: **BHU/15/465**
L5: BHU/15/511
L6: **BHU/15/512**
L7: **BHU/15/513**
L8:BHU/16/89
L9:**BHU/16/70**
L10:**BHU/16/8**
L11:BHU/16/9

Fig. 4: 2% Agarose Gel Electrophoresis for MYBPC3 having amplicon size 953 bp



L1: Ladder
L2: **BHU/15/408**
L3: BHU/15/423
L4: **BHU/15/450**
L5: BHU/15/453
L6: BHU/15/454
L7: BHU/15/455
L8: BHU/15/465
L9: **BHU/15/512**
L10: **BHU/15/608**
L11: BHU/16/8
L12: **BHU/16/9**
L13: **BHU/16/39**
L14: **BHU/16/58**
L15: **BHU/16/59**
L16: **BHU/16/70**
L17: **BHU/16/ 89**
L18: BHU/16/104
L19: **BHU/16/258**
L20: BHU/16/345

Fig. 5: 2% Agarose Gel Electrophoresis for MYBPC3 having amplicon size 953 bp

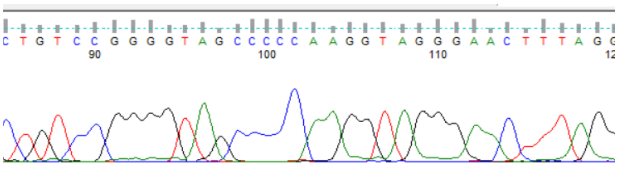


Fig. 6: Representative sequence electropherograms showing c.3624_3625insC substitution in patient BHU/15/450 in MYBPC3 gene

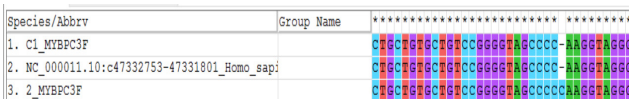


Fig. 7: Multiple sequence alignment of MYBPC 3 gene in patient BHU/15/450 with control sample.

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Table 1: Showing various synonymous and non-synonymous mutations

S. No.	Genomic position	Patient ID	Aminoacid change	Mutation taster
	MYBPC3			
1	Chr 11:47354160C>A	BHU/15/407	G1195V	rs730880595
2	Chr 11: 47354127>47354128 ins C	BHU/15/408	S1207	HMGD CM057198, CM068014
3	Chr11: 47354121G>C	BHU/15/408	P1208R	SINGLE BASE EXCHANGE,CDS
4	Chr 11: 47354072_4735407 ins C	BHU/15/408		INTRON VARIANT
5	Chr 11:47354038_47354039 ins G	BHU/15/408		INTRON VARIANT
6	Chr 11: 47353899C>T	BHU/15/408	SINGLE BASE EXCHANGE	rs11039186
7	Chr 11: 47353536_47353537 insA	BHU/15/408		INTRON VARIANT
8	Chr 11: 47353509_47353510 insA	BHU/15/408		INTRON VARIANT
9	Chr 11: 47353464_47353465 insA	BHU/15/408		INTRON VARIANT
10	Chr 11: 47353715C>A	BHU/15/423	R1241I	CDS
11	Chr 11: 47353647A>C	BHU/15/423	C1264G	CDS
12	Chr 11: 47353626G>T	BHU/15/423	CDS	HGMD CM086866
13	Chr 11: 47354102C>A	BHU/15/424		INTRON VARIANT
14	Chr 11: 47354090C>T	BHU/15/424		INTRON VARIANT
15	Chr 11: 47354019_47354020 insA	BHU/15/424		INTRON VARIANT
16	Chr 11: 47353977_47353978 insC	BHU/15/424		INTRON VARIANT
17	Chr 11: 47353948A>C	BHU/15/424		INTRON VARIANT
18	Chr 11: 47353536T>A	BHU/15/424		INTRON VARIANT
19	Chr 11: 47353502>A	BHU/15/424		INTRON VARIANT
20	Chr 11: 47353496G>C	BHU/15/424		INTRON VARIANT
21	Chr 11:47353464_47353465 insA	BHU/15/424		INTRON VARIANT
22	Chr 11: 47353453T>C	BHU/15/424		INTRON VARIANT
23	**Chr 11: 47354119_47354120 insG**	**BHU/15/425**	**K1209Q**	**rs397516029 FRAMESHIFT**
24	Chr 11: 47354095_47354096 insC	BHU/15/425		INTRON VARIANT
25	Chr 11: 47354068G>A	BHU/15/425		INTRON VARIANT rs3729802
26	Chr 11: 47354040G>T	BHU/15/425		INTRON VARIANT
27	Chr 11: 47353498G>A	BHU/15/425		INTRON VARIANT rs2290146
28	**Chr 11: 47354119_47354120 insG**	**BHU/15/450**	**K1209Q**	**rs397516029**

S. No.	Genomic position	Patient ID	Aminoacid change	Mutation taster
				FRAMESHIFT
29	Chr 11: 47354068G>A	BHU/15/450		INTRON VARIANT rs3729802
30	Chr 11: 47353536_47353537 insA	BHU/15/450		INTRON VARIANT
31	Chr 11: 47353498G>A	BHU/15/450		INTRON VARIANT rs2290146
32	Chr 11: 47353464_47353465 insA	BHU/15/450		INTRON VARIANT
33	Chr 11:47353434_47353435 insA	BHU/15/450		INTRON VARIANT
34	Chr 11: 47353464_47353465 insA	BHU/15/464		INTRON VARIANT
35	Chr 11: 47354090 delC	BHU/15/465		INTRON VARIANT
36	Chr 11: 47353502C>A	BHU/15/465		INTRON VARIANT
37	Chr 11: 47353464_47353465insA	BHU/15/465		INTRON VARIANT
38	Chr 11: 47353453_4735345 insT	BHU/15/465		INTRON VARIANT
39	Chr 11:47354090delC	BHU/15/608		INTRON VARIANT
40	Chr 11:47354065C>T	BHU/15/608		INTRON VARIANT
41	Chr 11:47353536_47353537insA	BHU/15/608		INTRON VARIANT
42	Chr 11:47353485_47353486 insA	BHU/15/608		INTRON VARIANT
43	Chr 11: 47353464_47353465insA	BHU/15/608		INTRON VARIANT
44	Chr 11: 47353457-47353458 insA	BHU/15/608		INTRON VARIANT
45	Chr 11: 47353745C>T	BHU/16/08	S1231N	HGMD CI014153
46	Chr 11:47353741C>T	BHU/16/08	CDS	
47	Chr 11:47353496G>C	BHU/16/08		INTRON VARIANT
48	Chr 11:47354093delG	BHU/16/39		INTRON VARIANT
49	Chr 11: 47354085G>A	BHU/16/39		INTRON VARIANT
50	Chr 11: 47353899C>T	BHU/16/39		rs11039186
51	Chr 11: 47353434_47353435insA	BHU/16/39		INTRON VARIANT
52	Chr 11: 47354038_47354039 insG	BHU/16/58		INTRON VARIANT
53	Chr 11: 47353536_47353537 insA	BHU/16/58		INTRON VARIANT
54	Chr 11: 47353498G>A	BHU/16/58		rs2290146
55	Chr 11: 47353464_47353465 insA	BHU/16/58		INTRON VARIANT
56	Chr 11: 47353874A>T	BHU/16/58		INTRON VARIANT
57	Chr 11: 47353864G>C	BHU/16/58		INTRON VARIANT
58	Chr 11: 47353756G>A	BHU/16/58	CDS	
59	Chr 11: 47353459_47353460 insA	BHU/16/58		INTRON VARIANT
60	Chr 11: 47353451_47353452 insC	BHU/16/58		INTRON VARIANT

S. No.	Genomic position	Patient ID	Aminoacid change	Mutation taster
61	Chr 11:47354093delG	BHU/16/59		INTRON VARIANT
62	Chr 11: 47354090C>G	BHU/16/59		INTRON VARIANT
63	Chr 11: 47353899C>T	BHU/16/59		rs11039186
64	Chr 11: 47353457_47353458 insA	BHU/16/59		INTRON VARIANT
65	Chr 11: 47353899C>T	BHU/16/59		rs11039186
66	Chr 11: 47354090deLC	BHU/16/70		INTRON VARIANT
67	Chr 11: 47354068G>A	BHU/16/70		rs3729802
68	Chr 11: 47353498G>A	BHU/16/70		rs2290146
69	Chr 11:47353457_47353458 insA	BHU/16/70		INTRON VARIANT
70	Chr 11: 47353434_47353435 insA	BHU/16/70		INTRON VARIANT
71	Chr11:47353418_47353419 insT	BHU/16/70		INTRON VARIANT
72	**Chr11:47354119_47354120 insG**	**BHU/16/89**	**K1209Q**	**rs397516029 FRAMESHIFT PRESENT**
73	Chr11:47354090_47354090 delC	BHU/16/89		INTRON VARIANT
74	Chr11:47354068G>A	BHU/16/89		rs3729802
75	Chr11:47353498G>A	BHU/16/89		INTRON VARIANT rs2290146
76	Chr11:47353434_47353435insA	BHU/16/89		INTRON VARIANT

DISCUSSION

Dilated cardiomyopathy is most commonly associated with various other heart disorders. The most common associated disorder is congestive heart failure. In DCM there is ventricular chamber enlargement, the ventricular walls become thin and there is depressed left ventricular systolic function. It is most commonly idiopathic or familial. There may be various modes of inheritance i.e. autosomal dominant, autosomal recessive, X-linked or mitochondrial mode of inheritance.

Present study combined with previous studies, provides extensive data of association with the genetic factors causing DCM. We used Sanger based sequencing which is still the gold standard for sequencing sensitivity and specificity. In present study **Exon 32-34 of MYBPC 3** gene was sequenced to identify possible genetic cause of dilated cardiomyopathy. Mutations in the gene for cardiac myosin-binding protein C account for main genetic cause of maximum cases of familial hypertrophic cardiomyopathy and dilated cardiomyopathy. In present study various synonymous and non-synonymous variations had been observed.

In MYBPC3 gene, several intronic variations observed. Total 76 intronic variations were reported. Intronic variation does not alter protein structure but it may change splice site that may affect the protein structure and function. In three (BHU/15/425, BHU/15/450, BHU/16/89) patients disease causing variant c3624_3625insC (rs397516029, HMGD CD0910628) was reported in MYBPC3 gene. Same variation was reported by Hershberger et al., 2010 [8].

Some novel variant reported may lead to DCM. Insertion of G at 47354119_47354120 position reported lead to a frameshift mutation in three subjects. Several missence variants were also observed 47354121G>C, 47353899C>T, 47353715C>A, 47353647A>C, 47353626G>T that are present in coding region and may lead to alteration in protein structure and function. Because of these variations are present in C terminal domain of protein that play very important role.

Cardiac myosin-binding protein C (MYBPC3) is arrayed transversely in sarcomere A-bands and binds myosin heavy chain in thick filaments and in elastic filaments. Phosphorylation of this protein appears to modulate contraction [9]. The prognosis in patients with dilated cardiomyopathy is considered to be poor as morbidity and mortality rates are very high. Moreover, DCM is the chief indication for heart transplantation. So, it is important to diagnose the disease early for better treatment.

Evidence from previous study reported that MYBPC3 play important role in cardiac contraction and responsible for pathogenesis of dilated cardiomyopathy [10].

CONCLUSION

The identification of frequent genetic transmission of dilated cardiomyopathy provides an important tool for the study of pathogenesis of this disease, which is a frequent cause of admission to the hospital and of heart failure. Molecular genetic techniques help to identify the gene causing familial dilated cardiomyopathy and can be used to study the effects of altered gene product whether it is frameshit mutation or missense variation. Whereas early features of dilated cardiomyopathy can be identified by echocardiography or electrocardiogram or magnetic resonance imaging. The genetic as well as imaging information together provides accurate status of disease which helps in the management and prognosis of dilated cardiomyopathy. As soon as the genetic cause of dilated cardiomyopathy is diagnosed, the patient should undergo genetic counselling. It includes family history, education regarding transmission, benefits of regular cardiac screening tests. Multidisciplinary medical care for DCM includes genetic counsellors, cardiologists, medical geneticists.

In present study, mutational analysis of 32-34 exons of MYBPC3 gene and 14-15 exon of TNNT2 gene had been done. Various synonymous and non-synonymous variations had been reported. Several intronic variation, frameshift mutation and missense variation are reported in both genes. That suggest these variation may be responsible for pathogenesis of dilated cardiomyopathy in patients of North Indian population. So, all families having familial transmission of dilated cardiomyopathy must have essential knowledge regarding the disease.

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REFERENCES

1. Goodwin JF: The frontiers of cardiomyopathy. Br Heart J. 1982; 48:1-18.
2. Mestroni L, Rocco C, Gregori D, Sinagra G, Lenarda A, Miocic S, Vatta M, Pinamonti B, Muntoni F, Caforio AL, McKenna WJ, Giacca M, Camerini. Familial dilated cardiomyopathy: evidence for genetic and phenotypic heterogeneity. Journal of the American College of Cardiology. 1999; 34(1): 181–190.
3. Taylor MRG, Carniel E, Mestroni L. Cardiomyopathy, Familial Dilated. Orphanet Journal of Rare Diseases. 2006; I:27; 1750-1172-1-27.
4. Henry WL, Gardin JM, Ware JH. Echocardiographic measurements in normal subjects from infancy to old age. Circulation. 1980; 62: 1054-1061.
5. Report of the WHO/ISFC Task Force on the definition and classification of cardiomyopathies. Br Heart J. 1980; 44:672–4.
6. Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, Gersh BJ, Ackerman MJ. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. J Am Coll Cardiol. 2004; 44:1903-10.
7. https://ghr.nlm.nih.gov/gene/MYBPC3#location.
8. Ray E. Hershberger, Ana Morales, and Jill D. Siegfried. Clinical and genetic issues in dilated cardiomyopathy: A review for genetics professionals. Genet Med. 2010; 12(11): 655– 667.
9. Lisa Dellefave and Elizabeth M. McNally. The genetics of dilated cardiomyopathy. Current Opinion in Cardiology. 2010; 25:198–204.
10. Mestroni L, Rocco C, Gregori D et al. Familial dilated cardiomyopathy: evidence for genetic and phenotypic heterogeneity. Heart Muscle Disease Study Group. Journal of the American College of Cardiology. 1999; 34 (1): 181–190.

Original Article

KNEE CAP: A MORPHOMETRIC STUDY IN DRY HUMAN PATELLA

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ABSTRACT

Introduction: Patella is the largest sesamoid bone. Knowledge of dimensions and of classification of patellae is very important anthropologically as well as clinically for total knee arthroplasty and harvesting techniques of patellar ligament grafts. Since there are very few studies on patella, present study aimed to do morphometry of patella.

Material & Methods: Morphometric measurements were done on 60 dry patellae of both sexes, using digital sliding Vernier caliper. All statistical analysis was done with help of standard SPSS software. Student t-test was used to ascertain whether significant difference exist between right and left patella. Patella was also classified into one of three categories according to Koyuncu's classification-Type A (WMAF=WLAF), Type B (WMAF <WLAF) and Type C (WMAF>WLAF).

Results: Out of 60 patellae, 34 patellae were of right side and 26 were of left side. Mean patellar height was 39.53$\pm$ 5.58; mean width of patella 41.27$\pm$3.67 and mean patellar thickness was 19.84$\pm$1.76. Width of medial articular facet was 17.50$\pm$1.88 and width of lateral articular facet was 20.82$\pm$2.72. There was no significant difference in measurements of right and left patella except width for lateral articular facet where p value was <0.01. In present study, according to Koyuncu's classification 86.66% patella were of class B type, 13.34% patella were of class C type while class A type patella was not found. The secondary ridge was found to be conspicuous in 23.3% patellae.

Conclusion: Present study on morphometry of the patella is obviously very important and concluded parameters can be variably used in anthropology and anthropometrics, comparative anatomy and evolutionary biology, patellofemoral unit prosthesis synthesis, patellar implants, arthroplasty and forensic sciences.

Keywords: Patella, sesamoid bone, knee arthroplasty.

INTRODUCTION

Patella is also known as Knee Cap. Patella is the largest sesamoid bone. It is found embedded in the tendon of quadriceps femoris. It lies anterior to distal femur (femoral condyles). It has two surfaces anterior and posterior, three borders superior, medial and lateral and an apex which points inferiorly (Fig. 1). The anterior surface is separated from skin by a prepatellar bursa and covered by an expansion of upcoming tendons of quadriceps femoris. The posterior surface is divided into two parts, superior (articulating) and inferior (non-articulating). The superior part of the posterior surface is crossed by a smooth vertical ridge, which fits into the intercondylar groove on femoral patellar surface, which divides this area into lateral and medial facet. The lateral facet is usually larger. Medial and lateral facets are further divided by faint horizontal lines into equal thirds. A seventh odd facet is present as a narrow strip along the medial border of patella; it contacts the medial femoral condyle in extreme flexion. The inferior part forms the apex of patella which provides attachment to the patellar ligament [1].

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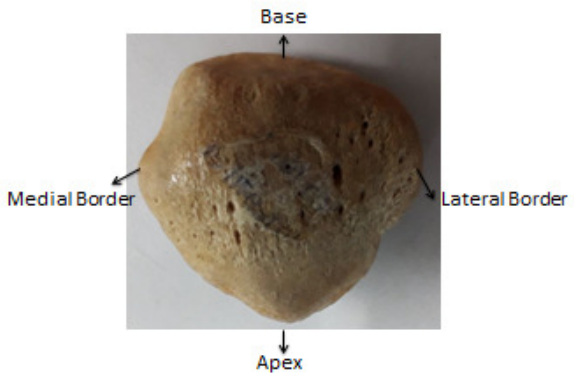

Fig. 1: Anterior view of left patella

Knowledge of dimensions and of classification of patellae is very important anthropologically as well as clinically. It is involved in the various movements of knee and methods of sitting and squatting. Therefore, there may be effect of cultural and ethnic variables on patellar morphology. It is to be assumed that the size of the patella, can be dependent on the strain generated by the quadriceps muscle as it lies within the tendon of quadriceps. But the absence of patella in some animals with powerful action of knee joint, led to controversy concerning with this concept [2].

It was revealed that the geometry of the patella and patellar tendon was significantly larger in males and other demographic factors including weight, height, body mass index well correlates with patellar thickness, but poor correlation with the length of patellar tendon [3,4]. Quadriceps tendon thickness also showed significant correlation with patellar height [5].

Koyuncu et al. (2011), studied the patellar development during the fetal life and found that there are no significant differences between genders or sides (right versus left patellae). However, a significant correlation was found between gestational age and all studied morphometric parameters of patella [6].

Many pathologies may affect the patellofemoral compartment of the knee joint including: osteoarthritis, chondromalacia, fractures, idiopathic and patellofemoral pain syndrome etc. [7]. Patients undergoing total knee arthroplasty (TKA) are routinely subjected to a pre-determined amount of post-surgical loading of the knee, to preserve patellar articular cartilage and subchondral bone trophism [8]. Other problems may occur during which TKA include patellofemoral instability and implant's failure [9]. Biomechanical disturbances can also affect the patellofemoral compartment of the knee [10,11].

Knowledge of morphology and dimensions of patella perform very important role in designing of prosthesis and development of surgical techniques.

MATERIAL AND METHODS

This study was carried out on 60 dry patellae of unknown sex, obtained from Department of Anatomy, Varun Arjun Medical College & Rohilkhand Hospital, Banthra and Rohilkhand Medical College & Hospital, Bareilly. Specimens having signs of fracture, pins and plates or the specimen having loss of bone density or bad erosion were excluded from study. All the measurements were taken by using digital sliding Vernier caliper up to 0.001mm accuracy. All measurements were taken twice at different time to exclude human error. Data was tabulated and analysis was done with help of standard SPSS software. Student t-test was used to ascertain whether significant difference exist between right and left patella.

Following parameters were measured by digital Vernier caliper (Fig. 2):

1. **Patella Height (PH):** Linear distance measured between the superior border and the apex of patella.
2. **Patella Width (PW):** Linear distance measured between the medial and lateral border of patella.
3. **Patella Thickness (PT):** measured as linear distance between the anterior surface of patella and the median ridge present on posterior surface of patella.
4. **Width of Medial Articular Facet (WMAF):** measured as maximum width from the medial border to medial ridge of patella.
5. **Width of Lateral Articular Facet (WLAF):** measured as maximum width from lateral border to median ridge of patella.
6. **Median Ridge Thickness (RT):** measured as width of median ridge.

Patella was also classified into one of three categories according to Koyuncu's classification as Type A (WMAF=WLAF), Type B (WMAF <WLAF) and Type C (WMAF>WLAF). The presence and predisposition of secondary ridge was also carefully noted.

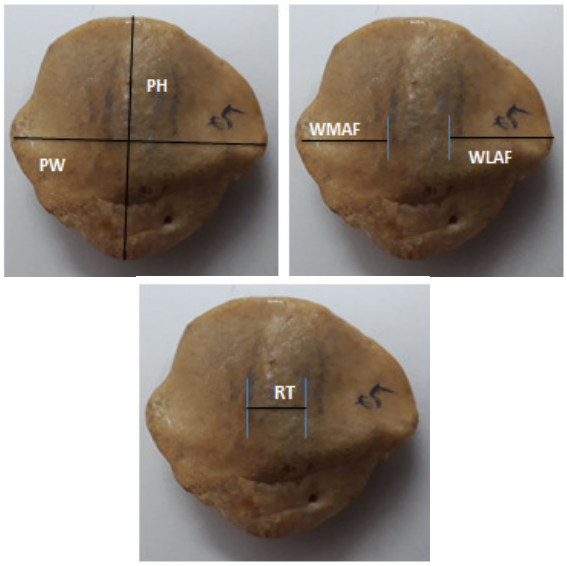

Fig. 2: Parameters measured by digital Vernier caliper. PH-Patellar height, PW-Patellar width, WMAF- width of medial articular facet, WLAF- width of lateral articular facet, RT-median ridge thickness

RESULTS

In the present study, out of 60 patellae 34 patellae were of right side and 26 were of left side. Mean patellar height was 39.53± 5.58; mean width of patella 41.27±3.67 and mean patellar thickness was 19.84±1.76. Width of medial articular facet was 17.50±1.88 and width of lateral articular facet was 20.82±2.72. Mean thickness of median ridge was 7.48mm±1.37 (Table 1).

Measurements of right and left patella, their t-value and p-value were analyzed. In present study, there was no significant difference in measurements of right and left patella except width for lateral articular facet where p value is <0.01 (Table 2, Fig. 3)). In present study, according to Koyuncu's classification 86.66% patella were of class B type, 13.34% patella were of class C type (Fig. 4 & 5)) while class A type patella was not found in present study. Secondary ridge was found prominent in 6 patellae (Fig. 6) and indistinct in 28 patella of right side whereas on left side it was distinct in 8 patellae and indistinct in 18 patellae (Fig. 7).

Table 1: Mean values of patellar measurements

Parameters	Mean (mm)	Standard Deviation (SD)
Patella Height	39.53	5.58
Patella Width	41.27	3.67
Patella Thickness	19.84	1.76
Width of medial articular Facet	17.50	1.88
Width of lateral articular Facet	20.82	2.72
Ridge Thickness	7.48	1.37

Table 2: Mean, maximum & minimum values along with t and p-values of different parameters of patella

Parameters	Right patella (N=34)			Left patella (N=26)			t-value	p-value (significant p<0.05)
	Mean ± SD (mm)	Maximum (mm)	Minimum (mm)	Mean ± SD	Maximum (mm)	Minimum (mm)		
Patella height	40.51±2.96	45.02	35.08	38.24±7.68	48.86	15.73	1.46	0.16
Patella width	42.04±2.69	46.70	38.06	40.26±4.51	47.13	33.53	1.74	0.10
Patella Thickness	20.10±1.48	23.10	18.14	19.49±2.05	23.42	16.43	1.25	0.22
Width of Medial Articular Facet	17.28±1.91	19.62	13.90	17.78±1.86	20.24	13.93	0.72	0.48
Width of Lateral Articular Facet	21.43±2.22	25.45	17.70	20.03±3.13	23.96	15.18	2.63	**0.01***
Ridge Thickness	7.65±1.46	9.91	4.67	7.25±1.22	9.32	5.68	1.08	0.30

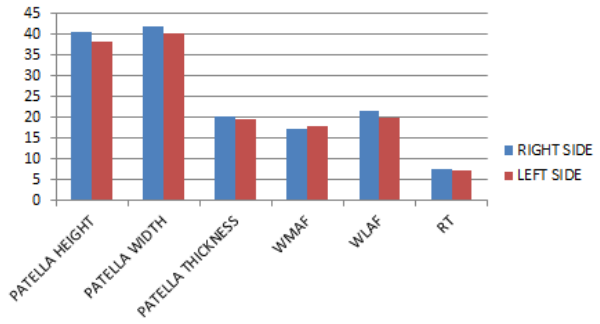

Fig. 3: Measurements of different parameters of patella on right and left side

Fig. 4: Class C type patella: width of medial articular facet is more than width of lateral articular facet

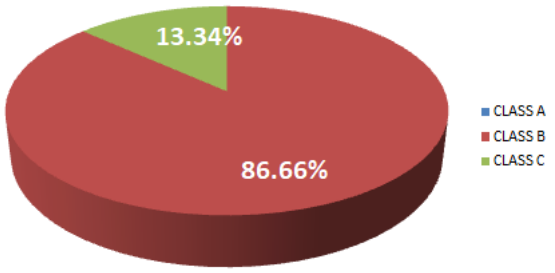

Fig. 5: Classification of patellae according to Koyuncu's classification

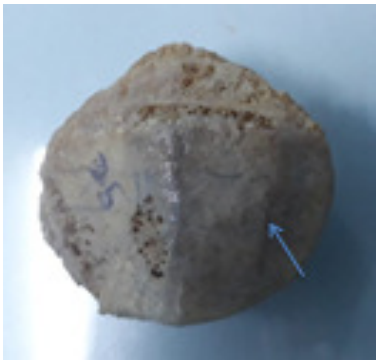

Fig. 6: Conspicuous secondary ridge (arrow)

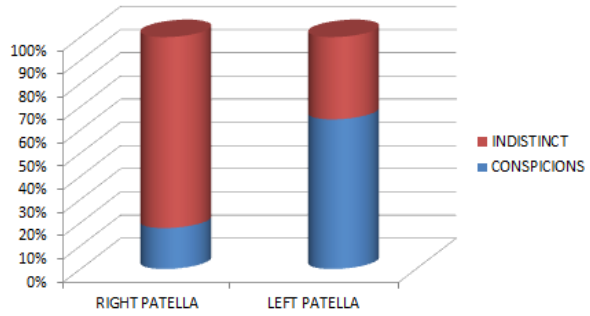

Fig. 7: Prominence of secondary ridge in patellae of right and left side

DISCUSSION

In previous studies, different measuring techniques were studied, like measurements taken with use of radiographs and measurements taken during knee arthroplasty. But all of these methods have some limitations and have more chances of errors. In our study, measurements have been taken on bones which are totally cleared from other muscles and tissues to avoid measurement errors. These measurements could be very beneficial for surgeries of knee and for anthropological records.

In present study, mean patellar height was 39.53mm±5.58, mean patellar width 41.27mm±3.67 and mean patellar thickness was 19.84mm±1.76 which was similar to values reported by Vohra (2017) [12] but less than the findings of Yoo et al. (2007) [4] Schlenzka and Schwesinger (1990) [13], Iranpour et al. (2008) [14], Oladiran et al. (2013) [15] and Kayalvizhi et al. [16]. Morphometric measurements of patellae of present study was higher than the study of Agnihotri et al. (2013) [2] (Table 3, Fig. 8).

Table 3: Comparison of patellar dimensions with previous studies

Authors	Patella Height		Patella Width		Patella Thickness	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Yoo et al. (2007) [4]	44.6	3.7	45.8	3.6	22.3	1.9
Schlenzka and Schwesinger (1990) [13]	54.4	3.5	50.3	4	-	-
Iranpour et al. (2008) [14]	34.3	4.8	44.8	4.8	22.4	2.3
Baldwin and House (2005) [17]			46.1		22.6	
Oladiran et al. (2013) [15]	43.7	3.6	45.1	3.9	23.9	2.1
Kayalvizhi et al. [16]	42.9	4.8	42.1	3.1	19.7	1.1
Agnihotri et al. (2013) [2]	35.8	-	37	-	16.95	-
Vohra P (2017) [12]	41.55	2.84	40.17	2.59	19.29	1.52
Present study (2018)	39.53	5.58	41.27	3.67	19.84	1.76

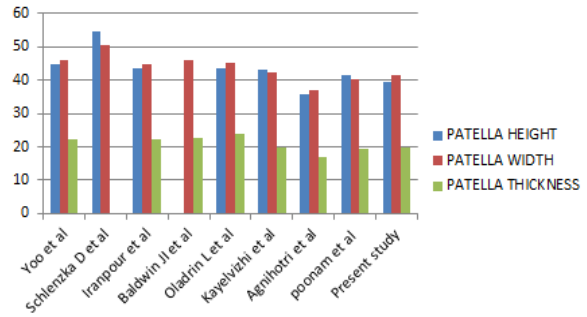

Fig. 8: Comparison of patellar dimensions with previous studies

In present study, mean of width of medial articular facet was less than the mean width of lateral articular facet. On comparison of WLAF of right and left side, significant difference was found ($p < 0.05$). WMAF < WLAF was also reported by Oladiran et al. (2013) and Murugan et al. (2017) [15,18] (Table 4).

Table 4: Comparison of width of medial and lateral articular facets

Authors	Width of medial articular facet			Width of lateral articular facet		
	Mean ± SD	t-value	p-value	Mean ± SD	t-value	p-value
Oladiran et al. (2013) [15]	20.38 ± 3.36	0.54	0.59	26.02 ± 2.68	0.54	0.59
Murugan et al. (2017) [18]	18.78 + 1.95	0.28	0.78	22.75 + 2.66	0.97	0.33
Present study (2018)	17.50 ± 1.88	0.72	0.48	20.82 ± 2.72	2.63	.01

In the present study, the width of the medial articular facet was 17.50 mm which was almost similar to the studies done on other populations like Chinese [19], Koreans [4] and Westerns [17] in whom the values of WMAF were 19.03mm, 18.4mm and 18.8mm respectively. Similarly the width of the lateral articular facet in present study was 20.82mm while 25.1mm in Chinese [19], 23.3mm in Koreans [4] and 25.3mm in Westerns [17] respectively.

In a study about the classification of patellae of fetal cadavers, Koyuncu et al. (2011) reported that 20% of patellae were in class A (width of medial articular facet and lateral articular facet were equal). Class B (the width of the MAF was smaller than the width of LAF) was reported as the most prevalent i.e. 50% while 30% of patella was of type C (width of MAF was greater than width of LAF) [6]. In the present study, it was found that the Type B patella was the most prevalent, which is in support of previous observations by Fucentese et al. (2006) and Murugan et al. (2017) [18, 20].

The secondary ridge was found to run obliquely in a generally longitudinal direction closer to the median ridge proximally than distally. This ridge may develop after birth in response to functional loads applied to knee. The secondary ridge was less prominent compared to the primary patellar ridge. There is considerable individual variation in prominence of the secondary ridge and in present study the secondary ridge was found to be conspicuous in 23.3% patellae while Agnihotri et al. (2013) reported it in 14% cases [2]. Variations in morphometric values of patella; reported by various authors, might be due to geographical and racial variation.

CONCLUSION

Present study on morphometry of the patella is obviously very important from a biomechanical perspective. Although the sample size of present study is less but the concluded parameters and their relevant statistical analysis, can be variably used in Anthropology and anthropometrics, comparative Anatomy and evolutionary Biology, patellofemoral unit prosthesis synthesis, patellar implants, arthroplasty, biomedical and biomechanical applications, Surgical and radiological indices, orthopedics and arthroscopy, rheumatology, and forensic sciences.

REFERENCES

1. Standring S. Gray's Anatomy. The Anatomical Basis of Clinical Practice.40th ed. p 1394.
2. Agnihotri G, Kaur R, Kalyan GS. Patellar shape, nose pattern and facet configuration in 200 North. Int J Curr Res Rev. 2013; 5 (14): 30-35.
3. Miller TT, Staron RB, Feldman F. Patellar height on sagittal MR imaging of the knee. AJR Am J Roentgenol. 1996; 167(2): 339-41.
4. Yoo JH, Yi SR, Kim JH. The geometry of patella and patellar tendon measured on knee MRI. Surg Radiol Anat. 2007; 29(8): 623-8.
5. Potage D, Duparc F, D'Utruy A, Courage O, Roussignol X. Mapping the quadriceps tendon: an anatomic and morphometric study to guide tendon harvesting. Surg Radiol Anat. 2015; 37(9):1063-7.
6. Koyuncu E, Cankara N, Sulak O, Özgüner G, Albay S. The morphometry of patella and patellar ligament during the fetal period. Clin Anat. 2011; 24(2):225-31.
7. Gunardi AJ, Brennan SL, Wang Y, Cicuttini FM, Pasco JA, Kotowicz MA, Nicholson GC, Wluka AE. Associations between measures of adiposity over 10 years and patella cartilage in population-based asymptomatic women. Int J Obes. 2013; 37(12): 1586-9.
8. Denaro V, Forriol F, Di Martino A, Papalia R. Modifications of patellar articular cartilage and subchondral bone following immobilisation and denervation: a histologic and morphometric in vivo study. J Orthop Trauma. 2004; 5(1): 44-9.
9. Kelly MA. Patellofemoral complications following total knee arthroplasty. Instructional course lectures. 2000; 50: 403-7.
10. Martín MD, Santiago FR, Calvo RP, Álvarez LG. Patellofemoral morphometry in patients with idiopathic patellofemoral pain syndrome. Eur J Radiol. 2010; 75(1): 64-7.
11. Lund F, Nilsson BE. Radiologic evaluation of chondromalacia patellae. Acta Radiol Diagn. 1979; 21(3): 413-6.
12. Vohra P. Morphometric study of patella and its role in sex determination. International Journal of Anatomy Physiology and Biochemistry. 2017; 4 (3):6-9.
13. Schlenzka D, Schwesinger G. The height of the patella: An anatomical study. E J Radiol. 1990; 11:19–21.
14. Iranpour F, Merican AM, Cobb JP, Amis AA. The width: thickness ratio of the patella: An aid in knee arthroplasty. Clin Orthop Relat Res. 2008; 466:1198-1203.
15. Oladiran I, Olateju OI, Philander I, Bidmos MA. Morphometric analysis of the patella and patellar ligament of South Africans of European ancestry. S Afr J Sci. 2013; 109(9-10): 01-6.
16. Kayalvizhi I, Arora S, Dang B, Bansal S, Narayan RK. Sex determination by applying discriminant functional analysis on patellar morphometry. MH; 42(4.8): 38-1.
17. Baldwin JL, House CK. Anatomic dimensions of the patella measured during total knee arthroplasty. J Arthroplasty. 2005; 20: 250–257.
18. Murugan M , Ambika S, Nim VK. Knee Cap: A morphometric study. Int J Anat Res. 2017; 5(1):3556-59.
19. Shang P, Zhang L, Hou Z, Bai X, Ye X, Xu Z, Huang X. Morphometric measurement of the patella on 3D model reconstructed from CT scan images for the southern Chinese population Chin Med J. 2014;127(1):96-101.
20. Fucentese SF, Von Roll A, Koch PP, Epari DR, Fuchs B, Schottle PB. The patellar morphology in trochlear dysplasia- A comparative MRI study. Knee, 2006; 13:145-150.

Original Article

FETAL LIVER MORPHOMETRY AT DIFFERENT CROWN RUMP LENGTHS

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ABSTRACT

Introduction: Normal morphometric parameters of liver in human fetus were studied at various stage of development with an aim to get insight into the normal development of fetal liver.

Material & Methods: Dissection of 29 human fetuses was performed. Anthropometric study was done and various parameters were measured.

Results: The development of liver is associated with increase in all the parameters although the increase is highly variable in different gestational age, indicating that liver development showed characteristic but highly regulated development to reach its fully developed stage.

Conclusion: The present study will be helpful in understanding the normal fetal liver development.

Keywords: Fetus, liver, development, anthropometry.

INTRODUCTION

Fetal liver is one of the most dynamic organs during gestational life arising from endodermal evagination of foregut and septum transversum mesenchyme [1]. It performs essential functions like haematopoiesis in fetus [2]. Fetal liver size is an important parameter of growth, showing linear increase in the lobes of liver during gestational period [3]. Liver growth gets deranged in numerous conditions. Large liver for gestational age was found in numerous conditions like gestational diabetes, isoimmunization and fetal anemias, intrauterine infections, fetal heart failure, tumours, certain metabolic diseases and fetal macrosomias [3,4]. Intrauterine growth retardation (IUGR) is associated with markedly retarded liver growth in comparison to other organs [2]. Since liver growth is a good indicator of fetal growth and outcome of pregnancy, our aim is to find out the development of fetal liver grossly, studying its various parameter [5] and contribute to available literature in understanding the liver development during gestation period.

MATERIAL AND METHODS

This prospective study was conducted in the Department of Anatomy, Government Medical College, Haldwani. Twenty nine dead fetuses with no external anomaly were obtained from Obstetrics and Gynecology Department, Dr Sushila Tiwari hospital over a period of two years (period commencing from 2011 to 2013), with due regard on ethical ground. Fetuses were preserved in formalin and various gross features were studied as per study design. The crown rump length (CRL) of the fetus was recorded. Fetuses were dissected and liver was displayed (Fig. 1). Various measurements were recorded using measuring tape, compass and vernier caliper in centimeter (cm) rounded off to second decimal place. The liver was exposed by making transverse incision extending from the umbilicus to the mid axillary line bilaterally, and two vertical incisions, extending from the costal arch to the iliac crest alongside the mid axillary line bilaterally [5].

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Liver was observed for any gross and congenital anomalies. The maximum height of right and left hepatic lobes were taken. The maximum transverse diameter of liver was taken along with maximum transverse length of right and left lobe of liver (Fig. 2). The liver was removed by dissecting inferior vena cava and excising ligaments of the liver and after that its weight was recorded before embalming. The length and breadth of quadrate and caudate lobe were also measured. The length and breadth of porta hepatis were measured too. The results were tabulated and analysed.

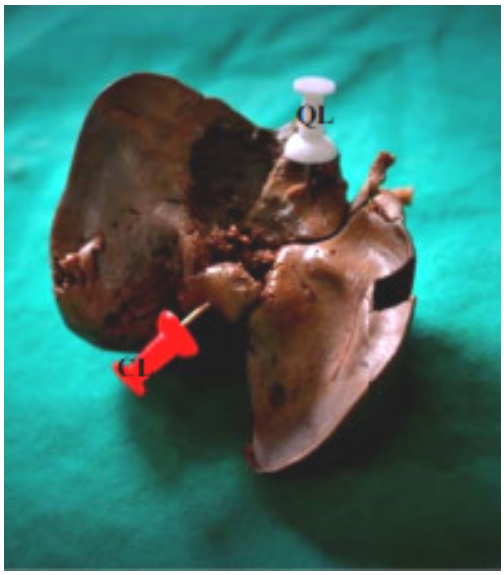

Fig. 1: Photograph of dissected fetal liver showing Quadrate lobe (QL) and Caudate lobe (CL)

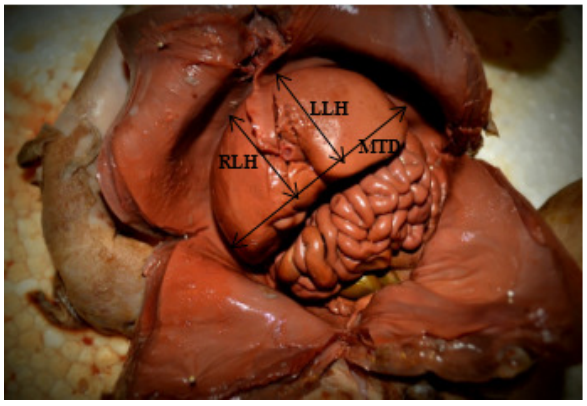

Fig. 2: Photograph of dissected fetal liver showing liver Right lobe height (RLH), Left Lobe height (LLH) and maximum transverse diameter (MTD)

OBSERVATIONS AND RESULTS

The various gross measurements of fetal liver were studied. The measurement showed marked variations of its length and breadth; however a tendency of increase in various parameters was established with increasing CRL.

The maximum liver transverse diameter (MLTD) was plotted against CRL and showed that the MLTD corresponded well to the increasing CRL (Fig. 3). The transverse diameter of right and left lobes and the height of the right lobe of liver increased in relation to CRL of fetus. When the transverse diameter of right lobe compared with that of left lobe in relation to CRL, it has been noticed that there was increasing tendency of transverse diameter of right lobe. The left lobe transverse diameter was almost similar from 13.5cm up to 28cm CRL and then it became considerably less as compared to the right lobe. This indicates that there is increasing tendency of right lobe enlargement as compared to left lobe (Fig. 3). When height of right lobe and left lobe was compared at 28cm CRL, height of right lobe was higher as compared to left lobe. Both right and left lobe height followed almost similar pattern from 6.5cm up to 25cm CRL, after that at 28 cm CRL, a difference of height of right and left lobe was observed.

When observation of length and breadth where graphed for quadrate, its breadth did show a variation till 18.5 cm CRL however, after 19 cm CRL both its length and breadth followed almost similar pattern running parallel in relation to CRL (Fig. 4). When its length and breadth were graphed, we did not notice much of the difference in early part of observation, however; its breadth was found to be increased subsequently. When length and breadth of caudate lobe were graphed together initially its length shows a tendency of increasing pattern, but after 17 CRL both length and breadth grew almost parallel to the base line. When measurement of length of quadrate and caudate lobes were compared together, marked variation was observed (up and down) in the caudate lobe length from 6.5 CRL to 16cm CRL, whereas a gradual rise in tendency was observed in the length of quadrate lobe as compared to the length of the caudate lobe. At 28cm CRL the quadrate lobe was slightly more as compared to caudate lobe length (Fig. 4).

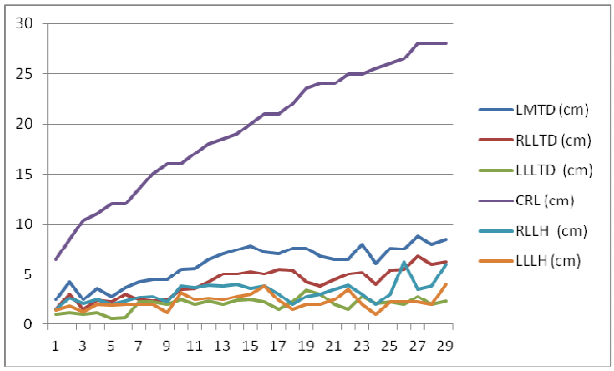

Fig. 3: Graph showing liver maximum transverse diameter (LMTD), right lobe liver transverse diameter (RLLTD), crown rump length (CRL), right lobe liver height (RLLH), left lobe liver transverse diameter (LLLTD), left lobe liver height (LLLH)

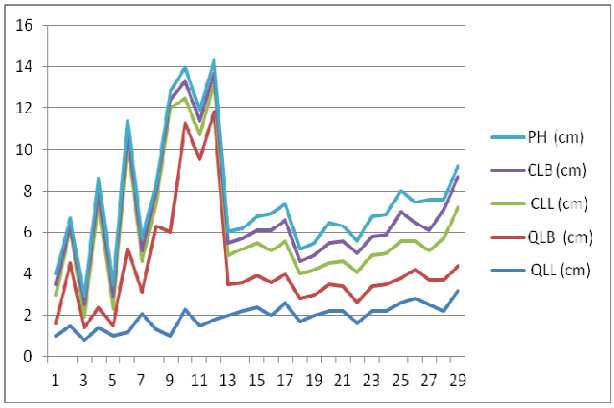

Fig. 4: Graph showing Caudate liver lobe length (CLL), Caudate liver lobe breadth (CLB), Quadrate liver lobe length (QLL), Quadrate liver lobe breadth (QLB), length of porta hepatis (PH)

DISCUSSION

Adult liver is an abdominal organ mainly localized to right hypochondrium with minimal extension on to left side however fetal liver is markedly different in different trimesters of fetal life. The liver shows different shape in different trimester varying from triangular, square, trapezoid to rectangular type, while near term fetus resembles adult shape [5]. Lee et al. (2003) measured the thickness, height and width of 12 late stage human fetus and found that the fetal liver shows great individual variation with fetal liver showing ellipsoid or oval surface while adult liver was triangular [6]. The marked variations in different parameters may occur because of expansion of abdominal capacities and organization of various viscera which require to be accommodated in the abdominal cavity. The present study has been done keeping in view of the development of liver during fetal period. Gross studies on fetal liver were found to be few in literature.

Various anatomical variations, congenital anomalies and pathologies are associated with intrauterine life. Knowledge of normal rate of growth of various liver parameters is essential to find out these causes and therefore has marked clinical significance. In previous studies, fetal liver length were studied using ultrasound on fetuses older than 2nd trimester [7,8]. In our study, however, we measured, height, transverse diameter of left, right, caudate and quadrate lobe between CRL corresponding to 2.5 and 5.6 weeks of gestation. All these parameters show largely a linear increase with gestational age.

In our study, height of both right and left lobe of liver showed almost similar pattern of increase from 6.5cm upto 25cm CRL, while at 28 cm right lobe of liver was found to have greater height. Fetal liver height shows marked difference in various pathologies, in fetuses with gestational diabetic mothers it was found to be higher than normal [4], while it was markedly lower in small-for gestational age (SGA) foetuses [9],emphasizing importance of knowing the normal range of the fetal liver size [5].

The maximum liver transverse diameter (MLTD) in our study corresponded well to the increasing CRL. Both right and left lobe transverse diameter increased in relation to CRL but in later stage, right lobe transverse diameter exceeded that of left side. Fetal liver length has marked clinical significance. Tongprasert et al. (2011) studied on 640 normal pregnant women between 14 and 40 weeks of gestation and found that fetal liver length was gradually increased with gestational age and fetal liver length may be a useful tool in assessment for some fetal pathologic conditions like fetal anemia [10].

Albay et al. (2005) detected a linear increase in the width of the left and right lobes with respect to gestational age and found that ratio between right and left lobe sizes did not show significant change throughout the gestation [5].

Our study showed increasing tendency of right lobe enlargement as compared to left lobe especially in later stage of fetal growth. Baruah & Choudhury (2013) stated that the left lobe of liver is large in fetal liver [1]. The height of both lobes increased during growth however right lobe showed more growth as compared to left lobe.

Quadrate lobe showed variation in breadth early in our study, as compared to length which almost showed almost similar pattern running parallel in relation to CRL. The length and breadth of caudate lobe showed a tendency of increasing pattern early up to 17cm CRL. Not much data was found on the anthropometric measurement of caudate and quadrate lobe measurement. Albay et al. (2005) demonstrated a high positive correlation between the size of the caudate and quadrate lobes and gestational age [5].

Fetal liver get markedly affected in various pathology like intrauterine growth retardation, gestational diabetes, infections and other conditions and remains an important parameter in there follow-up as shown by different studies [4,11]. This study highlighted that various liver parameters increase in relation to CRL but individual parameters show marked variation and their proper understanding is required to rule out any anatomical variation, congenital anomaly or pathology.

Conflict of interest

All authors have none to declare.

REFERENCES

1. Baruah P, Choudhury PR. Tongue-like elongation of the left lobe of liver. OA Case Reports. 2013; 2(17):161.
2. Murao F, Takamiya O, Yamamoto K, Iwanari O. Detection of intrauterine growth retardation based on measurements of size of the liver. Gynecol Obstet Invest. 1990; 29: 26-31.
3. Roberts AB, Mitchell JM, Pattison NS. Fetal liver length in normal and isoimmunized pregnancies. Am J Obstet Gynecol. 1989; 161: 42-6.
4. Fleischer AC, Manning FA, Jeanty P, Romero R. Sonography Principles of Obstetrics and Gynecology and Clinical Applications. In: Yuksel A, Turkish editor. Ankara, Turkey: 5th press; 2000. p. 109-130, 411-32.
5. Albay S, Malas MA, Cetin E, Cankara N, Karahan N. Development of the liver during the fetal period.Saudi Med J. 2005; 26(11):1710-5.
6. Lee SD, Kim CY, Cho YH, Fujiwara D, Murakami G, Mutsumura H, Lee SK, Kim JH, Jin ZW, Cho BH . Morphometrical data of size and shape of late stage human fetal liver, including these of intrahepatic vessel. Some prenatal and postnatal developmental consideration. Korean J HBP Surg. 2003; 7:12-18.
7. Murao F, Takamori H, Aoki S, Hata K, Hata T, Yamamoto K. Ultrasonographic measurement of the human fetal liver in utero. Gynecol Obstet Invest. 1987; 24: 145-150.
8. Murao F, Takamori H, Hata K, Hata T, Kitao M. Fetal liver measurements by ultrasonography. Int J Gynaecol Obstet. 1987; 25: 381-385.
9. Roberts AB, Mitchell JM, McCowan LM, Barker S. Ultrasonographic measurement of liver length in the small for-gestational-age fetus. Am J Obstet Gynecol. 1999; 180:634-8.
10. Tongprasert F, Srisupundit K, Luewan S, Tongsong T. Normal length of the fetal liver from 14 to 40 weeks of gestational age. J. Clin. Ultrasound. 2011; 39: 74–7.
11. Ghidini A, Sirtori M, Romero R, Yarkoni S, Solomon L, Hobbins JC. Hepatosplenomegaly as the only prenatal finding in a fetus with pyruvate kinase deficiency anemia. Am J Perinatol. 1991; 8: 44-46.

Original Article

ANATOMICAL STUDY ON PRESENCE OF MULTIPLE ACCESSORY WHARTON'S DUCT AND ITS CLINICAL IMPORTANCE

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ABSTRACT

Introduction: Submandibular salivary glands are paired salivary glands that lie below the mandible on each side. The gland is drained by a single submandibular duct or Wharton's duct. It opens in the summit of the sublingual papilla .The duct lies between the lingual nerve and hypoglossal nerve on hyoglossus muscle. The aim of this study is to describe the multiple accessory submandibular duct or Wharton's duct.

Material & Methods: A total of 28 submandibular regions were used during routine dissection classes of 1st year MBBS students in year 2016-2018, to demonstrate the anatomical variations and presence of multiple accessory Wharton's duct. This study was conducted inJaipur National University Institute for Medical Sciences and Research Centre, Jaipur,Rajasthan, India.

Results: In our study, total incidence of variation was 14.2%. Wharton's duct or submandibular duct was double in 7.1%, three submandibular ducts were present in 3.5% and four submandibular ducts were present in 3.5% specimens.

Conclusion: Appreciation of these variations is very important in diagnostic and therapeutic techniques. Awareness of variations of the accessory ducts can help surgeons during oral surgical procedures.

Keywords: Submandibular gland, submandibular duct, variation, accessory, sialolithiasis.

INTRODUCTION

Submandibular salivary glands are paired salivary gland that lie below the mandible on each side .They consist of a larger superficial and smaller deep part,continuous with each other around the posterior border of mylohyoid muscle. It is a seromucous but predominantly serous gland [1,2].

The gland is drained by a single submandibular duct or Wharton's duct [2-4] and is about 5 cm long.It begins from numerous tributaries in the superficial part of the gland and emerges from the medial surface of this part of the gland at the posterior border of the mylohyoid. It bends sharply at the posterior margin of mylohyoid to form the genu of submandibular ductthen it runs forwards between mylohyoid and hyoglossus to open in the anterior part of floor of the mouth (linguogingival) on the summit of the sublingual papilla on each side of the frenulum of the tongue [1]. The duct lies betweenthe lingual nerve and hypoglossal nerve on hyoglossus muscle but it presents an intimate relation with the lingual nerve.At first the lingual nerve lies above the duct then crosses its lateral side and finally ascends medially winding round the lower border of the duct [1,2].

Embryologically, the submandibular gland develops in fifth to sixth week of intrauterine life. Primordial cords arise in the anterior alveololingual groove and grow in the floor of the mouth. Primordial

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cells proliferate into the underlying mesenchymal tissue, the duct branches repeatedly during this evagination process. Condensation of the deep cervical fascia produces the external capsule [5].

Most of the accessory submandibular ducts are detected incidentally during sialography [6-8]. Sialography is a diagnostic procedure of choice for the detection of structural variations of submandibular gland and duct including trauma, inflammatory disorders and calculi [9,10].

In this study, these variations were found by dissection of the cadavers.The cadavers are important report sources that aim to demonstrate the structural differences inspecimensof the human body.

Awareness of variations of salivary gland duct can aid in the accurate diagnosis and treatment, it helps surgeons to avoid duct laceration during oral surgery and further complications.

The aim of this study was to describe the anatomical variation of multiple accessory submandibular duct and its morphological features.

MATERIALS AND METHODS

The present study was conducted in the Anatomy department of Jaipur National University Institute for Medical Sciences and Research Centre, Jaipur, Rajasthan, India, to demonstrate the anatomical variations and presence of multiple accessory Wharton'sduct.

A total of 28 submandibular regions ofadult embalmed cadavers were dissected and studied during routine dissection classes for 1st MBBS students in year 2016-2018. The cadavers had no signs of trauma or operation in submandibular region. First of all, marginal mandibular incision was made from mastoid process to the chin and midline skin incision in neck from chin to the sternum was given to reflect the skin inferolaterally [11].

Wharton's duct and multiple accessory Wharton'sducts were explored and examined for its emergence, course and its opening in oral cavity. The details of the variation were recorded and photographed.

OBSERVATIONS AND RESULTS

Incidence of multiple accessory submandibular ducts was found in 4 submandibular regions i.e. total incidence was14.2%.Two submandibular ducts were found in 2 cases (7.1%). Three submandibular ducts were found in 1 case (3.5%) and four submandibular ducts were also found in 1 case (3.5%). Variations were in the disposition and location of the multiple accessory submandibular ducts. Two submandibular ducts i.e. duplication of ducts was noted in two submandibular glands one on right side and one left side. Emergence, course and opening of accessory ducts were carefully noted. These ducts were almost parallel to each other, passing between the hyoglossus and mylohyoid muscles. In this, one duct was smaller and superior in position and second duct was larger and inferior in position. The superior duct was crossed laterally by the lingual nerve. Both ducts emerged independently from the deep part of the gland and opened separately into the floor of the mouth. The main duct opened at the sublingual papilla. The accessory duct opened adjacent to the main duct (Fig. 1). One submandibular salivary gland of left side had three submandibular ducts. These three ducts were arising independently from the deep part of submandibular gland. They were almost parallel to each other, passing between hyoglossus and mylohyoid muscles. The main duct (2nd) was middle in position and accessory ducts were located superior and inferior to it. The 3rd duct was narrowest, longest and inferior most in position. The lingual nerve was crossing lateral side of superior duct (1st) and middle duct (2nd) then passed between middle and inferior ducts.The upper two ducts opened into sublingual papilla and the inferior duct opened adjacent to the main sublingual papilla (Fig. 2). One submandibular salivary gland of left side had four submandibular ducts. These ducts were emerging separately from deep part of the gland. They were different in length and thickness. The 1st, 2nd and 3rdaccessory ducts were thinner and smaller. The location of 4th duct or main duct was inferior most and it was longest and thickest.The lingual nerve was crossing lateral side of 1st, 2nd and 3rd ducts then it passed between 3rd and 4th duct. The main duct was opening in the sublingual papilla and rest three accessory ducts opened independently into lingulogingival groove up to second molar tooth (Fig. 3).

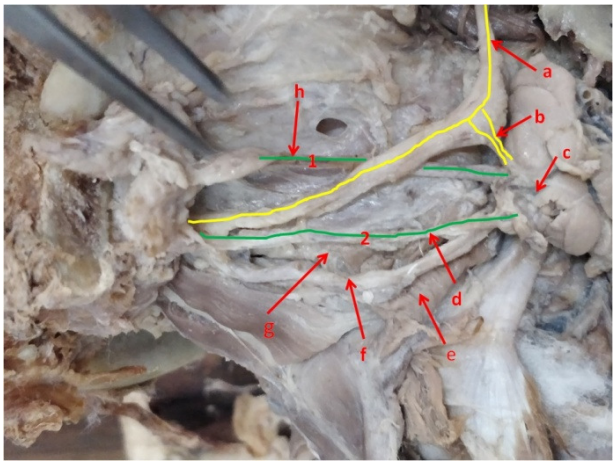

Fig. 1: Photograph showing submandibular region of left side, having two submandibular ducts (1 & 2). (a) Lingual nerve (b) Submandibular ganglion (c) Deep part of the submandibular gland (d) Accessory submandibular duct (2nd) from deep part of gland (e) reflected part of Mylohyoid muscle (f) Hypoglossal nerve (g) Hyoglossus muscle (h) Main submandibular duct (Wharton's duct) (1st) from deep part of the gland

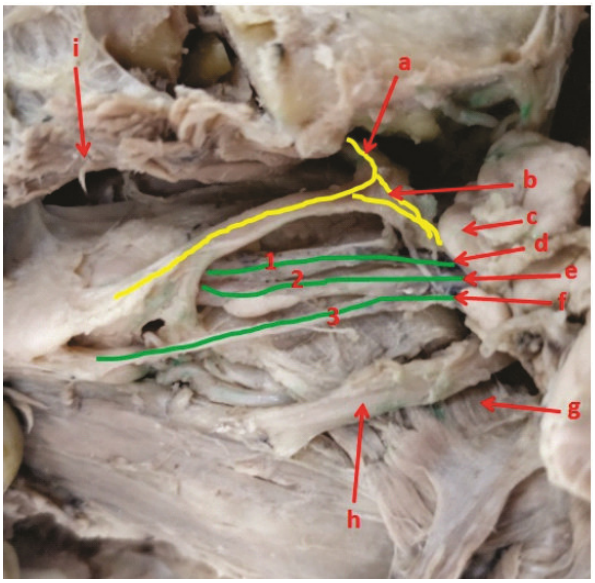

Fig. 2: Photograph showing submandibular region of left side, having three submandibular ducts (1,2 & 3). (a) Lingual nerve (b) Submandibular ganglion (c) Deep part of the submandibular gland (d) 1st is accessory submandibular duct (e) 2nd is Main submandibular duct (Wharton's duct) (f) 3rd is accessory submandibular duct (g) Hyoglossus muscle (h) Hypoglossal nerve (i) Reflected part of Mylohyoid muscle

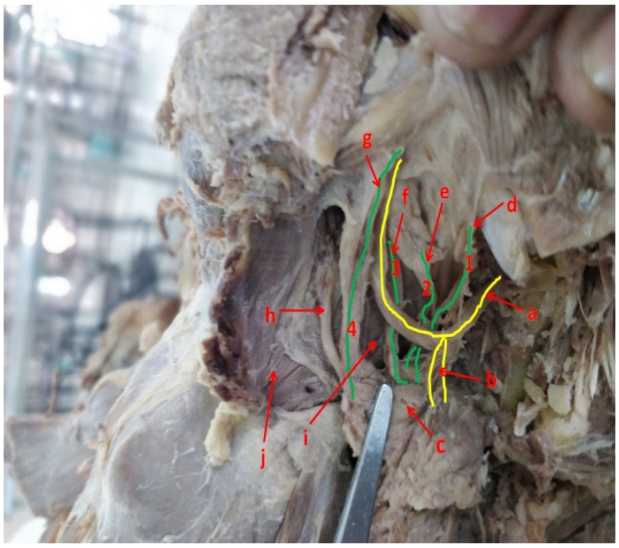

Fig. 3: Photograph showing submandibular region of left side, having four submandibular ducts (1-4) (a) Lingual nerve (b) Submandibular ganglion (c) Deep part of the submandibular gland (d) 1stwas accessory submandibular duct (e) 2nd was accessory submandibular duct (f) 3rd was accessory submandibular duct (g) 4th was Main submandibular duct (Wharton's duct) (h) Hypoglossal nerve (i)Hyoglossus muscle (j) reflected part of Mylohyoid muscle

DISCUSSION

The most frequent disorders that require surgical excision of the submandibular gland and submandibular duct are chronic inflammation of the gland with or without sialolithiasis and it requires anatomical knowledgeand careful protection of the surrounding structures.

Billakanti (2016) presented a case of accessorysubmandibular duct in which both the main and accessory ducts opened separately into the floor of the mouth on the right side of the frenulum of the tongue [12]. Kuroyanagi et al. (2007) observed duplication of the submandibular duct via sialographic examination [6]. In our study, two parallel submandibular ducts were found in two cases; both ducts emerged independently from the deep part of submandibular gland and opened separately into the floor of the mouth. In one case, the upper duct was crossed inferomedially by the lingual nerve and in second case the lower duct was crossed inferomedially by the lingual nerve.

Gaur et al. (1994) described a right submandibular gland with three separate ducts which opened independently into the oral cavity.The authors

considered that the duct branches in an arboreal fashion, increasing in number and decreasing in caliber. If multiple primordia develop it would lead to the formation of multiple ducts, the glandular tissue of these primordia if closely placed and compactly covered by connective tissue, should form a single submandibular gland having multiple ducts,each were opening separately in the mouth [13]. In our study, we found one submandibular gland with three separate ducts. These three ducts were arising independently from the deep part of the gland. The three ducts were almost parallel to each other. The main duct was larger and middle in position and accessory ducts were slightly narrower. The upper two ducts opened into sublingual papilla and the inferior duct opened adjacent to the main sublingual papilla.

Rare variations of duct arrangement may occur. Rose (1932) described a case in which Wharton's duct bifurcate, with one end opening into the sublingual papilla and a second into the mouth opposite the second molar [14].

Arquez (2017) observed the excretory duct of the left submandibular gland that had an external location that ascends and crosses vertically off the body of mandible. At its termination it was divided into four ducts that had separate openings into the oral cavity, upon a small papilla independently, opposite to the first and second lower molar crown [15]. In our study, we found one submandibular gland with four separate ducts. These ducts were emerging separately from deep part of the gland. They were different in length and thickness.The location of main duct was inferior most and it was longest and thickest. The main duct was opening as usual in the sublingual papilla and rest three accessory ducts opened independently into lingulogingival groove up to second lower molar tooth.

Awareness of the possibility of multiple ducts or variations in the position of ducts is important for surgeons and radiologists. Radiolucent stones or any other pathology present in the accessory duct may be overlooked if sialography is not performed into each of these ducts. Majority of salivary calculi are radio-opaque and are seen on plain X-ray film, about 20%of these are radiolucent [13]. Silography is regarded as diagnostic procedure of choice for the detection of various conditions of the salivary glands including trauma, inflammatory disorders and calculi [12].

The literature revealed previous reports of anatomical variations of the submandibular duct [6,16-18]. The presence of double duct is important for a technique in which transfer of double duct (microvascular autologus mandibular gland transfer) is done for treating bilateral dry eyes [19].

This study may be useful for the sialography, sialoendoscopy and submandibular gland transfer. The good knowledge of anatomy is important for surgeons to reduce the injury of nerve in this area.

CONCLUSION

The presence of anatomical variations and multiple accessory Wharton's ducts were studied. We found duplication of submandibular duct in two cases, three submandibular ducts in one case and four submandibular ducts in one case. Appreciation of these variations is very important in diagnostic and therapeutic techniques. Awareness of variations of the excretory ducts can help surgeons during oral surgical procedures.

REFERENCES

1. Standring S. Gray's Anatomy- The Anatomical Basis of clinical practice. Oral cavity. 41st ed. Spain, Elsevier Limited. 2016, pp. 527-531.
2. Datta AK. Essential of Human Anatomy- Head & Neck. Submandibular and Parotid Region. 5th ed. Kolkata, Current Books International. 2009, pp. 140-142.
3. Norman S. Williams. Bailey & Love's- Short practice of surgery. Disorder of the salivary glands. 26th ed. London, New York, CRC Press Taylor & Francis Group. 2013, p. 726.
4. Josef E. Fischer. Fischer's Mastery of Surgery. Surgery of the Submandibular and sublingual salivary glands. Vol. 1, 6th ed. Gurgaon, Wolters Kluwer India Pvt. Ltd. 2012, pp. 298-300.
5. Patten BM. Human Embryology. 2nd ed. New York, McGraw Hill. 1953.
6. Kuroyangi N, Kinoshita H, Machida J, Suzuki S, Yamada Y. Accessory duct in the submanibular gland. J Oral Maxillofac Surg Med Pathol. 2007; 19:110-12.
7. Codjambopoulo P, Ender-Griepekoven I, Broy H. Bilateral duplication of the submandibular gland and the submandibular duct. Rofo. 1992; 157:185-6.
8. Gadodia A, Seith A, Neyaz Z, Sharma R, Thakkar A. Magnetic resonance identification of an accessory submandibular duct and gland: An unusual variant. J Laryngol Otol. 2007; 1 21:e18.
9. Gates GA, Johns ME. Diagnostic radiology. In: Paparella MM, Shumrick DA, editors. Otolaryngology. 2nd ed. Philadelphia, Pennsylvania, USA: Saunders.1980, pp.1067-86.
10. Rice DH. Diagnostic imaging. In: Cummings CW, Fredrickson JM, Harker LA, Krause CJ, Schuller DE, editors. Otolaryngology. Head and Neck.1st ed. St Louis, Missouri, USA: Mosby. 1986, pp. 987-98.

11. Rachel K. Cunningham's Manual of Practical Anatomy. Head, Neck and Brain. Anterior triangle of the neck. Vol. 3, 16th ed. United Kingdom, Oxford University Press. 2018, pp. 51-56.

12. Billakanti PB. Accessory duct of submandibular duct of the submandibular gland. Sultan Quaboos Univ Med J. 2016; 17(1):e119-e120.

13. Gaur U, Chaudhry R, Anand C, Chaudhry S. Submandibular gland with multiple ducts. Surg Radiol Anat. 1994; 16:439-40.

14. Rose BH. Bifurcation of the submaxillary duct .Am J Surg.1932; 17:257-8.

15. Arquez HF. Anatomical variation of the submandibular gland duct. A unreported Anomaly. International Archives of Medicine. 2017; 10 (268).

16. Mori S, Wada T, Harada Y, Toyoshima S. Accessory duct in the submandibular gland. Oral Surg Oral Med Oral Pathol. 1986; 62:607-8.

17. Pownell PH, Brown OE, Pransky SM, Manning SC. Congenital abnormalities of the submandibular duct. Int J Pediatr Otorrhonolaryngol. 1992; 24:161-9.

18. Myerson M, Crelin ES, Smith HW. Bilateral duplication of the submadibular duct. Arch Otolaryngol. 1966; 83:488-90.

19. Horsburgh A, Massoud TF. The salivary duct of Wharton and Stenson: Analysis of normal variant sialographic morphometry and a historical review. Annals of Anatomy. 2013;195:238-242.

Original Article

CHRONIC EXPOSURE TO BISPHENOL A PRODUCES MORPHOLOGICAL DERANGEMENTS IN LIVER, KIDNEY AND HEART IN RATS

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ABSTRACT

Introduction: Bisphenol A (BPA), an estrogenic compound is used in the manufacturing of plastics and also as coating for the inner linings of food packaging containers. It is reported to be an endocrine disruptor and produce toxicity in various organs. Since BPA exposure mainly occurs in the form of oral ingestion in humans, the present study was performed to examine the effects produced by the chronic exposure to BPA orally on kidney, liver and heart in rats.

Material & Methods: Adult female rats of Charles Foster strain were used for the study. The rats were divided into two groups (n=6 in each group). In group 1, the rats were provided with food and water ad libitum whereas the rats of group 2 were fed with BPA containing pellets (2 µg/kg body weight/day) for 30 days. Thereafter the heart, kidneys and liver were excised and processed for histopathological study.

Results: The histopathological examination of the organs in BPA fed rats showed major structural changes which manifested as loss of normal cytoarchitecture in all the three organs. Further there was significant reduction in the number of glomeruli in kidneys, degenerative changes in liver in the form of damage to portal triad and in heart the intercalated discs were damaged.

Conclusion: Chronic exposure to BPA by oral route produces renal toxicity as loss of glomeruli; hepatotoxicity as cytoarchitecture loss and cardiotoxicity as damage of intercalated disc changes.

Keywords: Histopathological examination, **c**ytoarchitectural changes, BPA, lymphocytic infiltration.

INTRODUCTION

Bisphenol A (BPA), an endocrine disruptor is used in the manufacturing of poly epoxy resins and plastics. It is also used to coat the inner linings of food and beverage cans for preservation of edibles due to its fungicidal property [1]. BPA is a phenolic compound and is lipid soluble. It has been reported that BPA produces a number of toxic effects in experimental animals even at low doses [2-6]. BPA acts on estrogenic receptors and has been reported to produce numerous defects in experimental animals. These defects are manifested as infertility in both male and female mice [7,8], delayed onset of puberty [9], behavioural changes [10], predisposition to cancer in both male and female rats and mice [11], defects in growth and survival of neonatal mice [12] etc.

Further studies in human population have also shown the presence of BPA in the urinary samples of US and Chinese population [13-15]. Moreover studies have shown the co-relation of BPA levels with coronary heart diseases, liver defects and endocrine abnormalities in humans [16,17]. BPA can enter our body through various routes like oral ingestion, skin penetration, and nasal inhalation [18]. However,

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humans are mostly exposed to BPA through oral route by ingestion of food and beverages packed in plastic containers or cans. BPA ingested orally is absorbed from the intestine and is metabolised in liver. In liver, BPA is converted to BPA glucuronide which is water soluble and excreted through kidneys. However some of the free form of BPA which is not conjugated in liver is biologically active and being lipid soluble reaches various organs [19].

The dose of < 50 µg/kg bw/day is considered to be safe as per U.S. EPA (Environmental Protection Agency) [20]. However studies have reported toxic effects of BPA even at lower doses. In humans, the daily intake is reported to be within 50 µg/kg bw/day. It was therefore hypothesized that BPA after oral ingestion may get accumulated in the organs with time and produce changes at the cellular level in various vital organs which might affect the functioning of the body. Hence the present study was undertaken to examine the effect of chronic exposure of BPA orally on the morphology of vital organs like heart, liver and kidneys in rats.

MATERIAL AND METHODS

The present study was performed on adult female Albino rats of Charles Foster strain weighing 150–200 grams after obtaining permission from Ethical Clearance Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

The rats were kept in the animals room with temperature maintained at 25 ± 0.5 ºC, humidity (50% of RH) and light (12:12 hr light:dark). Food (Raj scientific Corporation, Varanasi) and water were provided ad libitum. The experiments were performed according to the guidelines given by Ethical Clearance Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. BPA was obtained from HiMedia Laboratory Pvt. Ltd. (Mumbai, India) and was dissolved in olive oil.

The animals were divided into two groups. In the first group (n = 6), the animals were allowed to drink tap water ad libitum for 30 days. In the second group (n = 6), the animals were fed with BPA filled food pellets (2 µg/kg/day per animal; orally) for 30 days. After 30 days, the animals were killed using excessive dose of anaesthesia (urethane) and dissected. The heart, liver and kidneys were excised and preserved in formalin (10%) for the study of histological changes.

Thereafter, the tissues were subjected to dehydration by exposing to increasing concentrations of alcohol (70% - 100%). Then the tissues were cleared and made translucent by placing them in xylol. This was followed by solidifying the tissues in molten paraffin and ribbon sections were made followed by slide formation. The slides prepared were subjected to Haematoxylin and Eosin stains. Further these slides were examined under microscope to observe for any histopathological changes.

Statistical Analysis

The semi-quantitative estimation of glomeruli was done by counting the number of glomeruli in 5 different fields and per field average was computed in both groups. All the data were pooled and mean ± SEM were calculated. The data were compared by Student's t-test for unpaired observations. p value < 0.05 was considered significant.

RESULTS

Changes in kidneys

In control group, normal cytoarchitecture of the nephrons showing numerous glomeruli in low power magnification (100x) was seen (Fig. 1a). Different diameter of tubules were also seen clearly. In higher magnification (400x), clear Bowman's capsule, vessels and healthy tubules were seen (Fig. 1b).

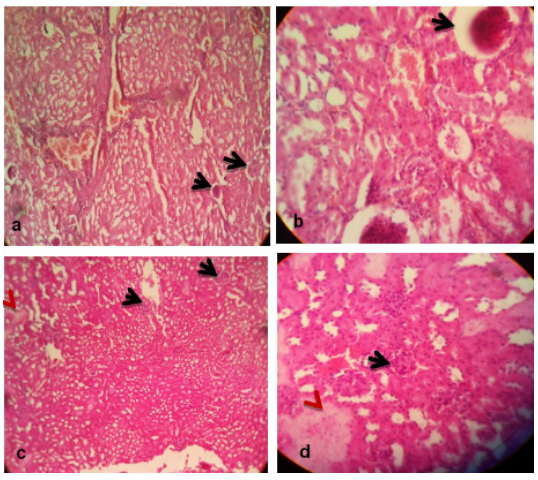

Fig. 1: Photomicrograph of HE stained kidney tissue of control rats and BPA treated rats. Normal cytoarchitecture is seen with presence of glomeruli (black arrows) in the control group (a&b). In BPA fed rats (c&d), loss of normal cytoarchitecture is seen. The glomeruli are less in number and the size of glomeruli is also reduced (black arrows). There are areas of hyaline deposits (red arrow). Magnification: 100x-a&c, 400x-b&d

In BPA treated group, in low power the glomerular morphology was not clearly seen. The glomeruli appear small in comparison to the control group and there was even reduction in the number of glomeruli along with areas of hyaline deposits. Tubular architecture was also lost (Fig. 1c). At higher magnification, hyaline deposition was clearly seen with lymphocytic infiltration and there was significant shrinkage and distortion in the structure of glomeuli (Fig. 1d).

Semi- quantitative estimation of the number of Glomeruli/field

The histological changes in the kidneys demonstrated decrease in the number of glomeruli in BPA fed animals as seen in low power. Therefore, the semi-quantitative estimation of glomeruli/field was done by counting the glomeruli in 5 different fields in both groups and then average value was taken to calculate per field number.

The observations revealed that treatment of the rats with BPA (2 µg/kg/day) caused a significant decrease in the number of Glomeruli/field as compared to the time matched control group of rats ($p < 0.05$; Student's t-test for unpaired observations) (Fig. 2).

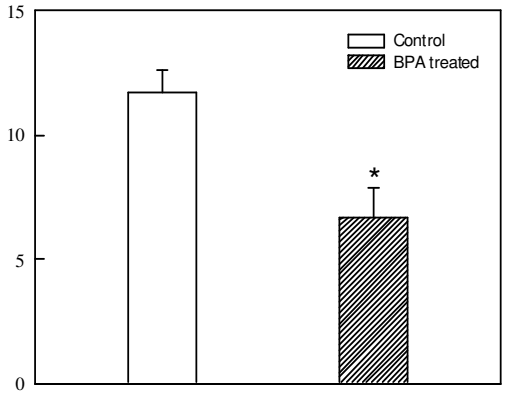

Fig. 2: Semi-quantitative estimation of gloweruli showing a significant reduction in the number of glomeruli in BPA fed rats. An asterisk represents $p < 0.05$ as compared to control group (Student's t-test for unpaired observations)

Changes in liver

In control group, the normal laminar pattern with central vein and the hepatocytes were seen under both low and high power (Fig. 3a&b). In case of BPA treated group, there was loss of the laminar pattern. There were also areas showing distinct vacuoles (Fig. 3c&d).

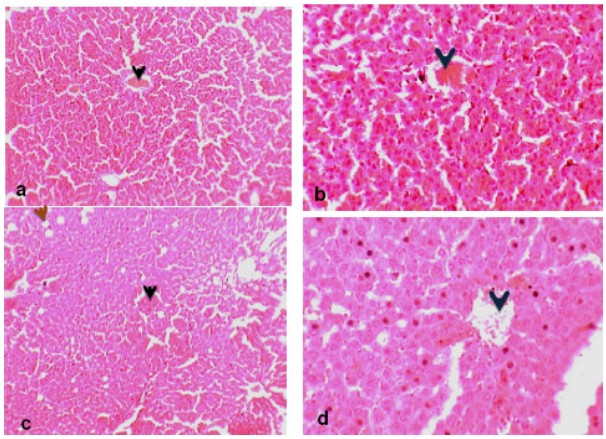

Fig. 3: Photomicrograph of HE stained liver **tissue of control rats and BPA treated rats. Central vein (black arrow) and the normal laminar pattern was seen in control group (a&b). In BPA fed group (c&d), number of small vacuoles was seen (red arrow). Loss of laminar pattern was also observed. Magnification: 100x-a&c, 400x-b&d**

Changes in heart

In control group, cardiac muscles with intercalated discs were seen (Fig. 4a). At higher magnification, striations with branched intercalated discs were seen (Fig. 4b). In the BPA treated group, broken myofibrils were seen (Fig. 4c&d).

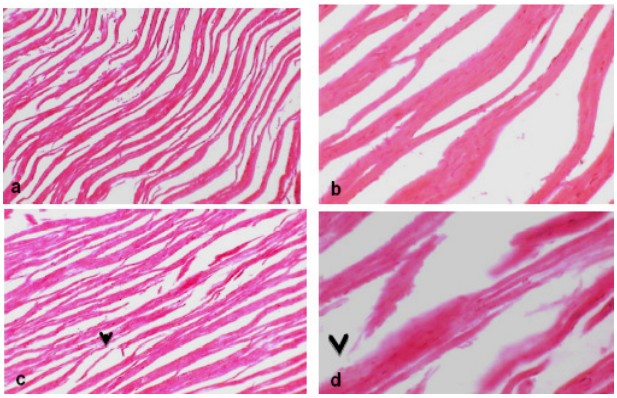

Fig. 4: Photomicrograph of HE stained heart **tissue of control rats and BPA treated rats. Control group shows normal branching pattern (a&b). In BPA fed group (c&d), there is rupture of myofibrils (black arrow) and loss of intercalated discs. Magnification: 100x-a&c, 400x-b&d**

DISCUSSION

Chronic exposure in the form of oral ingestion of BPA (2 µg/kg/day) for a period of 30 days produced extensive changes at organ level as seen on histological examination of heart, kidneys and liver. The dose selected for the present study was kept much lower than the recommended safe dose of < 50 µg/kg bw/day. Even this low dose of BPA produced cytoarchitectural changes in the vital organs in rats.

BPA after ingestion is known to be metabolised in liver by conjugation. The conjugated BPA is excreted out through the kidneys in the form of BPA-glucuronide/sulphate. The excess concentration of BPA which is not metabolised is the free BPA which is active and toxic [20].

Taking into consideration this background knowledge of BPA metabolism, the possible mechanism responsible for the cytoarchitectural changes in the rats exposed to BPA in the present study was proposed. Probably, the ingested BPA was metabolised/ conjugated by liver and excreted by kidneys in these rats. However, the free unconjugated BPA, which is lipid soluble, might have accumulated in different organs due to daily exposure of these rats to BPA. This might BPA produced cytoarchitectural damage along with colloid deposition in liver and kidneys. There was significant reduction in the number and size of glomeruli in the BPA treated rats. The morphological damage produced in the nephrons by BPA exposure is likely to decrease the excretion of BPA thereby increasing the BPA load in the treated rats. Further, damage to the hepatocytes along with degenerative changes in liver produced by BPA reduced the metabolism/conjugation of BPA. Involvement of both the kidneys and liver is expected to increase the BPA (free) load as conjugation and excretion are decreased in the treated rats which in turn might have also produced histological changes in heart seen as loss of myofibrils and breakage of intercalated disks in BPA treated rats.

In the present study, all the morphological changes occurred after exposure to BPA at a very low dose of 2 µg/kg bw/day. Exposure to BPA is increasing in the humans with increasing usage of plastic materials and packaged food and beverages. Even though the ingestion dose in humans is low, it is quite possible that this may lead to increased levels of free BPA which is toxic and may produce deleterious effects on the organs in humans similar to that in rats in the present study and lead to several diseases.

CONFLICT OF INTERESTS

The authors declare that they do not have any conflict of interests.

REFERENCES

1. Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V, Olea N. Xenoestrogens released from lacquer coatings in food cans. Environmental Health Perspectives. 1995; 103: 608-612.
2. Kiguchi M, Fujita S, Lee J, Shimizu N, Koshikawa N. Behavioral responses to methylphenidate and apomorphine in rats exposed neonatally to bisphenol A. Journal of Oral Sciences. 2007; 49: 311-318.
3. Toufexis D. Region and sex specific modulation of anxiety behaviours in the rat. Journal of Neuroendocrinology. 2007; 19: 461-473.
4. Wetherill YB, Akingbemi BT, Kanno J, McLachan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belchar SM. In vitro molecular mechanisms of bisphenol A action. Reproductive Toxicology. 2007; 24: 178-198.
5. Wetherill YB, Petra CE, Monk KR, Puga A, Knudsen KE. The xenoestrogen bisphenol A induces inappropriate androgen receptor activation and mitogenesis in prostate adenocarcinoma cells. Molecular Cancer Therapeutics. 2002; 1: 515-524.
6. Zoeller RT, Bansal R, Parris C. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. Endocrinology. 2005; 146(2):607-12.
7. Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K. Exposure to bisphenol A is associated with recurrent miscarriage. Human Reproduction. 2005; 20: 2325-2329.
8. Yamasaki K, Sawaki M, Noda S, Imatanaka N, Takatsuki M. Subacute oral toxicity study of ethynylestradiol and bisphenol A, based on the draft protocol for the "Enhanced OECD Test Guideline no. 407". Archives of Toxicology. 2002; 76: 65-74.
9. Tinwell H, Haseman J, Lefevre PA, Wallis N, Ashby J. Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. Toxicological Sciences. 2002; 68: 339-348.
10. Patisaul HB, Fortino AE and Polston EK.. Differential disruption of nuclear volume and neuronal phenotype in the preoptic area by neonatal exposure to genistein and bisphenol A. Neurotoxicology. 2007; 28: 1-12.
11. Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Research. 2006; 66(11):1–9.
12. NTP. Bisphenol A: reproduction and fertility assessment in CD-1mice when administered in the feed. NTP 1985; 85-192. Research Triangle Park, NC.

13. Calafat AM, Needham LL, Silva MJ. Lambert, G. Exposure to di-(2-ethylhexyl) phthalate among premature neonates in a neonatal intensive care unit. Pediatrics. 2004; 113: 429–434.

14. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environmental Health Perspectives. 2008; 116: 39-44.

15. He Y, Miao M, Herrinton LJ, Wu C, Yuan W, Zhou Z, Li DK. Bisphenol A levels in blood and urine in a Chinese population and the personal factors affecting the levels. Environmental Research. 2009; 109(5): 629-33.

16. Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. Journal of American Medical Association. 2008; 300: 1303–1310.

17. Melzer D, Rice NE, Lewis C, Henley WE, Galloway TS. Association of urinary bisphenol A with heart disease: evidence from NHANES 2003/06. 2010; PLoS ONE. 5(1): e8673.

18. Nisrin K, Catherine H, Henri D, Elizabeth M, Michel P. Cutaneous penetration of bisphenol A in pig skin. Journal of toxicology and environmental health Part A. 2008; 71 (8): 471-3.

19. Chapin R, Adams J, Boekelheide K, Gray L, Hayward S, Lees P, McIntyre B, Portier K, Schnorr T, Selevan S, Vandenbergh J, Woskie S. NTP-CERHR expert Panel Report on the reproductive and developmental toxicity of bisphenol A. 2008; Birth Defects Res B 83:157–395.

20. Zhuo C, Xuezhi Z, Dongliang H, Shibin D, Fangyi X, Huiqin Y, Xin J, Ying F, Li Y, Chong T, Chenjiang Y. Long-term exposure to a 'safe' dose of bisphenol A reduced protein acetylation in adult rat testes. Scientific Reports. 2017; 7: 40337.

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Original Article

LOWER RIGHT QUADRANT PAIN: A SONO ANATOMICAL PERSPECTIVE

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ABSTRACT

Introduction: Ultrasonography has been found to be highly precise in evaluation of various abdominal masses. It is highly sensitive, non-invasive in nature and has no radiation risk.

Material & Methods: A prospective study aiming at sonographic evaluation of 133 patients with right lower quadrant pain/mass which includes patients of all age groups and both sexes was planned. In this study, the efficiency of ultrasonography over clinical assessment in determination of the organ of origin was evaluated in a systematic manner according to anatomy of the region.

Results: The maximum number of cases belonged to gastrointestinal (GI) pathologies (54%) followed by genitourinary (GU) pathologies (31.6%) while 14% non-gastrointestinal and non-genitourinary origin.

Keywords: Appendicular mass, intussusception, gastrointestinal, carcinoma caecum, genitourinary, myoma, lymph node mass.

INTRODUCTION

Ultrasound has high diagnostic accuracy in diagnosing the pathological nature of right iliac fossa masses (overall accuracy 90%) [1] while it is 100% accurate in case of appendicular mass, in detecting normal cases, normal variants (clinically diagnosed as right iliac fossa masses), uterine mass, lymph-nodal mass and ileocolic intussusception. Differential diagnosis of lower right quadrant pain depends upon the affection of various organs present in right iliac and right lumbar region and their surrounding anatomical relations. Right lower quadrant pops up with plethora of pathological conditions which are either confined to this corner or encroach towards it and victimize a large number of patients. Differential diagnosis of lower right quadrant pain according to regional anatomy are gastrointestinal (GI) causes e.g. appendicular mass/abscess/mucocele, ileocaecal tuberculosis, intussusception, inflammatory bowel disease, carcinoma caecum. Genitourinary causes (GU) include tube ovarian masses and myomas. Non-gastrointestinal and Non-genitourinary causes include psoas abscess, ectopic kidney and post-traumatic masses. Ultrasonography (USG) has been found to be highly precise in evaluation of various abdominal masses [2] including the gastrointestinal, genitourinary and masses from various other systems. Because of its high sensitivity, non-invasive nature, lower cost, general accessibility and no radiation risk, it is widely used as a diagnostic tool. It not only provides image of mass independent of its and its vicinity organ function but also pioneers as method of choice for guided biopsies and fine needle aspirations [3]. It has been a boon to the pregnant patients by the virtue of lacking radiation risk.

MATERIAL AND METHODS

This is a prospective study of sonographic evaluation of patients with lower right quadrant pain/right iliac fossa (RIF) masses which includes patients of all age groups and both sexes. A total number of 133 patients

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referred from various clinical departments with pain/suspected RIF mass were included in the study and evaluated.

USG was done on ACCUSON 300 XE PREMIUM EDITION sonography machine with curvi-linear array transducer with frequency of 2-5 MHz, linear array transducer with frequency of 8-10 MHz and endovaginal probe of frequency 7 MHz in uterine and tuboovarian masses. Prior to performing USG, a verbal informed consent was obtained from patients. Detailed relevant history taking of patients was followed by thorough general, physical and abdominal examination before subjecting patients to USG examination.

Technique of Examination

Scanning was done in longitudinal and transverse directions covering all the areas of interest. For kidney and retroperitoneum, patients were also scanned in prone and lateral positions. Graded compression technique was utilized with exerting gentle compression with the high frequency transducer using both hands in same way when palpating abdomen. This technique displaced gas in bowel producing artifacts and precisely located the region of pathology by maximal tenderness if present. On gray scale sonography, following things of masses were evaluated: location, organ of origin, characteristics of mass (size, shape, margin echotexture, and calcification) and relation to adjacent organs.

This was followed by color Doppler examination with color flow mapping using low flow settings. The confirmation of data was done by fine needle aspiration cytology and biopsy, further radiological imaging (CT, barium study, intravenous urography, non-radiological tests, clinical and ultrasonographic) follow up.

Statistical Analysis

The sensitivity of detecting RIF mass by USG was 90.76% and it is 100% specific (accurate in detecting true negative). The positive predictive value of USG was 100% in detecting masses correctly i.e. 100% accurate in detecting true presence of mass. The negative predictive value was 20%. Shows predictive value of negative test is 20%.

OBSERVATIONS AND RESULTS

It was found that USG is 100% efficient in detecting psoas abscess, intra-abdominal abscess, parietal haematoma and lymph node mass whereas it is 75% accurate in detecting parietal abscess as compared to clinical examination which can detect psoas abscess to 75% accuracy and intra-abdominal abscess to 50% accuracy, but it can only detect one third of prietal abscess, parietal haemetoma and lymph node mass. In case of localized collection, where USG can detect one third of confirmed cases, clinical examination was unable to detect.

According to symptoms especially pain and presence/absence of mass in the affected area, clinical assessment and further sonographic evaluation was done to detect the organ of origin as per the anatomy of lower right quadrant of abdomen mainly right iliac region, partly right lumbar and right iliac region (Table 1-4, Figs. 1-8). In the present study, maximum number of cases was found in 30-45 years age group (39%) followed by 15-30 years age group (35.4%), 0-15 years age group (10%) and 45-60 years age group (12%). Least number of patients was seen above 60 years of age group (3%). Male to female ratio was found to be 1:1.5. The common presenting symptom in all patients was pain in abdomen followed by vomiting and fever in almost 44- 45%, rest in line were GI complaints followed by menstrual irregularities (Table 5). Though the mass was palpable in 68.4% cases.

Table 1: Comparison of efficiency of USG and clinical assessment in correctly detecting the organ of origin in gastrointestinal pathologies in RIF

Type of mass	No. of confirmed cases	Percentage	No. of cases detected by organ of origin	
			By USG	By Clinical Evaluation
Appendicular mass	52	72.2%	52	42
Carcinoma caecum	2	2.7%	2	0
Colitis	10	13.8%	9	8
Ileocolic intussusception	3	4.16%	3	2
Ileocaecal tuberculosis	3	4.16%	2	1
Carcinoma colon	2	2.7%	1	0
Acute mesenteric lymphadenitis	2	2.7%	2	2
Total	72	100%	70	55

Table 2: Comparison of efficiency of USG and clinical assessment in correctly detecting the organ of origin in genitourinary pathologies in RIF

Type of mass	No. of confirmed cases	Percentage	No. of cases detected by organ of origin	
			By USG	By Clinical Evaluation
Right sided ovarian mass	22	51.1%	22	12
Uterine mass	7	16.2%	7	4
Renal mass	2	4.65%	2	0
Left sided ovarian mass	1	2.32%	1	0
Tuboovarian mass	5	11.6%	4	1
Right sided ectopic pregnancy	2	4.65%	2	2
Ruptured graffian follicle	2	4.65%	2	0
Ectopic iliac right kidney	2	4.65%	2	0
Total	43	100%	42	19

Table 3: Comparison of efficiency of USG and clinical examination in correctly detecting the organ of origin in non-GI and non-GU pathologies in RIF

Type of mass	No. of confirmed cases	Percentage	No. of cases detected by organ of origin	
			By USG	By Clinical Evaluation
Right psoas muscle abscess	4	100%	4	3
Intra-abdominal abscess	4	100%	4	2
Parietal abscess	4	100%	4	1
Parietal haematoma	3	100%	3	1
Lymph nodal mass	2	100%	2	1
Localized collection	1	100%	1	0
Total	18	100%	1	7

Table 4: Detection of palpable RIF masses by USG

	Mass Present	Mass Absent	Total
Ultrasound +ve	118	0	118
Ultrasound –ve	12	3	15
Total	130	3	133

Table 5: Distribution of frequency of symptomatology in patients with RIF mass

Symptom (n=133)*	No of cases	Percentage
Pain in abdomen	100	100%
Fever	44	33.2%
Mass discovered by the patient	29	21.8%
Vomiting	44	33.0%
Gastrointestinal complaints (diarrhea, constipation, blood in stools)	37	27.8%
Menstrual irregularities	18	13.5%
Loss of appetite	23	17.2%
Weight loss	10	7.5%
Abdominal distension	6	4.5%
Bladder complaints (increase or decrease frequency of micturition, burning micturition, hematuria)	8	6.0%

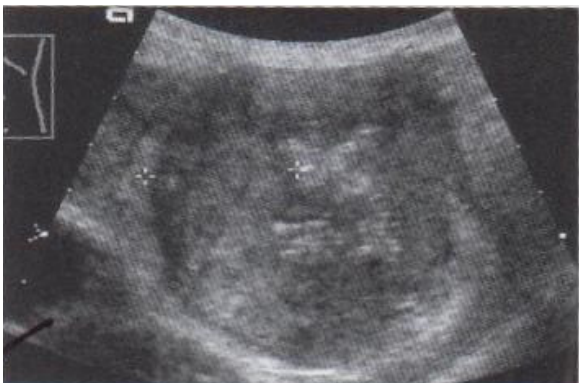

Fig. 1: USG showing Carcinoma caecum

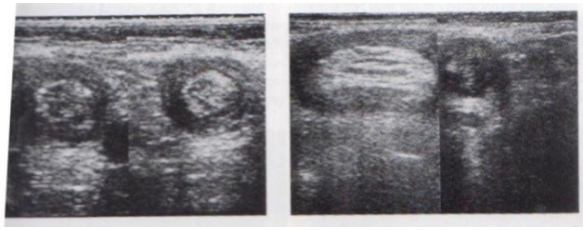

Fig. 2: USG showing intussusception & Gut signature intussusception

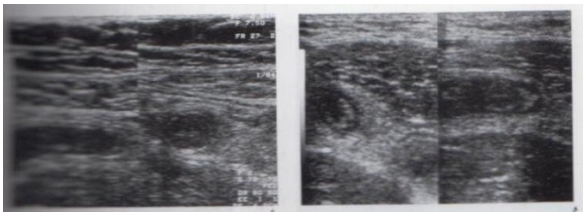

Fig. 3: USG showing appendicitis

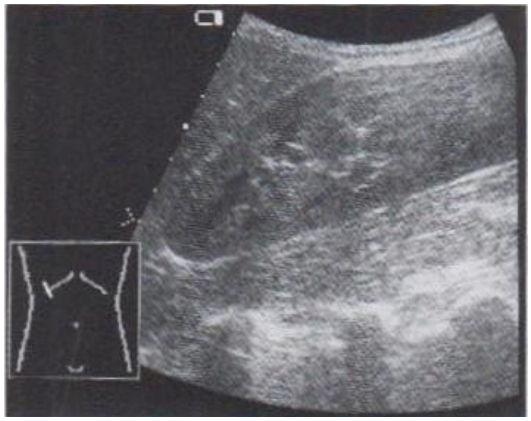

Fig. 4: USG showing caudally fused horse shoe kidney

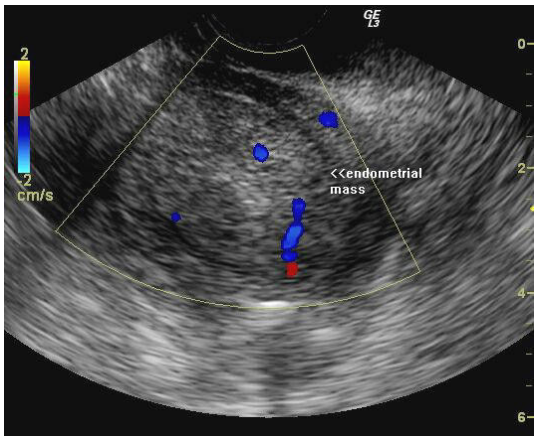

Fig. 5: USG showing Rt. lateral uterine wall myoma

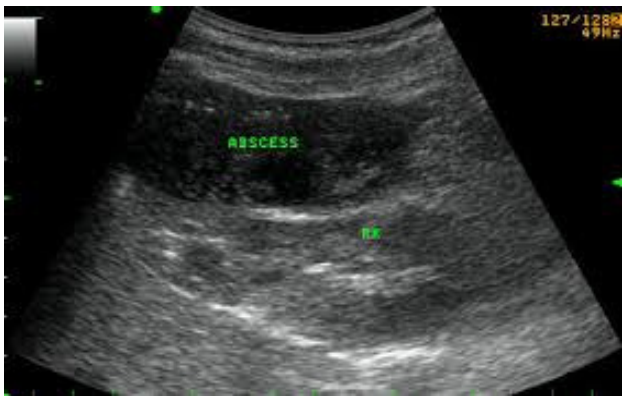

Fig. 6: USG showing Psoas abscess

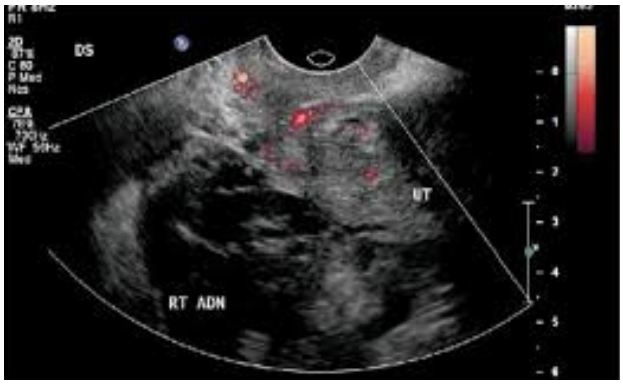

Fig. 7: USG showing heterogenous collection in pelvis secondary to ruptured ectopic gestation

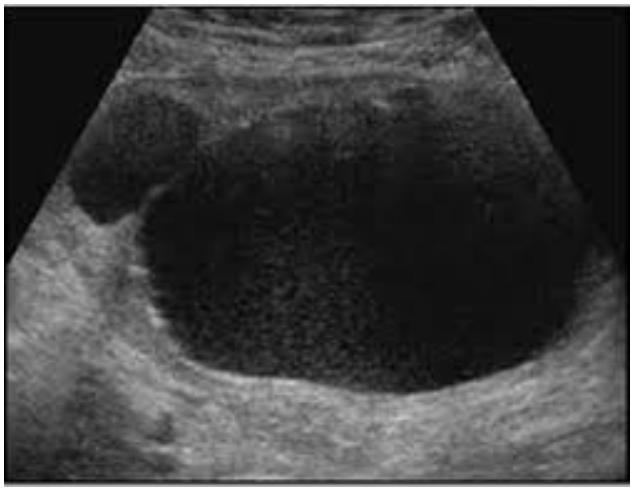

Fig. 8: USG showing Rt. ovarian echo filled cyst

DISCUSSION

The maximum number of cases belonged to gastrointestinal pathology (54%) followed by genitourinary pathologies (31.2%) and 14% cases belonged to non-GI non-GU origin. Majority of the lesions in the present study were found to be infective or inflammatory in origin followed by neoplastic cases, traumatic and congenital [2]. Other lesions include ileocolic intussuception [4], incisional hernia, ectopic pregnancy and normal cases.

Out of total of 72 cases of gastrointestinal pathologies, 52 cases were appendicular masses (Fig. 3) followed by 10 cases of colitis. Two cases of carcinoma colon and 2 of carcinoma caecum (Fig. 1) were found. Jadvar et al. (1997) noted that out of 10 cases of colitis, 9 were diagnosed as infectious and 1 was tubercular in nature [5]. Seven cases of infectious colitis showed positive stool culture for E. histolytica and 2 had positive blood culture for Campylobacter jejuni in a child [6], while remaining one case of tubercular colitis was finally diagnosed on colonoscopic biopsy and histopathology. In all the cases diagnosed as appendicular pathology, appendicitis was found to be most frequent finding which is similar to previous studies [7,8]. There were also 3 cases of ileocolic intussuception (Fig. 2) and 3 cases of ileocaecal tuberculosis. In GU pathologies out of 22 ovarian masses (Table 3), 5 were malignant, 14 benign and 3 were haemorrhagic cyst (Fig. 8). All were right sided GU pathologies except one was a huge left ovarian haemorrhagic cyst. There were 7 uterine leiomyomas extending to right iliac fossa (Fig. 5). Two cases of right adnexal ectopic pregnancy with loculated hematoma was seen extending in RIF (Fig. 7). There were 2 cases of congenital etiology which comprised of left sided caudal crossed fused renal ectopy and 1 right ectopic iliac kidney (Fig. 4). Next in number was psoas pathology which included two cases of psoas abscess (Fig. 6). USG in cases of psoas abscess revealed hypoechoic collection in right psoas muscle and bulky psoas muscle [9].

Aspiration of pus in case of abscess and haemorrhagic fluid in case of hematoma confirmed the diagnosis. The culture of the pus revealed the growth of staphylococcus. Three cases that had no masses in RIF on sonographic examination and were considered normal but clinically masses were palpated [10]. One case clinically suspected of ileocaecal tuberculosis but sonography revealed no lesion. The present study constituted appendicular masses mainly acute appendicitis [11] as maximum number of cases and

diagnosed correctly, which shows efficiency of ultrasound in diagnosing appendicular masses especially in gravid patients with symptoms of appendicitis [12]. The second most common pathology was right ovarian mass which was almost similar to previous studies [13,14] extending to RIF. Barker & Lindsell [2] and Millard et al. [3] in their studies stated that in any patient with palpable RIF mass, USG should be the primary investigation. The initial consideration in evaluation of right lower quadrant pain or mass is whether the lesion is present or not. In the present study, 3 masses were normal variant (i.e. low lying kidney) [7]. The sensitivity and specificity of ultrasound for detection of right iliac fossa mass was found to be 100% which is similar to previous studies [2,3] which also showed high sensitivity and specificity of ultrasound for detection of presence of abdominal and RIF masses respectively.

CONCLUSION

USG can detect pathologies which are inaccessible to clinical examination. It lacks hazards of radiation. It has high diagnostic accuracy in diagnosing the pathological nature of RIF masses (over all accuracy 92%) while it is 100% accurate in appendicular mass. It is also highly accurate (100%) in detecting normal cases and normal variants (low lying kidney) clinically diagnosed as RIF masses, uterine mass, lymph-nodal mass and ileocolic intussusception. USG had 100% sensitivity, specificity, positive predictivity [4] and negative predictivity and accuracy in detecting presence of mass in RIF in present study.

REFERENCES

1. Aspelin P, Hildell J, Karlsson S, Sigurjonson S. Ultrasonic evaluation of palpable abdominal masses. Acta Chir Scand, 1980; 146(7):501-506.
2. Barker CS, Lindsell DRM. Ultrasound of the palpable abdominal mass. Clinical Radiology. 1990; 41:98-99.
3. Millard FC, Collins MC, Peck RJ. Ultrasound in the investigation of right iliac fossa mass. Br J Radiol. 1991; 64: 17-19.
4. Verschelden P, Filiatrault D, Garel L, Grignon A, Perreault G, Boisvert J et al. Intussuscetion in children: Reliability of US in diagnosis-A Prospective Study. Radiology. 1992; 184:741-744.
5. Jadvar H, Mindeizun RE, Olcott EW, Levitt DB. Still a great mimicker: Abdominal Tuberculosis. AJR, 1997; 168:1455-1460.
6. Puylaert JB, Vander Zant FM, Mutsaers JA. Infectious ileocecitis caused by Yersinia, Campylobacter, and Salmonella; clinical, radiological and US findings. Eur Radiol. 1997;7(1):3-9.
7. Das S. A Manual on Clinical Surgery. 5th edition, S. Das Publishers, Calcutta, 2000; 392-394.
8. Andersson RE. Meta-analysis of the clinical and laboratory diagnosis of appendicitis. Br J Surg. 2004; 91:28-37.
9. Yadav RP, Agrawal CS, Adhikary S, Kumar M, Regmi R, Amatya R, Gupta RK. Iliopsoas abscess: Analysis and perspectives from an endemic region of Eastern Nepal. Kathmandu University Medical Journal. 2007; 5(20):497-500.
10. Standring S. Gray˝s Anatomy: The Anatomical Basis of Clinical Practice. 40th Ed., Churchill Livingstone Elseviers Publishers, London, 2008; 1125-1162.
11. Toorenvliet BR, Wiersma F, Bakker RF. Routine ultrasound and limited computed tomog raphy for the diagnosis of acute appendicitis. World J Surg. 2010; 34(10):2278-85.
12. Lehnert BE, Gross JA, Linnau KF et al. Utility of ultrasound for evaluating the appendix during the second and third trimester of pregnancy. Emerg Radiol. 2012; 19(4):293-9.
13. Malhotra A, Tarafdar S, Tayade A. Benign versus malignant adnexal masses: Does addition of Color and Spectral Doppler over and above the Gray Scale Ultrasound improves efficacy? Sch J App Med Sci. 2016; 4(1A):62-74.
14. Tarafdar S, Malhotra A, Tayade A. Acute Abdomen: Role of Ultrasonography in differentiation of common clinical mimics of appendicitis. GJRA. 2016; 5(4):20-23.

Original Article

HISTOLOGICAL VARIATIONS IN FALLOPIAN TUBE DURING VARIOUS PHASES OF OESTRUS CYCLE

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ABSTRACT

Introduction: The fallopian tube is the vital part of reproductive system where the fertilization of ova takes place. It also acts as a transporting channel for passing the ova and the products of conception to the uterus. To perform these vital functions fallopian tubes undergo various histological variations during various phases of oestrus cycle.

Material & Methods: Histology of fallopian tubes of 50 female rabbits were studied by using H & E staining. Study of the cytology of vagina of the animal decided the phase of the oestrus cycle i.e. proestrus, oestrus, metaestrus and diestrus phases by Papaniculou's staining.

Observations and Results: The study revealed histological changes in relation to various phases of oestrus cycle. Epithelial surface area was maximum in oestrus phase. Secretory cells were most active in metaestrus and diestrus phases. Cilliary cells were prominent in proestrus and oestrus phases. The vascular activity was found maximum in oestrus phase.

Keywords: Fallopian tube, oestrus cycle, proestrus, oestrus, metaestrus, diestrus, phases.

INTRODUCTION

The fallopian tube was first identified as a distinct entity from the uterus by Greek anatomist Fallopius (1561) in sheep and human and so named after him. However the detailed discussion of the intricacies of oviduct was only taken up by Williams (1891). Since then studies of fallopian tube have made a steady progress. An extensive and detailed study of the human reproductive biology has attracted worldwide attention with a view to stop, check or control the population explosion in the world. About 30% of infertile women all over the world have associated fallopian tube pathology. Now-a-days with the introduction of IVF, this aspect of infertility has been neglected [1].

The fallopian tube is an important part of female reproductive system that receives the ovum, provides appropriate environment for its fertilization and transports it to the uterus. Not only this, the tubal fluid provides nutrition and conducive environment to the sperm for fertilization. To perform these vital functions the fallopian tube undergoes histological and histochemical changes during various phases of oestrus cycle viz proestrus, oestrus, metaestrus and diestrus. The histological variations in these phases were studied and evaluated in relation to their functional aspect.

MATERIAL AND METHODS

Assessment of different phases of estrus cycle was done by studying vaginal smears. Vaginal smears were made from the experimental animals. The external genital parts of the female rabbit was prepared in the morning from the normal saline washings, the saline moistened cotton swab was inserted in vagina. The smear was made on the clean sterilized glass slide. It was then fixed in ethyl alcohol

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and solvent ether (1:1) for two hours and finally stained with the Papaniculou's staining. The stained slides were studied under light microscope for their cytological appearance to ascertain various phases of sexual/ estrus cycle.

For histological study, the fallopian tubes were obtained from fifty female rabbits and arranged in different phases of estrus cycle. The fallopian tubes were fixed in 10% formal saline for 24 hours. The tubes were processed and transverse thin micro sections of four different parts of the fallopian tube i.e. from medial to lateral - intramural, isthmus, ampulla and infundibulum was taken. Slides were studied under light microscope after H & E staining.

OBSERVATIONS AND RESULTS

The histological changes in various cyclic phases were most marked in the infundibulum and ampulla and least in isthmus and intramural segments of fallopian tube.

Pro-Oestrus Phase (Fig. 1 & 2):

In pro-oestrus phase, the epithelium showed a papilliferous pattern. The lining cells were low columnar and cylindrical with scanty eosinophilic cytoplasm and elongated nuclei. No evidence of secretion was seen in the living cells. The submucosa showed few blood vessels. The muscle layer consisted of spindle shaped muscle fibers.

Oestrus Phase (Fig. 3 & 4):

The sections of the fallopian tube showed a qualitatively similar histological appearance in various parts of the tube. Most pronounced changes were seen in the lining mucosa, which showed an arborescent pattern with frond's and papillae. The lining cells were tall cylindrical and hypertrophied with eosinophilic cytoplasm and elongated oval, round and spheroid nuclei. At some places the prominent hypertrophied ciliary cells were discemable. No evidence of secretion was seen in the cells. The submucosa showed vascularized stromal tissue. The muscular layer consisted of elongated spindle cells.

Meta-Oestrus Phase (Fig. 5 & 6)

In this phase, the lining mucosa showed columnar cells with cytoplasm showing bleb formation and protrusions towards the luminal surface. The luminal border of the lining epithelium showed fraying with secretion. The lamina propria showed mild vascularization. The muscular coat showed no significant changes.

Di-Oestrus Phase: (Fig. 7 & 8)

The lining mucosa showed low columnar and cuboidal cells with scanty cytoplasm and bare nuclei, suggestive of secretory activity. The ciliary cells were not discernable. The rest of the changes resembled with that of meta-oestrus phase.

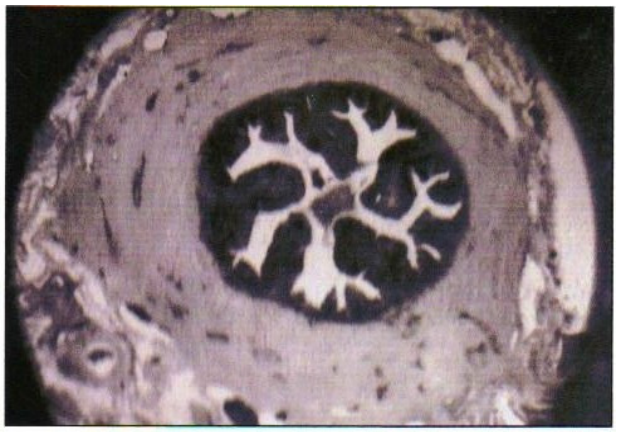

Fig. 1: Photomicrograph showing coarser bundles of circular smooth muscle fibers and poorly formed primary folds in intramural part of fallopian tube in proestrus phase (H&E X I5O)

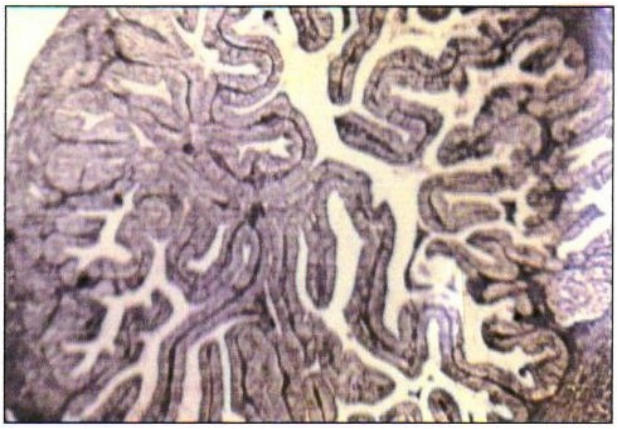

Fig. 2: Photomicrograph showing few isolated smooth circular muscle fibers, arborising pattern primary, secondary and tertiary folds with height proportionally greater than width, slender appearance and larger epithelial surface area in infundibular part of fallopian tube in proestrus phase (H&E X I5O)

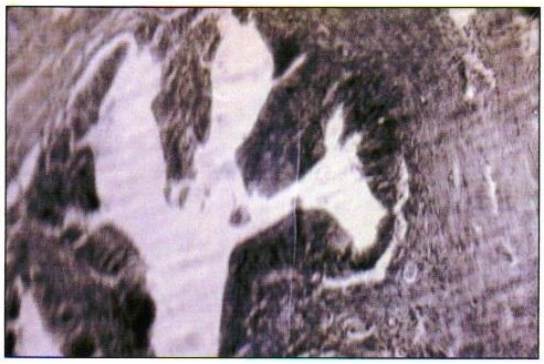

Fig. 3: Photomicrograph showing dense, packed and abundant serosal connective tissue with minimal epithelial surface area in intramural part of fallopian tube in oestrus phase (H&E X 270)

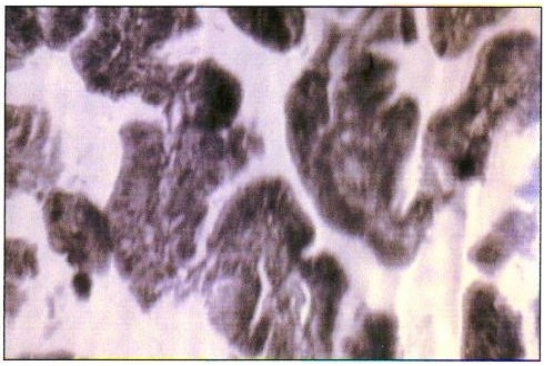

Fig. 4: Photomicrograph showing loose and scanty serosal connective tissue, epithelium is highly developed with extensive surface area, tall columnar secretory cells are inactive, ciliary cells are prominent in infundibular part of fallopian tube in oestrus phase (H&E X 270)

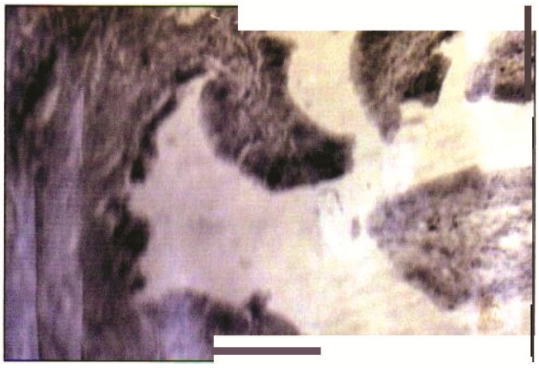

Fig. 5: Photomicrograph showing coarser bundles of circular smooth muscle fibres, dense packed serosal connective tissue and poorly formed primary folds in intramural part of fallopian tube in metaoestrus phase (H&E X 270)

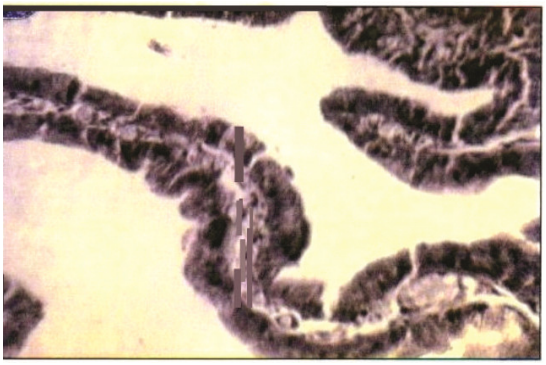

Fig. 6: Photomicrograph showing isolated smooth circular muscle fibers, arborising pattern, with moderate epithelial surface area and active secretory tall columnar cells in infundibular part of fallopian tube in metaoestrus phase (H&E X 270)

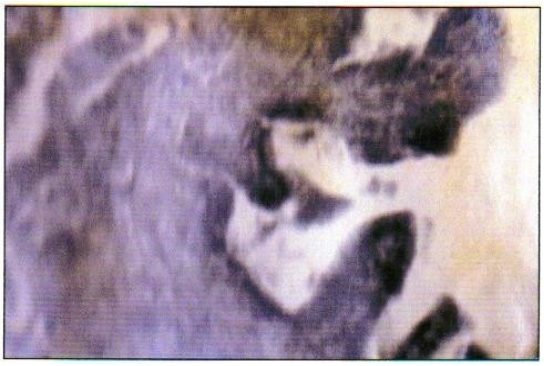

Fig. 7: Photomicrograph showing packed serosal connective tissue, coarser bundles of circular muscle fibres with minimal epithelial surface area in intramural part of fallopian tube in diestrus phase (H&E X270)

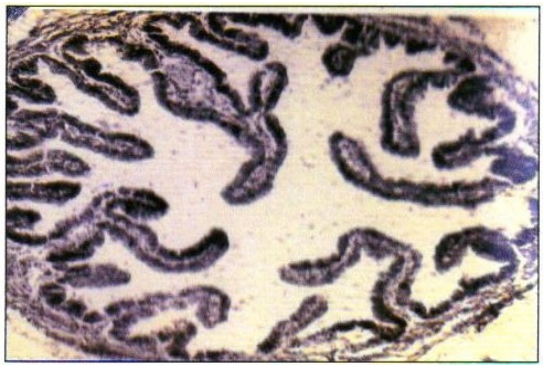

Fig. 8: Photomicrograph showing few isolated circular muscle fibers, arborising pattern primary, secondary and tertiary mucosal folds with smaller epithelial surface area and active secretory cells in infundibular part of fallopian tube in diestrus phase (H&E X120)

Histological parameters showed different findings indifferent phases of sexual cycle (Table 1).

Table 1: Histological changes in the tubal structure in different phases of oestrus cycle

Histological Parameter	Phases of Oestrus cycle			
	Pro-Oestrus	Oestrus	Meta-Oestrus	Di-Oestrus
Epithelial surface area	Large	Extensive	Moderate	Small
Secretory Cells	Inactive	Inactive	Active	Active
Ciliary Cells	Prominent	Prominent	Insignificant	Insignificant
Vessel	Vascularised	Highly Vascularised	Significant	Insignificant

DISCUSSION

The survey of literature showed that the proportion and character of secretory cells differ in different animal species and even in animals of same species and these differences depend upon the portion of tube studied and phase of the reproductive cycle represented. The present study done in rabbit showed that the epithelial surface area was greater in proestrous and oestrus phases in all segments of the tube. Kumar and Srivastava (1995) reported arborising pattern primary, secondary and tertiary mucosal folds with height proportionately greater than width and slender appearance in infundibulum [2]. So the area proportionately increased with greater arborisation of the epithelial folding especially in infundibulum. This implied increased functional activity of tubal epithelium in proestrus and oestrus phases of sexual cycle.

The secretory cells in tubal epithelium were found active in metaestrus and diestrus phases when progesterone activity is high. In this regard, tubal secretory cells and endometrial cells behaved alike to progesterone. Some authors observed a cyclic variation in activity of secretory cells in the tube [3-5]. The secretory cells appeared low columnar and often their bare nuclei were extruded out of cytoplasm which was noticed in metaestrus phase. This finding runs parallel with that of Allen (1922) who also noted the same feature [6]. The secretion formed by secretory cells might have nutritional importance for egg. That is why the secretory cells became active after oestrus phase, when ovulation has occurred. Since embryologically tubal and endometrial epithelium are related both structurally and functionally, their identical response to the ovarian hormones is reconcilable. Changes in the shape and density of secretory granules in different phases of sexual cycle was noted by different authors [7,8]. Bjorkmann and Fredrickson (1961) observed changes in size of secretory cells in luteal phase [9].

The ciliary cells were prominent in proestrus and oestrus phases of sexual cycle. Such a periodic alteration in ciliary cells have been recorded by Iwata (1929) [10]. In human fallopian tubal epithelium, these specialized ciliated cells have hair-like projections on the free surface. With help of this structure, they move the secretions of secretory cells along the free surface of the epithelial membrane.

Any abnormality in ciliary activity of cells will affect propagation of ovum to primed uterus and might result in tubal implantation. Sowmya et al. (2014) has reported that the incidence of tubal gestation varies from 1 in 300 to 1 in 150 pregnancies and it contributes significantly to the maternal mortality and morbidity [11]. Lack of secretory activity due to any cause might lead to lack of supply of nutrition and proper milieu to fertilized ovum, thereby interfering with its maturation and development. A detail knowledge of phasial behavior will help in understanding the propagation and implantation of fertilized ovum. Early diagnosis and intervention will help in reducing the maternal mortality and morbidity due to ectopic pregnancy.

CONCLUSION

The histological study of fallopian tube revealed structural and functional changes in relation to various phases of sexual cycle. During proestrus and oestrus phases, the mucosa showed structural cellular growth attesting relation with estrogen. Likewise the functional secretory activity was greatest during metaestrus and diestrus phase suggesting relation with progesterone. This secretory activity seems to bear nutritional importance for passing ova through the tube. The ciliary cells, prominent during oestrus and proestrus

phases, may be helpful in flushing the secretions along the tube.

REFERENCES

1. Briceag I, Costache A, Purcarea VL, Cergan R, Dumitru M, Briceag I, Sajin M, Ispas AT. Fallopian tubes - Literature review of anatomy and etiology in female in infertility. J Med Life. 2015; 8(2): 129-131.
2. Kumar V and Srivastava A. Histological differences in various segments of rabbit's fallopian tube. J Anat Sci. 1995; 14 (2): 22-28.
3. Casida LE and Mc Kenzie FF. The oestrus cycle in the ewe: Histology of the genital tract. Mo Agr Exp Stat Res Bull No. 170; 1932
4. Mc Kenzie FF and Terrill CE. Estrus, ovulation and related phenomena in the ewe. Univ. Miss Agr Exp Stn Res Bull No. 264; 1937.
5. Roark DB and Herman HA. Physiological & Histological phenomena of the bovine estrual cycle with special reference to vaginal - cervical secretions. Miss Agric Exp Stn Res Bull No. 455; 1950.
6. Allen E. The estrous cycle in the mouse. Am J Anat. 1922; 30: 297-348.
7. Nilsson 0. Influence of oestradiol on the ultrastructure of the mouse uterine epithelium. Exp Cell Res. 1958a; 14:434-435
8. Nilsson 0 and Reinius, S. The mammalion oviduct. 57th ed. Hafez ESE and Blandon RJ. The University of Chicago press. Chicago, London, 1969.
9. Bjorkmen N and Frederickson. The bovine oviduct epithelium and its secretary process as studied with the electron microscope and histochemical test. Z Zellforsch Mikrosk Anat.1961; 55: 500-513.
10. Iwata M. Beitrage Zur Morphology der Menschlich en Tub. Mschhr. Geburtsh.Gynak. 1929; 81: 283-299.
11. Sowmya S, Priya R, Meenakshi P, Shwetha B. Study of trophoblasts and histological changes of fallopian tube in tubal pregnancy and anatomical considerations for its early rupture. Int J Anat Res. 2014; 2(4):609-613.

Original Article

VARIATIONS OF RENAL ARTERY: A CADAVERIC STUDY

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ABSTRACT

Introduction: Renal arteries are the lateral branches of abdominal aorta, which vary in number, origin and course. The aim of this study was to document the incidence of renal artery variations in number and position, which can be advantageous to urosurgeons and imaging experts.

Material & Methods: The study was conducted on 36 formalin fixed embalmed cadavers. The renal artery variations were observed and noted during routine posterior abdominal wall dissection for medical undergraduates in dissection hall in the Department of Anatomy, BRD Medical College Gorakhpur and IMS, BHU Varanasi.

Results: We observed accessory renal artery (ies) in 30.56% of cases with unilateral incidence in 27.78% and bilateral in 2.67% cases. Accessory hilar renal artery (ies) were observed in 16.68% and accessory polar renal artery (ies) in 13.89% cases. Accessory arteries were more common on left (16.68%) than on the right side (11.12%). Inferior polar arteries were more common than superior polar arteries.

Conclusion: Knowledge of the variations of renal artery is of immense importance to the urosurgeons during renal transplantation, partial nephrectomy, laparoscopic surgery and angiographic interpretation by radiologists.

Keywords: Embalmed cadaver, accessory hilar renal artery, accessory polar renal artery.

INTRODUCTION

The kidney is a retroperitoneal organ, usually supplied by single renal artery, which is a lateral branch of abdominal aorta below the origin of superior mesenteric artery. Usually one renal artery supplies each kidney and one renal vein drains the kidney. Normally, renal artery enters into the kidney through the hilum and divides into five segmental branches. Graves (1954) gave the first detailed account of the primary pattern of renal vascular segmentation[1]. Accessory renal artery is an addition to normal single renal artery. It is called accessory hilar renal artery if it enters through the hilum in addition to the normal single renal artery and called accessory polar renal artery if entering the respective kidney through the sites other than the hilum, usually near the pole. These accessory renal arteries commonly arise from the abdominal aorta inferior to the normal level of origin of renal artery but it may also arise from the phrenic, superior mesenteric, inferior mesenteric and common iliac artery. The variations of renal artery are common in their number, positions and course[2,3]. The presence of an accessory renal artery was reported in approximately 30% of cases by many researchers[4-6] and this presence of accessory renal arteries is found associated with high failure rate and post-operative complications in renal transplantation[5]. There is a high incidence of urinary tract obstruction due to the accessory renal arteries running anterior or posterior to uretro-pelvic junction leading to hydronephrosis[7]. It is important to be aware that accessory renal arteries act as end arteries; therefore, if an accessory renal artery is ligated in the donor kidney and not vascularized subsequently, the part of the kidney

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supplied by it is likely to become ischemic[4].

Thus a detailed knowledge of variations of renal artery is helpful in preventing the failure and complications in renal vascular surgery, partial nephrectomy and misinterpretation of angiography by radiologists.

MATERIAL AND METHODS

The study was conducted in thirty six (36) formalin fixed embalmed cadavers during routine posterior abdominal wall dissection for teaching of medical undergraduates in the Department of Anatomy, BRD Medical College, Gorakhpur and Institute of Medical Sciences, BHU, Varanasi, Uttar Pradesh, India. Out of 36 cadavers, 31 were male and 5 were female. The renal area was explored upto the pelvis and the findings were noted regarding the variations of renal artery in their number, origin and course in both right and left kidney.

OBSERVATIONS AND RESULTS

The accessory renal arteries were observed in 30.56% (11/36) cases and out of these, 16.68% were accessory hilar renal arteries entering into kidney through the hilum and 13.89% accessory polar renal arteries entering into the kidney at sites other than the hilum i.e. at or near the respective poles. All sites of entry of accessory polar arteries were found on the anterior surface of the respective kidney. Double hilar arteries were reported in 11.12% cases of which 5.56% on right and 2.78% on left side and bilateral in 2.78% cases. Triple hilar arteries were reported in 5.56% cases on the left side only. Accessory superior polar artery was reported in 5.56% cases and inferior polar artery in 8.33% cases. Unilateral renal artery variations was reported in 27.78% and bilateral in 2.67% cases (Table 1, Fig. 1-4). Out of 5 females, only in one female (20%) double hilar renal artery was noted and that too on the right side.

Table 1: Incidence of accessory renal artery in present study (Out of 36 cases)

Renal artery	Right	Left	B/L	Total
Single hilar artery (Normal)	32/36 (88.89%)	30/36 (83.33%)	35/36 (97.22%)	25/36 (69.44%)
Accessory double hilar arteries	2/36 (5.56%)	1/36 (2.78%)	1/36 (2.78%)	4/36 (11.12%)
Accessory triple hilar arteries	0/36 (0%)	2/36 (5.56%)	0/36 (0%)	2/36 (5.56%)
Accessory SPA	1/36 (2.78%)	1/36 (2.78%)	0/36 (0%)	2/36 (5.56%)
Accessory IPA	1/36 (2.78%)	2/36 (5.56%)	0/36 (0%)	3/36 (8.33%)

***SPA**-superior polar artery, **IPA**- inferior polar artery*

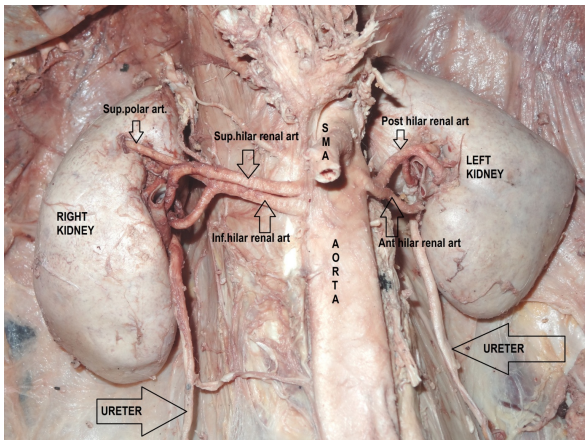

Fig. 1: Photograph showing bilateral double hilar renal arteries and inferior hilar renal artery giving a branch to superior pole of right kidney known as accessory superior polar artery (SMA-superior mesenteric artery)

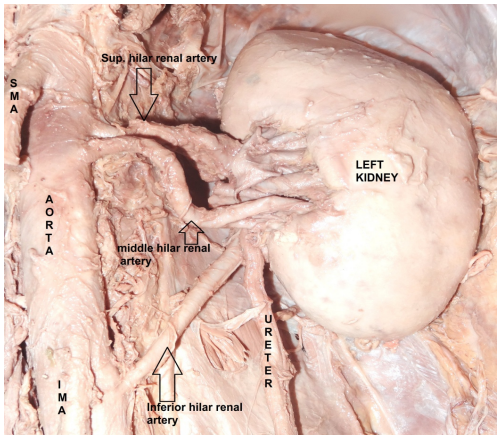

Fig. 2: Photograph showing triple hilar renal artery on left side, inferior hilar renal artery arising below the origin of inferior mesenteric artery (SMA-superior mesenteric artery, IMA-inferior mesenteric artery)

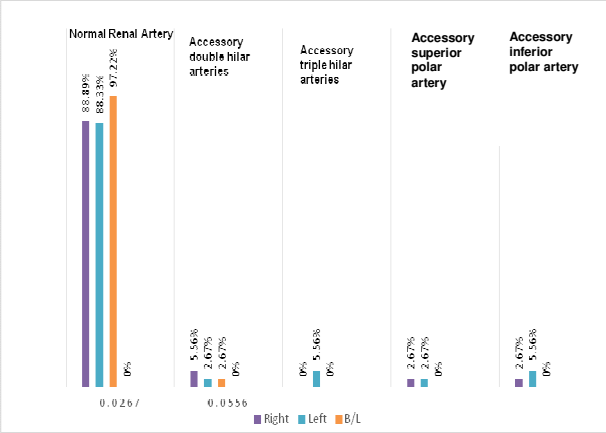

Fig. 3: Incidence of renal artery on right and left side

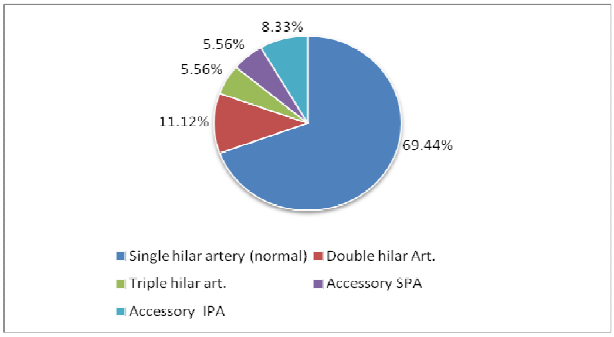

Fig. 4: Pie diagram showing incidence of renal artery on right and left side

DISCUSSION

Embryological explanation of these variations has been presented and discussed by Felix (1912) in an 18 mm fetus [8]. The developing mesonephros, metanephros, suprarenal glands and gonads are supplied by nine pairs of lateral mesonephric arteries arising from the dorsal aorta. Felix divided these arteries into three groups. The 1st and 2nd arteries as the cranial, the 3rd to 5th arteries as the middle, and the 6th to 9th arteries as the caudal group. The middle group gives rise to the renal arteries. Persistence of more than one artery of the middle group results as multiple renal arteries. Initially the metanephric kidney lies in the sacral region and subsequently with the differential growth of the abdominal wall, it ascends via the iliac fossa to its final destination in the lumbar region. The caudal arteries degenerate and one of the more proximal vessels which are closer to the final position of the kidney persist as a single renal artery on either side [9].

The failure of regression of these arteries result in accessory renal arteries. Thus, the multiple renal arteries in our study may be a result of persisting lateral mesonephric arteries from the middle group.

The various types of accessory renal arteries, their number, position and the way of entry into the kidney and its segmentation were studied extensively [2]. Bordei et al. (2004) reported 54 cases of double renal artery supplying one kidney. Out of 54 cases, 6 cases were bilateral [10]. There are reports of additional renal arteries in literature [11-13]. In our study we have observed double renal artery in 11.12% (3/36) cases, of which only one (2.78%) was bilateral.

Extra hilar artery (ies) originating from hilar renal artery (ies), have vertical trajectory in comparison to polar arteries taking origin from the aorta and this vertical trajectory can lead to polar infarction [14]. Accessory arteries to the inferior pole, running anterior to the renal pelvis or ureter may be the cause of hydronephrosis due to the obstruction to the flow of urine at pelvic-ureteric junction or the proximal part of the ureter. Reported incidence of inferior polar arteries is 15.1% and that of superior polar arteries 9.6% cases [15-17]. Kumar and Prabha (2016) observed superior polar arteries in 13.09% and inferior polar in 5.95% cases in South Indian population [18]. In our study, accessory inferior polar artery were present in 8.33% cases and superior polar artery in 5.56% cases.

Brannen et al. (1982) and Gupta et al. (2010) were in opinion that, presence of multiple renal arteries have chances of rejection and poor graft functions [19,20] but Benedetti et al.(1995) didn't find significant difference with regard to acute rejection rate in grafts with single or multiple arteries [21]. However, allografts with multiple renal arteries have risk of renal artery stenosis [22]. In renal transplantation, polar vessels can increase the failure rate due to thrombosis of renal vessels and urinary leakage [23]. It is important to be aware that the accessory renal arteries act as an end artery and if ligated, the part of kidney supplied by it is likely to become ischemic [4]. The presence of bilateral accessory renal arteries makes it technically difficult to procure the donor kidney for transplantation and also they result in increased incidence of subsequent complications.

The incidence of bilateral accessory renal arteries have been ranging from 1.66% to 10%. Studies of Dhar and Lal (2005), reported bilateral accessory renal

arteries in 5% cases [24] while in our study we noted them in 2.67% cases. Pollak et al. (1986), reported triple renal arteries in 4% and quadruple in 1% cases [25]. In our study, triple renal arteries were present in 5.56% cases and that too on left side.

Different studies showed that left side shows more variations than right [24-26]. In our study also, the left side renal artery variations were more common which is in concordance with the previous studies [24-16]. Saldarriaga et al. (2008) found no difference on left and right side [27].

CONCLUSION

The incidence of accessory renal artery are very high and more common on left side in number as well as in position. If an accessory artery cut or ligated, it will lead to damage of the respective segment of the kidney supplied by it. Thus a urosurgeon needs to be aware of the detailed knowledge of the variations of renal artery of the donor as well as of the recipient during renal transplantation. Knowledge of aforesaid variations does appear mandatory during nephrectomy, laparoscopic surgery and angiographic interpretations by radiologists.

REFERENCES

1. Graves FT. The anatomy of the intrarenal arteries and its application to segmental resection of the kidney. Brit J Surg. 1954; 42:132–139.
2. Sykes D. The arterial supply of the human kidney with special reference to accessory arteries. Brit J Surg. 1963; 50: 368-374.
3. Anson BJ, Daseler EH. Common variations in renal anatomy affecting blood supply, form and topography. Sur Gyn Obstet. 1961;112:439–449.
4. Satyapal KS, Haffejee AA, Singh B, Rasmsaroop L, Robbs J V, Kalideen JM. Additional renal arteries: Incidence and morphometry. Surg Radiol Anat. 2001; 23: 33-48.
5. Bergman RA, Afifi AK, Miyauchi R. Illustrated encyclopedia of human anatomic variations: Opus II Cardiovascular System: Arteries: Abdomen: Renal and Gonadal Arteries.
6. Vasbinder GB, Nelemans PJ, Kessels AG, Kroon AA, Maki JH, Leiner T, Beek FJ, Korst MB, Flobbe K, de Haan MW, van Zwam WH, Postma CT, Hunink MG, de Leeuw PW, van Engelshoven JM. Renal artery diagnostic imaging study in hypertension (RADISH) study group: Accuracy of computed tomographic angiography and magnetic resonance angiography for diagnosing renal artery stenosis. Ann Intern Med. 2004;141: 674–682.
7. Rao M, Bhat SM, Venkataramana V, Deepthinath R, Bolla SR. Bilateral prehilar multiple branching of renal arteries: a case report and literature review. Kathmandu Univ Med J. 2006;4:345–348.
8. Felix W. Mesonephric arteries (aa. mesonephrica). In Keibel F and Mall FP (Eds.). Manual of Human Embryology. 2nd ed. Philadelphia: Lippincott, 1912. vol. 22, pp 820-825.
9. Bremer JL. The origin of the renal arteries in mammals and its anomalies. Am J Anat. 1915;18:179–200.
10. Bordei P, Sapte E and Iliesc D. Double renal artery originating from aorta. Surg Radiol Anat. 2004;26 (6): 474-479.
11. Krishnasamy N, Rao M, Somayaji SN, Koshy S, Rodrigues V. An unusual case of unilateral additional right renal artery and vein. International Journal of Anatomical Variations. 2010; 3: 9-11.
12. Ilke, AG, Aysin K, Ozcan G, Bulent B, Ahmet U, Mahul EK, Zafer ARI. Bilateral variations of renal and testicular arteries. International Journal of Anatomical Variations. 2009; 2: 45-47.
13. Patasi B and Boozary A. A case report: accessory right renal artery. International journal of Anatomical variations. 2009; 2:119-121.
14. Beyer RW and Daily PO. Renal artery dissection associated with GZ acceleration. Aviation, Space and Environmental Medicine. 2004; 75 (3): 284-287.
15. Pick JW, Anson BJ. The renal vascular pedicle. An anatomical study of 430 body halves. J Urol. 1940; 44:411–434.
16. Shoja MM, Tubbs RS, Shakeri A, Ardalan MR, Ardabili BR, Ghabili K. Asymptomatic bilateral ureteropelvic junction obstruction due to supernumerary renal arteries. Saudi Journal of Kidney Disease and Transplantation. 2008b; 9 (5): 806-808.
17. Weld KJ, Bhayani SB, Belani J, Ames CD, Harubi G, Landman J. Extrarenal vascular anatomy of kidney: assessment of variations and their relevance to partial nephrectomy. Urology. 2005; 66:985-989.
18. Kumar U, Prabha R. Study of prehilar branching pattern of renal artery in human cadaveric kidneys. Nat J Clin Anat. 2016; 5(2):86–90.
19. Brannen GE, Bush WH, Correa RJ, Gibbons RP, Cumes DM. Microvascular management of multiple renal arteries in transplantation. The Journal of Urology. 1982; 128 (1): 112-115.
20. Gupta V, Kotgiwar S, Trivedi S, Deopujari R, Singh V. Bilateral variation in renal vasculature. International journal of Anatomical variations. 2010; 3: 53-55.
21. Benedetti E, Troppmann C, Gillingham K, Sutherland DE, Payne WD, Dunn DL, Matas AJ, Najarian JS, Grussner RW. Short and long term outcomes of kidney transplants with multiple renal arteries. Annals of Surgery. 1995; 221 (4): 406-414.
22. Kamali K, Abbasi MA, Ani A, Zargar MA, Shahrokh H. Renal transplantation in allografts with multiple versus single renal arteries. Saudi Journal of Kidney Disease and Transplantation. 2012; 23 (2): 246-250.
23. Bayazit M, Gol MK, Zorhutuna Y, Tasdemir O, Bayazit K. Bilateral triple renal arteries in a patient with iliac artery occlusion: a case report. Surg Radiol Anat. 1992; 14:81–83.

24. Dhar P, Lal K. Main and accessory renal arteries – a morphological study. Int J Anat Embryol. 2005; 110(2):101–110.

25. Pollak R, Prussak BF, Mozes MF. Anatomic abnormalities of cadaver kidneys procured for purposes of transplantation. Am Surg. 1986; 52:233–235.

26. Patil UD, Ragavan A, Nadaraj, Murthy K, Shanker R, Helical CT. angiography in evaluation of live kidney donors. Nephrol Dial Transplant. 2001;16: 1900–1904.

27. Saldarriaga B, Perez AF, Ballesteros LE. A direct anatomical study of additional renal arteries in a colombian mestizo population. Folia Morphol. 2008; 67 (2):129–134.

Case Report

THYRO-LINGUO-FACIAL TRUNK OF EXTERNAL CAROTID ARTERY: A RARE VARIATION

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ABSTRACT

The complicated process of angiogenesis and remodelling which includes annexation and regression of vessels may give rise to variations in the branches of external carotid artery. The knowledge of variations in the branching pattern of external carotid artery (ECA) is important for surgical procedures in the neck region, such as radial neck dissection, catheterization, reconstruction of aneurysm, carotid endarterectomy and intervention radiology. The anatomical consequences of anomalous branching pattern of external carotid artery may have important clinical implications. A rare, common branch of the ECA, a thyro-linguo-facial trunk was found on right side in a 65 years old male cadaver during routine dissection. After giving a branch to thyroid gland as superior thyroid artery, this trunk ran forwards and medially as linguo-facial trunk and divided into lingual and facial arteries. The hypoglossal nerve was crossing the linguo-facial trunk.

Keywords: External carotid artery, thyro-linguo-facial trunk, variation, hypoglossal nerve.

INTRODUCTION

The common carotid artery bifurcates into internal carotid and external carotid arteries at upper border of thyroid cartilage in the carotid triangle. On right side, the common carotid artery arises from brachiocephalic trunk, on left side it arises directly from the arch of aorta. The superior thyroid, lingual, and facial arteries arise from its anterior surface, the occipital and posterior auricular arteries arise from its posterior surface and ascending pharyngeal artery arise from its medial surface [1]. The rich vascularity of most parts of head and neck (except brain and eye) is mainly maintained by the external carotid artery through its branches. The external carotid artery has numerous important anastomoses with the internal carotid artery and the vertebrobasilar system, thus ensuring blood circulation in case of disturbed cerebral blood flow. Like other great vessels of neck, the external carotid artery and its branches have numerous variations and their exploration is more than interesting for a better anatomical knowledge of neck. These variations pose a dangerous situation during surgeries like thyroidectomy, laryngectomy facio-maxillary surgeries, tonsillectomy, glossectomy and other neck surgeries. It is important for the elevation of various cutaneous and myocutaneous flaps for plastic and reconstructive surgeries of the head, neck and face, which depend on the external carotid artery for their blood supply [2].

CASE REPORT

A rare, common branch of the ECA, a thyro-linguo-facial trunk was found on right side in a 65 years old male cadaver during routine dissection in the Department of Anatomy, King George's Medical University, Lucknow, UP. The history of the individual and the cause of death was not known. The topographic details of the external carotid artery was examined by casual dissection and photographed. The thyro-linguo-facial trunk was 1.6mm below the carotid bifurcation from anterior surface of right common carotid artery. After giving a branch to thyroid gland as superior thyroid artery, this trunk ran forwards and medially around 1.7mm as linguo-facial trunk and divided into lingual and facial arteries. The hypoglossal nerve was crossing the linguo-facial trunk. The lingual artery ascends vertically up crossed the internal laryngeal nerve, taking an oblique course it passed

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underneath the hypoglossal nerve and anterior belly of digastric muscle to enter digastric triangle. The facial artery passed upwards and forwards and reached posterior part of the submandibular gland (Fig. 1).

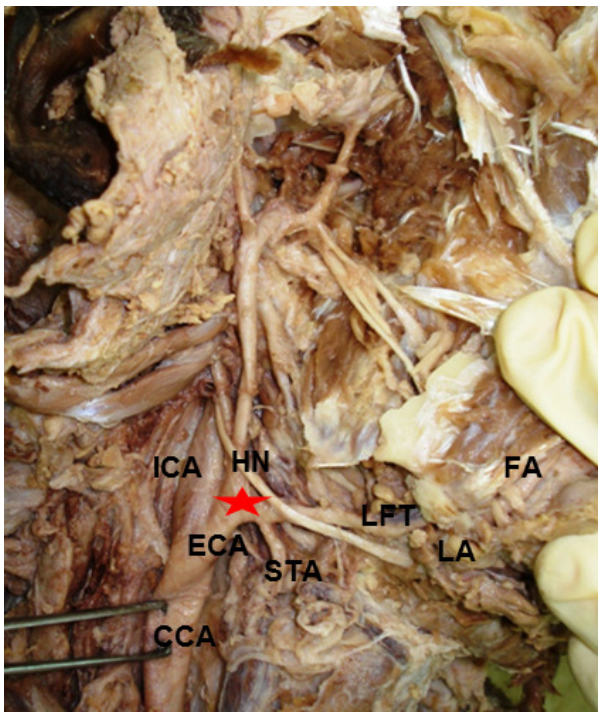

Fig. 1: Photograph showing thyro-linguo-facial trunk* (CCA-common carotid artery, ECA-external carotid artery, ICA-internal carotid artery, STA-superior thyroid artery, LFT-linguo-facial trunk, LA-lingual artery, FA-facial artery, HN-hypoglossal nerve)

DISCUSSION

External carotid artery provides the major source of blood to the head and neck region. Variations in the branching pattern of it has been reported earlier. Aaron et al. (1970) reported the percentage of variants in the branching pattern of external carotid artery based on 113 specimens dissections. He described three different types of branching pattern i.e. “ladder division”, “grouped division” and “bouquet division”. He reported that linguofacial trunk was present in 20% of the cases, thyrolingual trunk in 5.9%, thyrolinguo-facial trunk in 2.7%. Out of these “bouquet division” was present in 2.7% and “ladder division” in 27.7%” [3]. Zumre et al. (2005) observed linguo-facial trunk in 20%, thyro-lingual trunk in 2.5%, thyro-linguo-facial trunk in 2.5% and occipito- auricular trunk in 12.5% of cases in the human fetuses [4]. Anil et al. (2000) reported that lingual artery arises from a common trunk with the facial as a linguo-facial trunk in 10-20 % of cases [5]. Yildrim et al. (2001) observed total 6 (15%) linguo-facial trunk in 40 neck side in human cadavers [6].

Ozgur et al. (2008) classified the origins of these arteries which were arising from the external carotid artery in 4 types and reported their incidences. The separate origins of the arteries were defined as type 1 (90% of cases), the linguo-facial trunk as type 2 (7.5%), the thyrolingual trunk as type 3 (2.5%), and thyrolinguofacial trunk as type 4 [7].The development of external carotid artery system is a complicated process of angiogenesis and remodelling which includes annexation and regression of vessels. The development of hypostapedial artery which links the neural crest arterial system to the ventral pharyngeal artery marks an important event in the development of external carotid artery system [8].

The knowledge of variation in the origin of ECA is important for surgical procedures in the neck region such as radical neck dissection, catheterization, reconstruction of aneurysm, and interventional radiology. These variations pose a dangerous situation during various neck surgeries.

REFERENCES

1. Standring S, Johnson D, Ellis H, Collins P (eds). Gray’s Anatomy, 39th ed. Churchill Livingstone: London. 2005, pp. 543-544.
2. Strauch B, Vasconez LO, Hall Findlay EJ. Grabb’s Encyclopedia of flaps. 2nd ed. Vol (1). Head and Neck. Philadelphia: Lippincott-Raven, 1998, pp. 989-997.
3. Aaron C, Doyon D, Fischgold H, Metzger J, Richard J. Arteriographie de la Carotide externe. Masson, Paris, 1970.
4. Zumre O, Salbacak A, Cicekcibasi AE, Tuncer I, Seker M. Investigation of the bifurcation level of the common carotid artery and variations of the branches of the external carotid artery in human foetuses. Ann Anat. 2005; 187(4): 361-369.
5. Anil A, Turgut HB, Peker T, Pelin C. Variations of the branches of the external carotid artery. Gazi Med J. 2000; 11(2):81-83.
6. Yildrim M, Tangeli E, Soyluogh A, Tuna Y. Truncus linguofacialis siklig.I. Morfologi Der; 2001 (Links): 9(1)33-34.
7. Ozgur Z, Govsa F, Ozgur T. Assessment of origin characteristics of the front branches of external carotid artery. J Craniofac Surg. 2008; 19(4): 1159-66.
8. Larsen WJ. Human Embryology. 2nd ed. Churchill Livingstone: New York, Edinburgh, London. 1997, pp. 191- 195.

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