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Study of Sex Chromatin in Primary Amenorrhoea Patients and their First Degree Relatives

Isha Jaiswal[1], A P Kasote[2], M P Fulpatil[3], A D Patil[4]

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Author's Affiliation: [1]Assistant Professor, [4]Professor and Head, Department of Anatomy, Byramjee Jeejeebhoy Government Medical College, Pune, Maharashtra 411001, India, [2]Professor and Head, Department of Anatomy, Govt Medical College, Jalgaon, Maharashtra 425001, India, [3] Officer on Special Duty, Department of Anatomy, Govt Medical College, Nagpur, Maharashtra 440003, India.

Corresponding Author: Isha Jaiswal, Assistant Professor, Department of Anatomy, Byramjee Jeejeebhoy Government Medical College, Pune, Maharashtra 411001, India.

E-mail: jaiswalisha89@gmail.com

Abstract

Introduction: The mean age of menarche has become younger this century. Primary amenorrhoea is defined as the absence of menses by 13 years of age when there is no visible secondary sexual characteristic development or by 15 years of age in the presence of normal secondary sexual characteristics. In all cases of primary amenorrhoea, there is a need to determine the sex chromatin pattern in the nuclei of epithelial cells obtained by buccal scrapings.

Aims and objectives: To study the sex chromatin among patients of primary amenorrhoea and their first degree relatives.

Materials and Methods: 32 patients having primary amenorrhoea along with their first degree relatives were studied (total 64). Buccal scrapings were taken from both the patient and relative and stained with Giemsa stain for study of various parameters like presence or absence, number, size, shape and staining intensity of Barr body.

Results: Total numbers of patients studied were 32. 56.25% of the patients were negative for sex chromatin. 37.5% of the patients had absent Barr bodies in their cells. The shape of Barr body was found to be planoconvex in all patients and relatives. 1 patient was found to have a smaller sized Barr body and 1 had a larger sized one. The staining intensity of Barr body was equally dark in both patients and their first degree relatives. Mean % of Barr bodies among patients was 13.41 ± 14.30% (p value <0.0001, HS).

Conclusion: Primary amenorrhoea is an extremely stressful problem for a young girl and her parents. The clinician should handle the case with great sensitivity. Patient awareness and proper counselling of parents is of great importance regarding the treatment options available and the need of follow up.

Keywords: Primary Amenorrhoea; Sex Chromatin; Barr Body; First Degree Relatives.

Introduction

Adolescence is the milestone of womanhood. Because a woman is not born as woman, she becomes woman with the attainment of reproductive maturity which starts with puberty or the beginning of adolescence. Of all the changes of puberty, initiation of menstruation (menarche) is the most important.[1] Amenorrhea or absence of menstruation is a symptom and not a disease.

As the term denotes, it is one of the prime causes for female infertility and can be either primary or secondary in nature.[2]

The mean age of menarche has become younger this century. Primary amenorrhoea is defined as the absence of menses by 13 years of age when there is no visible secondary sexual characteristic development or by 15 years of age in the presence of normal secondary sexual characteristics.[3]

What should be the clinical approach to a case of amenorrhea? Fundamentally it is to determine the cause. When the complaint is primary amenorrhea, cryptomenorrhea has to be excluded. Thereafter the clinical features of the case deserve the closest study.

In all cases of primary amenorrhoea, there is a need to determine the sex chromatin pattern in the nuclei of epithelial cells obtained by buccal scrapings. On obtaining the buccal smears for Barr bodies, the diagnostic possibilities can be divided into two groups:

1. those that are sex chromatin positive
2. those that are sex chromatin negative

Also, when facilities are available, the chromosome complement should be studied by the appropriate examination of cultures of leucocytes and other tissues.[4]

The present study will also be carried out on first degree relatives of cases, on which not much literature is available. So, a detailed study on them will be quite helpful for the family members and the physician to predict any abnormalities in them and accordingly, proper counselling and management can be done.

Aims and Objectives

To study the sex chromatin in primary amenorrhoea patients and their first degree relatives.

Materials and Methods

32 patients having primary amenorrhoea were studied along with their first degree relatives (total 64). After asking the patients and relatives to rinse their mouths with water, buccal scrapings were taken from both of them and smeared on different glass slides and fixed in 95% alcohol overnight. Next day, the slides were stained with Giemsa stain for about 20 minutes, washed off to get rid of excess stain and were observed under oil immersion objective.[5] Various parameters of Barr body were studied like presence or absence, number, size, shape and staining intensity. 100 cells were counted each for the patient and relative and the sample was considered as 'sex chromatin positive' if ≥20% of the cells showed the presence of Barr body and 'sex chromatin negative' if cells showing Barr body were <20%.[6] The size was determined by observing the Barr body under ocular micrometer having a pitch length of 1μ under oil immersion objective.[7] The staining intensity of Barr bodies was compared by observational method and experience.

Results

Number of patients and their first degree relatives studied were 32 each (total 64). The Barr body was studied among cases and controls for the % of sex chromatin (positivity or negativity), number of Barr bodies per cell, size, shape, staining intensity and statistical analysis of Barr bodies was done.

56.25% of the patients had a sex chromatin percentage of below 20 (i.e. negative sex chromatin), whereas 43.75% showed positive sex chromatin (≥20%). Among the controls (relatives), all were positive for sex chromatin (Table 1 and Diagram 1). We found 1 case (3.12%) in which 4 out of the 20 cells which were positive for Barr body, contained 2 Barr bodies (Fig. 1 and 2). Among the controls, all showed only 1 Barr body in a cell (Table 2 and Diagram 2). Table 3 and Diagram 3 reveal that 30 patients (93.75%) had a normal sized Barr body, 1 patient each had a size of >1μ (3.12%) (Fig. 4) and <1μ (3.12%) (Fig. 5). Among the controls, all showed a normal sized Barr body (100%). Fig. 3 shows a normal sized Barr body. In all 32 cases and the controls, the shape of Barr body was found to be planoconvex (Table 4). None of the cases or controls had lighter or paler staining Barr bodies. All showed darkly stained Barr bodies (Table 5). The mean % of Barr bodies among the patients was 13.41 ± 14.30%, while among the first degree relatives, it was 30.63 ± 4.47%. On applying the Mann Whitney test, the percentage of Barr bodies in cases was found to be significantly lower as compared to the controls, the p value being <0.0001, which is highly significant (Table 6).

Table 1: Distribution according to % of sex chromatin.

Sex chromatin	NP	%	NR	%
Positive (≥20%)	14	43.75	32	100
Negative (<20%)	18	56.25	0	0

(NP – No. of patients; NR – No. of relatives)

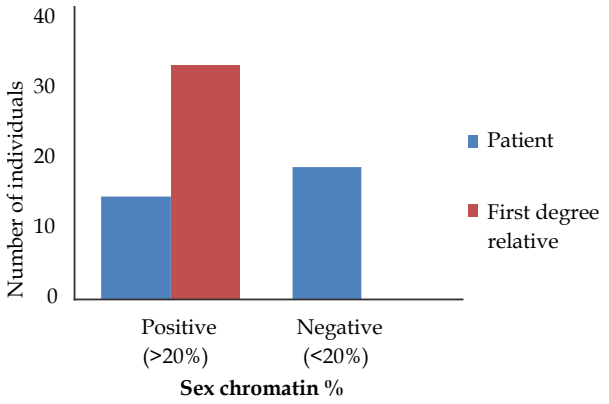

Diagram 1: Comparison of positivity or negativity of sex chromatin.

Table 2: Distribution according to number of Barr bodies per cell.

No. of Barr bodies per cell	NP	%	NR	%
0	12	37.5	0	0
1	19	59.37	32	100
>1	1	3.12	0	0

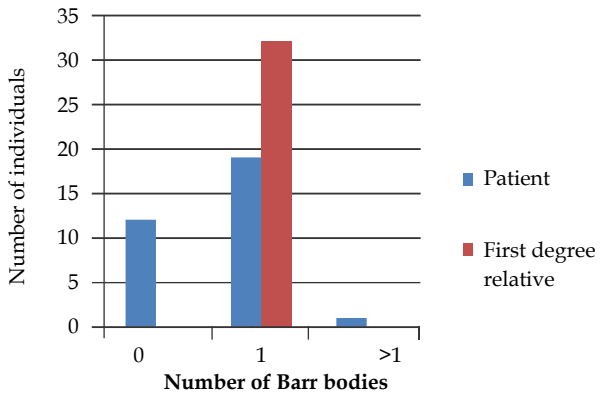

Diagram 2: Comparison of no. of Barr bodies per cell.

Table 3: Distribution according to size of Barr body.

Size of Barr body	NP	%	NR	%
<1 μ	1	3.12	0	0
1 μ	30	93.75	32	100
>1 μ	1	3.12	0	0

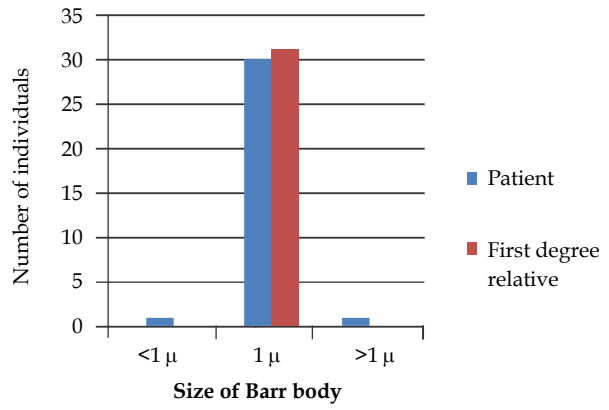

Diagram 3: Comparison of sizes of Barr body.

Table 4: Distribution according to shape of Barr body.

Shape of Barr body	NP	%	NR	%
Planoconvex	32	100	32	100
Triangular	0	0	0	0

Table 5: Distribution according to staining intensity of Barr body.

Staining intensity of Barr body	NP	%	NR	%
Light	0	0	0	0
Dark	32	100	32	100

Table 6: Statistical analysis of % Barr bodies.

Parameter	Cases	Controls
Mean	13.41%	30.63%
SD	14.30%	4.47%
Range	0-35	20-36
Z – value	4.593	
p value	<0.0001, HS	

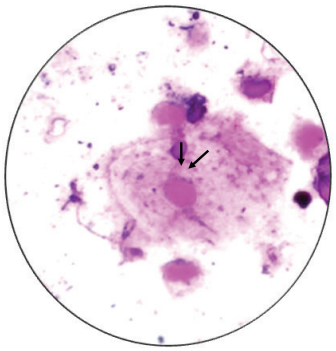

Fig. 1: A cell showing 2 Barr bodies at 12 and 1o'clock positions in the nucleus.

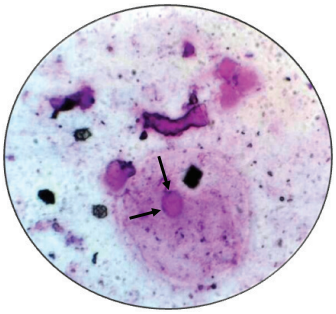

Fig. 2: A cell showing 2 Barr bodies at 8 and 12o'clock positions in the nucleus.

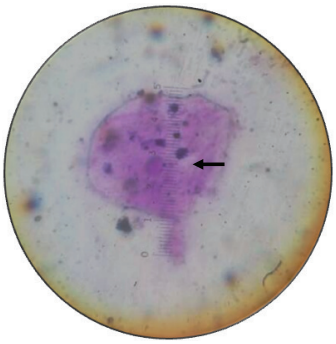

Fig. 3: A cell with Barr body at 3 o'clock observed with the ocular micrometer. The Barr body corresponds to a single pitch length (1 μ) of the ocular micrometer scale as depicted by the arrow.

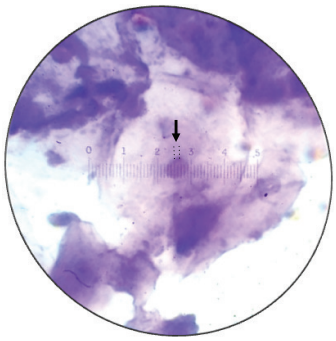

Fig. 4: A cell showing Barr body at 12o'clock position of larger size (approximately 2 μ).

The arrow is pointing towards the Barr body and the dotted lines are corresponding to the 2.5 and 2.7 mark of the ocular micrometer, which is equal to two pitch lengths, which is equal to 2 μ when observed under the 100X objective lens.

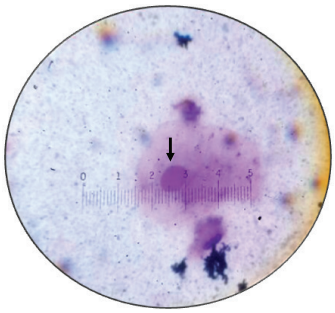

Fig. 5: A cell showing smaller sized Barr body (<1 μ) at 12 o'clock position seen as a small dot.

Discussion

The patients in our study positive for sex chromatin were 43.75% and those negative for it were 56.25%, whereas among controls, all were positive for it. The present study is similar to the study done by Coco R and Bergada C[8] in which 45.60% of the patients were positive and 54.40% were negative for sex chromatin. The present study is contrasting to a similar study conducted by Chhabra V. et al.[9], a correlation study of sex chromatin and primary amenorrhea in which they compared the percentage of cells showing Barr bodies in primary amenorrhoea patients and normal fertile Indian women of different age groups. In their study, 80% of the patients were chromatin positive while 20% were chromatin negative. In a study done by Lacramioara Butnariu et al.[10], the sex chromatin was abnormal in 40.8% and normal in 59.2% of the cases. Our study is also in contrast to the study done by Chryssikopoulos A. and Grigoriou O.11 and V. Lakshmi Kalpana et al.[12] in which 76.62% and 91.43% of the patients were positive and 23.37% and 8.57% of them were negative for it, respectively. Our study is also contrasting to the study done by Naushaba Rizwan and Razia Mustafa Abbasi[13] in whose study, buccal smears of 73.68% patients were positive and 26.31% were negative for Barr bodies (Table 7). These contrasting results could be due to the underlying hormonal imbalances in the patients. In general, PA is associated with hormonal disturbances. Stoyan I. Dokumov and Spas A. Spasov[14] performed a study on the influence of sex hormone administration on the incidence of nuclear sex chromatin in women. According to their study, testosterone uniformly led to a marked reduction in the incidence of sex chromatin body while progesterone produced a reduction in the majority of cases, whereas diethylstilbestrol (oestrogen) administration resulted in a significant increase in the nuclear sex chromatin material in all the cases. In our study, we found only 1 patient with 2 Barr bodies, whereas Lacramioara Butnariu et al.[10] found 16 patients with 2 Barr bodies (Table 8). This difference can be attributed to the great difference in the sample size taken for study. We studied only 32 patients whereas they studied 531 patients with primary amenorrhea. Only 2 patients in the present study showed abnormalities in size of Barr bodies. One patient each, had a Barr body of size >1μ and <1μ. In the study done by Lacramioara Butnariu et al.[10], 10 patients were found to have Barr body with a size >1μ (Table 8). These differences in the size of Barr bodies may be due to numerical and stuructural abnormalities of X chromosome (<1μ suggesting deletion of a part of X chromosome, and >1μ suggesting an isochrosome). The shape of Barr bodies of all the patients and first degree relatives in the present study was found to be planoconvex. We found no studies in which the different shapes of Barr bodies were taken as a parameter under study. Barr bodies have several distinct shapes. Many appear to be planoconvex or wedge shaped, with the plane side resting against the nuclear membrane and the convex part pointing towards the cytoplasm. Barr bodies in the centre of the nucleus appear to be rectangular, and some rectangular Barr bodies may also be observed at the periphery of the nucleus.[15] In an X-chromatin survey done by Janet K. Lyman,[16] the cell was considered positive for X-chromatin body only if the nucleus contained a condensation of chromatin material that was planoconvex to triangular in shape and closely applied to the nuclear membrane. However, we did not find any triangular or rectangular shaped Barr bodies in our study. In the present study, the staining intensity of sex chromatin was equally dark in all cases and controls. We did not find lighter stained sex chromatin in any of the individuals. Reitalu J.[17] conducted a sex chromatin study

Table 7: Comparison of % of Barr bodies of present study with other studies.

	% of Barr bodies (Positivity or negativity of sex chromatin)						
	Present study	Coco R. and Bergada C[8]	Chhabra V et al.[9]	Lacramioara Butnariu et al.[10]	Chryssikopoulos A. and Grigoriou O.[11]	V Lakshmi Kalpana et al.[12]	Naushaba Rizwan and Razia Mustafa Abbasi[13]
+ve	43.75%	45.60%	80%	59.2%	76.62%	91.43%	73.68%
–ve	56.25%	54.40%	20%	40.8%	23.37%	8.57%	26.31%

Table 8: Comparison of number of Barr bodies per cell and size of Barr body of present study with other study.

Parameter	No. of patients	
	Present study	Lacramioara Butnariu et al.[10]
> 1 Barr body per cell	1	16
Size of Barr body (>1μ)	1	10

Table 9: Comparison of mean % of sex chromatin of present study with other studies.

	Mean % of sex chromatin		
	Present study	Chhabra V. et al.[9]	Priseila G. Otto[18]
Patients	13.41 ± 14.30%	21.7%	18.63%
Controls	30.63 ± 4.47%	35.82%	–

Table 10: Comparison of range of Barr bodies of present study with other studies.

	Range of Barr bodies
Present study	0–35%
Chhabra V et al.[9]	0–40%
Jacobs et al.[19]	0–60%
Lakshmy et al.[12]	15–45%
Moore K L[20]	20–70%
Beaver D L and Douglas L E[21]	2–23%
Pansegrau D G and Peterson R E[22]	97.6%

on liver cells of female rats in which he noted a variation in the staining ability of the sex chromatin with age. It was rather pale in the nuclei of young female rats and more intensely and darkly stained in the nuclei of older animals. We tried to find out a similar correlation, if any, exists in the staining ability of sex chromatin in human also. However, we could not find any such variation. The mean % of sex chromatin in our study among the patients was 13.41 ± 14.30%, whereas among the first degree relatives (control group of fertile females), it was 30.63 ± 4.47%, which is highly significant ($p < 0.0001$). This is similar to the study by Chhabra V. et al.,[9] in which a significant difference was found among the mean percentage of sex chromatin in patients (21.7%) and control group (35.82%), p value being <0.01 (highly significant). Our study is also quite similar to the study done by Priseila G. Otto et al.[18] in which the average frequency of Barr bodies among the patients was 18.63% (Table 9). Regarding the range of Barr bodies, in the present study it ranged between 0 – 35% in the patients and in the range of 20 – 36% in the control group. Our study is similar to the study by Chhabra V. et al.[9] in which the range was 0 – 40%. Our study is in contrast to the study done by Jacobs et al.[19] and Lakshmy et al.[12] whose studies showed the ranges of 0 – 60% and 15 – 45% respectively. Moore K. L.20 estimated the percentage of Barr bodies in normal XX chromosome complement females to be between 20 – 70%; however, values above 60% are rare. Beaver D L and Douglass L E[21] examined 257 buccal smears and estimated the percentage of positive cells in normal females to be 2 – 23%. Their lower X chromatin values resulted from the inflexible use of more rigid criteria than previously used by others. Pansegrau D G and Peterson R E[22] reported an average X chromatin frequency of 97.6% in female buccal cells (Table 10). They, unlike the previous studies mentioned above, included both peripheral and central chromatin bodies in their counts; this would explain the elevated frequency of Barr bodies.

Conclusion

Primary amenorrhoea is an extremely stressful problem for a young girl and her parents. The clinician should handle the case with great sensitivity. Detection of sex chromatin by buccal smear in the present study proved to be an important tool for the clinicians in making right decisions for further evaluation and management of primary amenorrhoea patients. Patient awareness and proper counselling of parents is of great importance regarding the treatment options available and the need of follow up. Though different treatment modalities are available, outcome regarding regular menses and fertility potential are not so satisfactory. With continuing advancement of technologies of artificial reproduction, there is still hope for some patients with primary amenorrhoea to have their genetic offspring.

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Original Article

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Morpho–Metrical and Stereological Analysis of Human Fetal Kidney During Mid-Gestation Period Ranging from 12th to 35th Weeks to Study Growth Pattern of Kidney in Qualitative and Quantitative Aspects

Kirti Sudhakarrao Solanke[1], Rajan Bhatnagar[2], Boruah Dibyajyoti[3], Tandon Aseem[4]

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Author's Affiliation: [1]Associate Professor, Department of Anatomy, Smt. Kashibai Navale Medical College and General Hospital, Narhe (Ambegaon), Pune 411041, Maharastra, India, [2]Professor and Head, Department of Anatomy, Ram Manohar Lohia Institute of Medical Sciences, Vibhuti Khand, Gomtinagar, Lucknow 226010, India, [3]Scientist E, Department of Pathology, [4]Professor, Department of Anatomy, Armed Forces Medical College, Pune 411040, Maharastra, India.

Corresponding Author: Kirti Sudhakarraoo Solanke, Associate Professor, Department of Anatomy, Smt. Kashibai Navale Medical College and General Hospital, Narhe (Ambegaon), Pune 411041, Maharastra, India.

E-mail: drkirtipawarsolanke@gmail.com

Abstract

Morpho–metrical and stereological analysis of human fetal kidney during mid-gestation period ranging from 12th to 35th weeks of gestation to study growth pattern of kidney in qualitative and quantitative aspects.

Aims and objective: Carry out Morpho-metrical Analysis of human fetal kidney during mid-gestation period ranging from 12th to 35th week and evaluating following parameters:

a. Physical dimensions and weight of each kidney.

b. Glomerular area for each kidney at different gestational ages.

c. The numerical density of glomeruli in the cortex of each kidney at different gestational ages.

d. Volume of glomeruli in the 3 different zones of each kidney at different gestational ages.

Methods: For study purpose we divided 31 aborted fetuses into six groups according to gestational age. After measuring external parameters such as CRL of fetuses; they were injected with 10% formalin and fixed in 10% formalin for 3 days. Kidneys were procured; physical dimensions, volume and weight of kidney were measured. After tissue processing, blocks were cut; sections were mounted and H&E stained. Using software analyzer density, area, volume of glomeruli at three different zones i.e. cortex, juxtramedullary and medullary regions were calculated. Glomerular area and volume of cortical, juxtamedullary and medullary region for each kidney at different gestational ages was estimated by measuring the area and volume of 100 glomeruli per fetal kidney under 20X objective lens of image analyzer. Comparison was done for three different zones.

Keywords: Kidney; Glomeruli; Density; Steriology; Volume; Area; Cortex; Medulla.

Introduction

With prevalence of end stage kidney disease on the rise worldwide and facilities of kidney transplantation being available in a large number of centres; demand for donor kidneys are increasing at the rate unrecorded in the history. There have been some approaches to development of kidney or kidney like structures in vitro.

The size[1] of kidney is dependent on the number[2] and size of nephrons[3] and is presumably influenced both by genetic and environmental factors and so are the number of glomeruli at birth.[4]

The total filtration surface area of kidney depends on the glomerular density and the glomerular surface area.[5] Any variation in these factors alters the total filtration area which is thus a useful indicator of renal development and function. Many methods have been used to derive glomerular sizes and all of them have their own shortcomings.[6] The estimation of renal glomerular volume is a useful technique with clinical, diagnostic and prognostic relevance in several conditions including renal artery stenosis, glomerulosclerosis and glomerulomegaly.[7] Some authors have presumed that the retardation of renal development, as occurs in individuals of low birth weight, gives rise to increased postnatal risk of systemic and glomerular hypertension, as well as enhanced risk of expression of renal diseases such as aplasia, hypoplasia, cystic diseases or renal agenesis.[8] Thus, in utero detection of anomalies will prevent delay in postnatal diagnosis and enable early surgical repair of significant lesions.[9]

One in three adults worldwide is suffering from high blood pressure. Forty percent of adults worldwide aged 25 and above had raised blood pressure in 2008. Theme by WHO for 2013 on world health day is "The Global brief on hypertension: silent killer, global public health crisis".[10] One of the causes for high blood pressure is reduced nephron number during fetal life. Studies showed that retarded intrauterine growth may be associated with significant reduction in nephron number and size.[11,12] Knowledge about number, size, density, volume and distribution of kidney thus renders significant knowledge about organization of kidney.

In the era of modern technology and equipments developed for diagnostic and therapeutic procedures in the field of medical sciences and improved survival of premature neonates in recent years, it is worthwhile to investigate the effects of premature delivery on the kidney, in which nephrogenesis is still ongoing during the third trimester.[13] In fetuses with no abnormality; nephrogenesis is completed between 32 weeks to 36 weeks, nephrons are not formed after birth.[3] It was found that nephron number in stillborn infants with intra uterine growth retardation (IUGR) was significantly reduced compared to infants that were appropriately grown-for gestational age.[14] Intra uterine growth retardation affects nephrogenesis. In case of preterm infant, nephrogenesis is seen even after birth and is evidenced by active nephrogenic zone which is observed along with increased number of immature glomeruli.[15,16] Thus, it becomes important to have sound knowledge of the basic human morphology and developmental anatomy.

Though reports pertaining to histogenesis of human foetal kidney do exist; not much work has been carried out covering both morphological and stereological aspects of development.[17] The present study aims to study the histogenesis as well as carry out morpho-metrical analysis of density, area and volume of glomeruli of human foetal kidney during intrauterine period ranging from 12th to 35th week.

Aim and Objectives

Carry out Morpho-metrical Analysis of human foetal kidney during mid-gestation period ranging from 12th to 35th week and evaluate following parameters:

a. Physical dimensions and weight of each kidney.
b. Glomerular area for each kidney at different gestational ages.
c. The numerical density of glomeruli in the cortex of each kidney at different gestational ages.
d. Volume of glomeruli in the cortex of each kidney at different gestational ages.
e. The total volume of each kidney to be estimated using Archimedes' Principle.

Materials and Methods

Collection of Material: Thirty one aborted human foetuses were collected from labour room of Obstetrics and Gynaecology department of a tertiary care hospital in, Wanvorie, Pune, after taking informed consent of parents and following all ethical norms and clearances. Foetuses with different gestational age ranging from 12 weeks to 35 weeks which showed no abnormalities on macroscopic examination were taken.

These foetuses included spontaneous abortions and stillborn foetuses. Twins and foetuses with gross anomalies of urogenital system were omitted from our study. Ten % formalin was injected in the body cavities and soft tissue of foetuses and immersed and fixed in 10% formalin for a minimum

period of 3 days. The kidneys were retrieved by dissection through anterior approach. Bilateral subcostal (rooftop) incision was taken to open the abdomen. Median umbilical ligament was cut; posterior peritoneum separated aside, to expose the kidneys which were then removed by cutting pedicle of all vessels and ureter at hilum.

Measurement of external parameters: Crown Rump length (CRL) of these foetuses was measured using digital sliding vernier calliper with accuracy of 0.01mm and an osteometric board with millimetre scale and data were tabulated (Table 1). True gestational age of foetuses was collected from the medical records. Crown-rump length was correlated with gestational age chart as per text book of Embryology by Hamilton, Boyd and Mossman (Table 2) to ensure there was no intra uterine growth retardation (IUGR). Thus selection of normal for gestational age foetuses was assured.

The physical dimensions and weight of each kidney were measured using a linear scale, vernier callipers, divider, thread and a balance. Volume of each kidney was ascertained by Archimedes's principle. Kidneys were preserved in 10% formal saline.

Estimation of total renal volume: Total volume of each kidney was measured by Archimedes principle. Whole kidney was immersed in measuring flask and displacement of water was seen and measured.

After measuring volume, fixed enblock kidneys were processed to prepare paraffin blocks. Sections were cut with rotary microtome 5 to 7 micron thick then mounted and stained.

Morphological Analysis: The histological sections of kidneys belonging to foetuses of different age of gestation were studied using a Olympus binocular light microscope under 4X, 10X, 40X objective lenses with the aid of an interactive image analyzer and observations were noted down.

Stereological Procedures

Estimation of numerical density of glomeruli in cortex, juxtamedullary and medullary regions: The numerical density of glomeruli in cortex, juxtamedullary area and medulla of each kidney at different gestational ages was estimated by observing 10 microscopic fields per foetus under 20X objective of image analyzer with aid of specific software package Dewinters Biowizard 4.2.

Microscopic field area was kept constant and the counting was done for all fields using grids to reduce counting errors. The average particle count was estimated and numerical density was calculated with the aid of software Dewinters Biowizard 4.2.

Estimation of glomerular area and volume: Glomerular area and volume of cortical, juxtamedullary and medullary region for each kidney at different gestational ages was estimated by measuring the area and volume of 100 glomeruli per fetal kidney under 20X objective lens of image analyzer with the aid of software package Dewinters Biowizard 4.2.

Steps using Dewinters Biowizard 4.2 software: Steps of estimating density, area and volume of glomeruli using software package Dewinters Biowizard 4.2 are given below:

1. Photograph of H and E stained slide was taken:

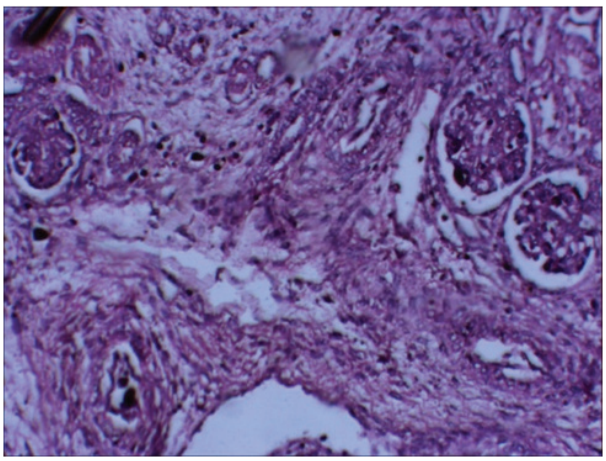


Fig. 1: Photograph of H and E Stained Slide.

2. Photograph was edited in paint. We marked thin lines with white colour around each glomerulus in each photograph as depicted in photograph below. After this photographs were opened with the help of software package Dewinters Biowizard 4.2.

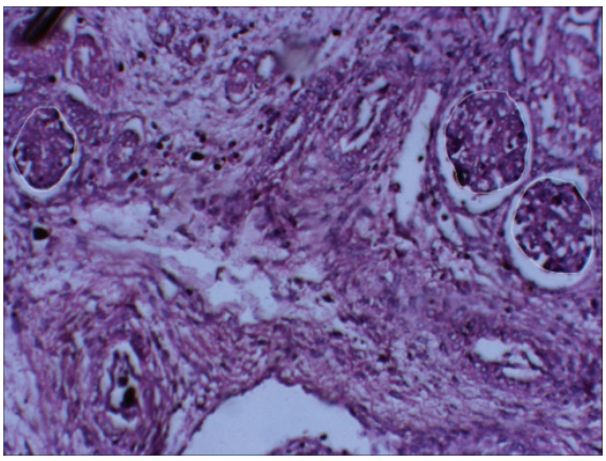


Fig. 2: Glomeruli Marked with White Color.

3. Software read glomeruli and gave all calculations about it as shown below:

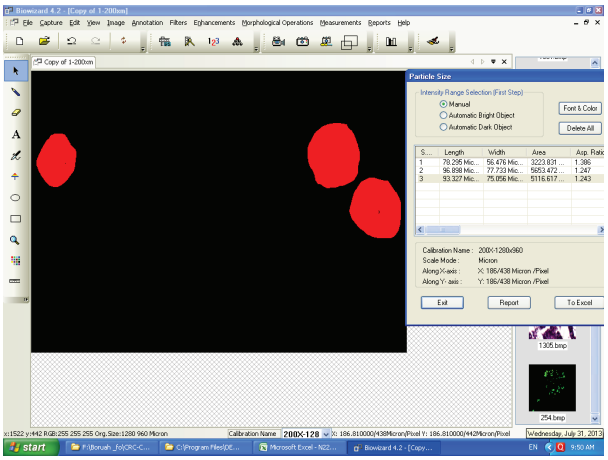


Fig. 3: Image on Software with Particulers.

Observations and Results

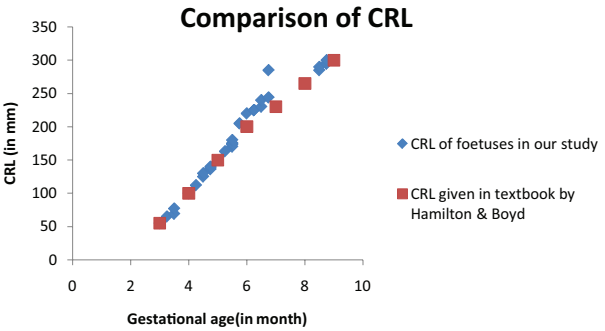
Physical Dimensions: The CRL was correlated with the gestational age of the foetuses and is shown in Table 1.

Table 1: The CRL was correlated with the gestational age of the foetuses.

Serial number	Crown rump length(mm)	Gestational age(weeks)
1	55	12
2	65	13
3	69	14
4	77	14
5	100	16
6	112	17
7	125	18
8	130	18
9	136	19
10	138	19
11	140	19
12	163	21
13	170	22
14	173	22
15	175	22
16	175	22
17	180	23
18	205	24
19	220	27
20	225	27
21	225	27
22	225	28
23	230	28
24	240	29
25	244	29
26	285	34
27	285	34
28	290	35
29	295	35
30	295	35
31	300	35

Table 2: From Human embryology by Hamilton, Boyd and Mossman.

Age(in lunar months)	Crown rump length(in mm)
3	55
4	100
5	150
6	200
7	230
8	265
9	300
10	335



Graph 1: Crown-rump length (CRL).

Crown-rump length (CRL) of foetuses in our study was correlated with CRL given in text book. Foetuses which were small for age were removed from the study (Graph 1).

We measured weight with digital weighing machine and volume by Archimedes principle. All data was tabulated in annexure-1. Width, thickness were measured at upper pole, hilum and lower pole and height was measured using vernier calliper and collected data was depicted in Table 3.

Steriological Observations

We divided our study samples in six groups for summarising the findings:

Table 3: Morphological Observations.

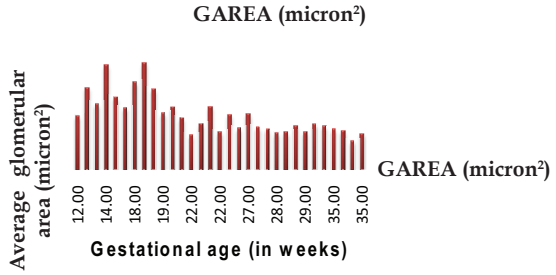
Age in weeks	Kidney width(mm)			Kidney thickness(mm)			Height (mm)
	At upper pole	At hilum	At lower pole	At upper pole	At hilum	At lower pole	
Group1(12-14 weeks)	3.75-6.04	3.38-5.69	3.49-5	3.64-3.86	3.06-3.86	2.73-4.65	6.93-9.3
Group 2(16-18 weeks)	5.56-6.15	4.9-5.9	5.6-7.04	4.41-6.43	4.51-7.83	3.83-7.29	12.82-12.87
Group 3(19-21 weeks)	8.83-11.37	8.12-10.12	9-9.55	8.55-9.18	7.45-8.82	7.03-8.62	18.86-20.52
Group 4(22-27 weeks)	11.37-15.7	10.1213.45	9.55-14.48	8.55-10.71	7.45-13.19	7.03-12.7	20.52-27.22
Group 5(28-29 weeks)	11.91-17.97	14.07-18	13.51-17.7	13.09-14.78	14.13-17.13	14.5-14.51	28.15-31.9
Group 6(34-35 weeks)	18.24-18.29	12.88-18.28	15.15-17.87	11.34-13.12	9.97-17	10.09-13.88	33.66-34

Table 4: Steriological Observations.

Age in weeks	Average glomerular area (micron^2)	Estimated average glomerular volume (micron^3)	Average glomerular density(GD)(per mm^2)
Group1(12–14 weeks)	3814.52	198.990 x 10^3	34.93
Group 2(16–18 weeks)	4094.36	205.955 x10^3	30.26
Group 3(19–21 weeks)	2485.60	150.366 x10^3	35.43
Group 4(22–27 weeks)	2298.20	101.003 x 10^3	35.99
Group 5(28–29 weeks)	1945.15	73.397 x 10^3	44.66
Group 6(34–35 weeks)	1929.69	73.797 X 10^3	38.13

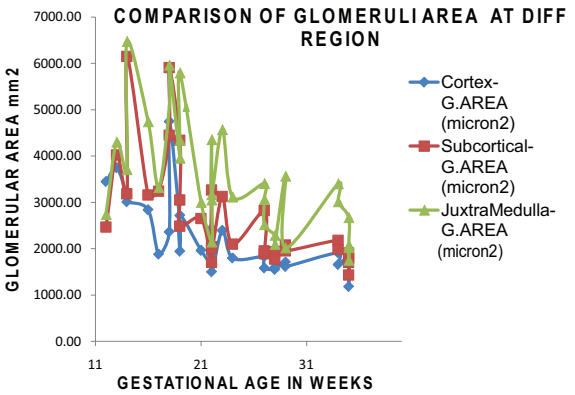
Table 4, annexure 3, 4 and 5 show glomerular area varied from 2000–4000 micron^2, estimated glomerular volume varied from 75.000 X 10^3 to 200.000 X 10^3 micron^3 and glomerular density ranged between 30–45 per mm^2. In group 2 i.e. from 16–18 weeks, minimum estimated glomerular volume was 61.632 X 10^3 micron 3 at 17 weeks and maximum 361.267 X 10^3 micron 3 at 18 weeks and average density 30.26 per mm^2. In group 3 i.e. from 19–21 weeks minimum glomerular area was 1940.94 micron 2 and maximum was 5796.60 micron 2 with average of 2485.60 micron 2 that of group 4 (22–27 weeks) minimum glomerular area was 1499.94 micron^2 and maximum was 4570.53 micron^2. In group 5 (28–29 weeks) minimum density was 22.62 per mm^2 and maximum was 72.40 per mm^2.

Glomerular Area



Graph 2: Showing changes in average glomerular area with gestational age.

Glomerular area showed initial increase up to 16-18 weeks and there after gradual reduction. The average was around 2000–4000 micron^2 (Graph 2).

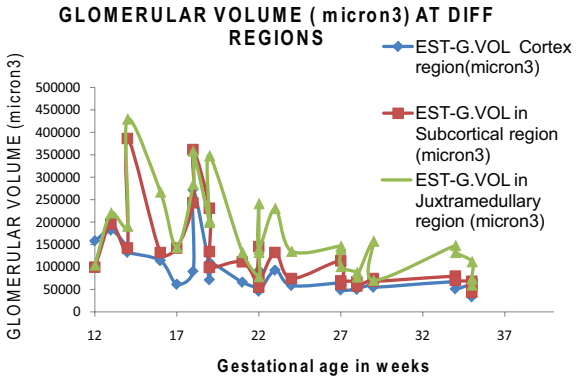


Graph 3: Showing comparisons of glomerular area at three different regions with gestational age.

The above graph 3 depicts comparison of glomerular areas at 3 different regions. It was found that glomerular area was largest in juxtamedullary region with maximum area being 6477.77 micron^2 and least at cortical region as 1181.14 micron^2. Glomerular area of subcortical region was in between. Glomeruli develop in cortical area and are then pushed towards medulla, thus area at juxtamedullary region was maximum. With increasing age, glomerular area decreases with exception at 14–18 weeks of age.

Estimated Volume of Glomeruli

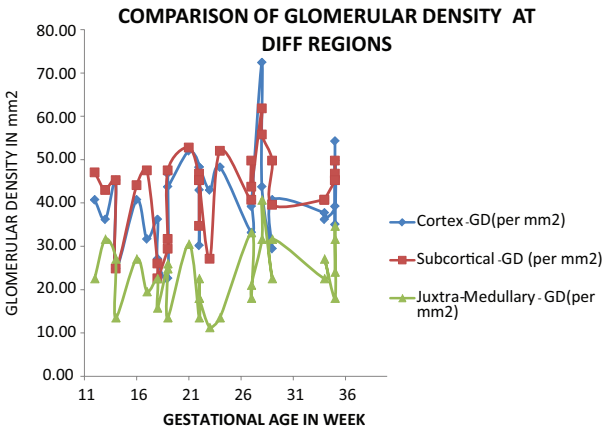
The volume of glomeruli showed gradual decrease from 12 weeks to 35 week with spurt at 14, 18, 19,24,28,34 weeks at more or less in all regions but most prominently in juxtamedullary region. Volume of glomeruli was largest with maximum of 429.991 X 10^3 micron^3 at juxtamedullary glomeruli and lest at cortical glomeruli as 33.715 X 10^{-3} micron.^{3} Subcortical estimated glomeruli volume was in between (Graph 4).



Graph 4: Showing comparison of glomerular volume at three different regions with gestational age.

Glomerular Density

Graph 5 shows glomerular density constantly increases from 12 to 35 weeks, with exceptions at 14, 18 and 23 weeks and sudden spurts at 28 and 35 weeks of gestation. At 14, 18 and 24 weeks estimated volume of glomeruli increases suddenly hence decrease in glomerular density. Glomerular density was maximum at cortical and subcortical regions with maximum being 72.40 per mm^2 and was lowest being at juxtamedullary region as 11.31 per mm^2.



Graph 5: Showing comparison of glomerular density at three different regions with gestational age.

When we compared estimated average glomerular volume with that of average glomerular density, it was found that whenever estimated average glomerular volume increased, the average density of glomeruli decreased. Overall glomerular density exhibited a fluctuating trend with abrupt fall at 14, 18 and 22 weeks of gestation. Accordingly the these estimated glomerular volume showed abrupt spurt at similar weeks of gestation (Graph 6).

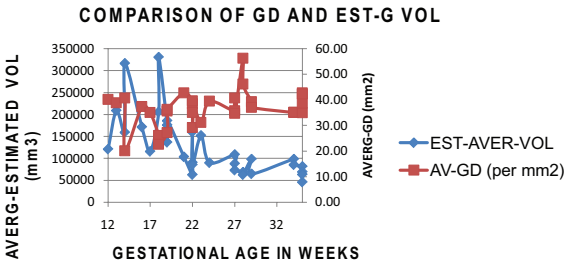


Table 5: Comparison of Glomerular Area Obtained in Present Study with Others.

Gestational Age (Weeks)	12Weeks			22 Weeks			28 Weeks			34 Weeks		
Authors	**Souster et al**	**Sabita Mishra**	**Present study**	**Souster et al**	**Sabita Mishra**	**Present study**	**Souster et al**	**Sabita Mishra**	**Present study**	**Souster et al**	**Sabita Mishra**	**Present study**
Cortical area (micron^2)	4891.1		3450.33	4086		2429.67	4492.7		1590.18	4383.3		1909.64
SD	1565		1408.19	276.7		1085.03	856.4		529.68	497		840.24
Subcortical area (micron^2)	7031		2464.82	4528	15313.71	3263.10	4578.8	6280.76	1773.21	4889.7		2175.48
SD	1525.9		1024.18	676.7		1320.18	745.7		781.19	764		1060.77
Juxtamedullary area (micron^2)	9736.5		2744.76	6961		4357.23	7537		2096.37	6951		3401.44
SD	1549.3		1916.71	214.4		1430.94	1059		754.83	1176		1822.48

Graph 6: Showing comparison of glomerular density (GD), estimated glomerular volume with gestational age in weeks.

Discussion

Stereological

Sabita Mishra et al. in 2006 stated volume of kidney showed linear growth from 14th week to 28th week with growth spurts during 22nd and 28th week. The present study delineated similar findings but a growth spurt was not appreciated. This might be because of difference in methods used for measuring volume of kidney. Same author also stated that glomerular density exhibited a fluctuating trend with abrupt fall during 16th, 22nd and 28th week. Present study showed similar fluctuating trend with abrupt fall during 14th, 18th, 23rd week and an abrupt rise during 28th and 35th week. This variation could be because of the number of foetuses studied. Sabita Mishra et al measured glomerular area with average being around 6000–7000 sq. microns. Glomerular area showed initial increase and then gradual fall. In the present study average glomerular area was around 2000–4000 sq. microns and showed a similar trend as that noted by Sabita Mishra et al. 18.

Conclusion

We studied 31 human foetuses from 12 to 35 weeks of gestation and found that nephrogenic zone was present beneath the capsule; glomeruli were seen at different stages of development. S shaped tubules were observed at 20 – 24 weeks and C shaped and crescent shaped in later weeks of development. With increasing gestational age kidney weight and volume were seen increasing gradually and glomerular area and glomerular volume were seen decreasing with some exceptions. Glomerular density showed a fluctuating trend.

Clinical correlation

The Present work is an attempt to study and analyse the growth pattern of kidney in human foetuses in qualitative and quantitative aspects which may be helpful in defining foetal kidney diseases such as agenesis, hypertension, hypoplasia, renal artery stenosis.[3]

Comparison of Glomerular Area Obtained in Present Study with Others

Volume measured by Archimedes' principle was seen constantly increasing with gestational age; these findings are in accordance with the study by Sabita Mishra et al.[18]

In the present study, kidney weight and kidney volume were seen increasing steadily but the same could not be seen with respect to glomerular volume and glomerular area. Nyengaard JR et al stated, that number of glomeruli and size of glomeruli showed significant negative correlation to age and a significant positive to kidney weight [19]. Reinaldo Manalich et al. 2000 measured the volume of glomeruli at 36 to 38 weeks as 158.8+/–49.89 μ^3 X 10^{-3} and stated that there was an inverse correlation between the number of glomeruli and volume of glomeruli and weight at birth and glomerular volume 11. Negative correlation between weight and glomerular volume was appreciated in our study as well.

Souster L P and Emery J L described that juxtamedullary areas were larger than cortical and subcortical region. Juxtamedullary and subcortical glomeruli showed initial decrease from 12 to 20 weeks but superficial glomeruli remained of same size from 12 to 40 weeks of gestation. In the present study juxtamedullary glomerular area was found to be larger.[6]

In the present study volume and weight of kidney increased with gestational age whereas glomerular area and volume were seen decreasing constantly with some exceptions at 14 and 18 weeks. Glomerular density showed a fluctuating trend in all three different regions i.e. cortical, subcortical and juxtamedullary. Area and volume of juxtamedullary glomeruli was largest while that of cortical regionthe smallest. Density was highest at cortical region.

Limitations: Foetuses included in current study were of gestational age between 12 weeks to 35 weeks. Foetuses of earlier weeks of gestation (first trimester) were not included because of scarcity of such aborted foetuses.

Conflicts of interest: The authors declare no conflicts of interest.

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Original Article

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Study of Anatomical Variations in Suprascapular Notch and Its Clinical Importance in Nerve Entrapment

Kumari Suman[1], Anjali Prasad[2], Binod Kumar[3]

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Author's Affiliation: [1]3rd Year Student, [2]Assistant Professor, [3]Associate Professor and Head, Department of Anatomy, Shri Krishna Medical College, Muzaffarpur, Bihar 842004, India.

Corresponding Author: Anjali Prasad, Assistant Professor, Department of Anatomy, Shri Krishna Medical College, Muzaffarpur, Bihar 842004, India.

E-mail: anjaliprasad28@gmail.com

Abstract

Introduction: The scapula is a large flat, triangular bone which lies on the posterolateral aspect of the chest wall having suprascapular notch (SSN), a variable depression on the superior border of scapula, near the root of coracoid process. The SSN is converted into a foramen by the superior transverse scapular (suprascapular) ligament, it is sometimes ossified. The suprascapular nerve traverses the foramen and is the main site for injury and entrapment of the nerve. Anatomical variations of the SSN are a possible cause of nerve entrapment. So the objective of the present study was to see variations in the shape of Suprascapular notch on gross examination.

Materials and Methods: 150 dried human scapulae were studied in the department of Anatomy of Govt. Medical College. The Scapulae were examined for different shapes of SSN including absence of notch.

Results: Out of total 150 scapulae 28% scapulae had indentation at the site of Suprascapular notch most common variation. 25.33% had U shaped notch, 20% had J shaped notch, 8% had V shaped notch. Absence of notch was also noted.

Conclusion: In our study indented notch is most common, which has a lower chance of nerve entrapment as compared to V shaped notch, which is least common in our study. Detail knowledge of SSN variations may be essential for surgeons performing suprascapular nerve decompression.

Keywords: Scapula; Supra scapular notch; Suprascapular nerve; Nerve entrapment.

Introduction

The scapula is a large flat, triangular bone which lies on the posterolateral aspect of the chest wall, covering parts of the 2nd to 7th ribs. It has costal and dorsal surfaces, superior, lateral and medial borders, inferior, superior and lateral angles, and three processes, the spine, its continuation the acromian and the coracoids process. The suprascapular notch (SSN) is a variable depression on the superior border of scapula, near the root of coracoid process.[1]

The SSN is converted into a foramen by the superior transverse scapular (suprascapular) ligament, it is sometimes ossified. The suprascapular nerve traverses the foramen and the suprascapular vessels cross above the ligament.[1]

The suprascapular nerve is a large branch of the superior trunk that runs laterally deep to the two muscles and enters the supraspinous fossa through

the SSN, inferior to the superior transverse scapular ligament.[1]

The SSN is one of the most important point along the course of the suprascapular nerve because this region is the main site for injury and entrapment of the nerve.[2,3] Anatomical variations of the SSN are a possible cause of nerve entrapment, especially in individuals involved in repetitive and forceful overhead activities (e.g. Players).[4,5] Etiology was first described by Andre Thomas in 1936.[6]

Suprascapular nerve entrapment is characterised by chronic, poorly localised pain in the posterior or/ and lateral region of the shoulder, which may radiate down the arm or up into the neck, weakness of abduction and increased external rotation of the arm, with atrophy of the supraspinatus and infraspinatus muscles.[7] Males are more likely to suffer from suprascapular nerve entrapment than females.[8,9]

Anatomical variations in shape and size of SSN are very common. A narrow SSN may predispose a patient to neuropathy. A V-shaped notch is more likely to be associated with nerve entrapment 10. So this study was conducted to see variations in shape of suprascapular notch.

Objective

To see variations in the shape of Suprascapular notch on gross examination.

Materials And Methods

150 dried human scapulae were studied in the department of Anatomy of Shri Krishna Medical College, Muzaffarpur. The Scapulae were examined for different shapes of notches. Absence of notch was also taken into consideration.

Results

A total of 150 scapulae were analysed in the present study. 85 were of right side 65 of left side. Out of total 150 scapulae 38 (25.33%) had U shaped notch (Fig. 1A), 30(20%) had J shaped notch (Fig. 1C), 12 (8%) had V shaped notch(fig 1D). Absence of notch was noted in 28 (18.67%) scapulae (Fig 1B). 42 (28%) scapulae had indentation at the site of Suprascapular notch (Fig 1E).

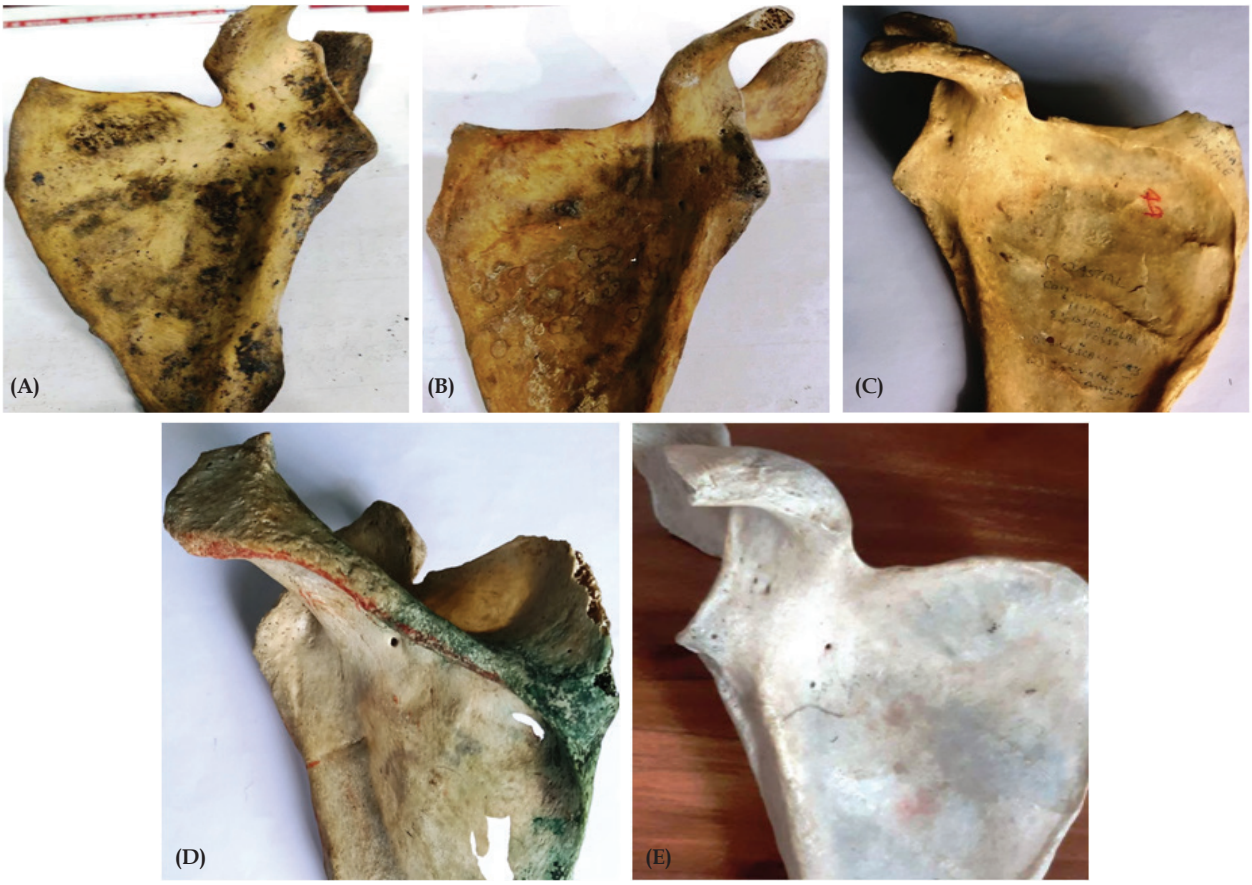

Fig 1: Showing variations in the shape of SSN: (A) U Shape; (B) Notch Absent; (C) J Shape notch; (D) V shape; (E) Indented Notch.

Discussion

Since past, many classifications of Suprascapular notch variations has been done in various scientific literatures.[10,11,12] Hadricka et. al[12] divided the SSN into into five types based on visual observation: shallow (type II), medium (type III) and deep (type IV). In type I, the SSN was absent, and in type V, a complete foramen was formed. Rangachary et al.[10] studied 211 American scapulae in 1979 and classified suprascapular notch into six types (Type I – Type VI). This classification was on the basis of following criteria's (a) Depth of notch, (b) Width at superior border of notch and (c) Widest point within the notch. They concluded that a small notch have greater chance of nerve impingement than a large one. Bayramoglu et al.[13] in 2003 classified the scapulae on the basis of classification of Rengachary et al.[10] and described two main types of notches i.e U- and V-shaped. Various Authors[14,15] found in their study that V shaped notches have lesser area than U shaped notches, so this is a causative factor for suprascapular nerve entrapment syndrome, but no direct clinical correlation was found. Iqbal et al 2010[16] found three different types of notch i.e. U, V and J in their study on Pakistani population. In their study, J shaped was most common type. In present study indented notch is most common and V shaped notch is least common. Vashudha TK et.al[17] studied 115 Indian dried scapulae for different shapes of suprascapular notch and degree of ossification of suprascapular ligament.They found eight different shapes of suprascapular notch. Vandana R et al[18] studied 134 dried scapulae and classified the SSN into six different types which are U, J, V, W, Indentation and absent SSN. In their study they found that U shaped SSN was most common and W shaped was least common. In present study indented notch is most common and V shaped notch is least common. Patel P et al[19] studied on 80 dried scapulae and found only three different types of SSN i.e. U, J and V shaped, in which U shaped was most common and V shaped was least common.

Conclusion

Our Study has very precisely described the SSN variations in a limited population. The size of the SSN is considered to play a part in the predisposition of nerve entrapment. A small notch has a larger tendency of nerve impingement than a larger notch.[10] In our study indented notch is most common, which has a lower chance of nerve entrapment as compared to V shaped notch, which is least common in our study. Detail knowledge of SSN variations may be essential for surgeons performing suprascapular nerve decompression.

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Clinical Anatomy of The Superficial Peroneal

Narendra A Patil[1], Mohammed Khaleel Ahmed[2]

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Author's Affiliation: [1]Assistant Professor, Department of Anatomy, Dr Patnam Mahendra Institute of Medical Science, Chevella, Telangana 501503, India, [2]Associate Professor, Department of Anatomy, Shadan Institute of Medical Sciences and Research, Hyderabad 500086, India.

Corresponding Author: Narendra A Patil, Assistant Professor, Department of Anatomy, Dr Patnam Mahendra Institute of Medical Science, Chevella, Telangana 501503, India.

E-mail: dr.mohammedkhaleel786@gmail.com

Abstract

Background: Superficial perineal nerve (SPN): It is the nerve of lateral compartment of leg. It is one of the two terminal branches of the common perineal nerve given at the neck of the fibula, it arises in the substance of peroneus longus on the lateral side of the neck of fibula.

Methods: A dissection was done in ten lower extremities (five from femaleand five from male) that had been well-maintained in a formalin arrangement. The subcutaneous layer was cautiously dissected in every preparation so that distortion of neural structures and their connections would be maintained a strategic distance from quite far. When the nerve structures had been recognized, the accompanying estimations were done: a) distance (in millimeters) from the upper end of the fibular head to the lower prominence of the lateral malleolus; b) number of branches from SPN when it arises through the superficial sural belt; c) distance (in millimeters) from the SPN rise highlight to the lateral malleolus; d) distance (in millimeters) from the SPN division to the lateral malleolus.

Results: There were two primary discoveries from these dissections. To begin with, we noticed varieties in the quantity of branches passing through superficialanterior fascia of the leg. A few cases indicated a solitary trunk rise, later partitioning into two branches, while others demonstrated two diverse recently separated cutaneous branches. Second, in both anatomical sorts, a checked variety was seen in the relative distance from the lateral malleolus, emerging at a low, medium or elevated level. Regarding the compartment from which the SPN pierces the superficial fascia, the contrast between single-trunk and two branches ought to be considered.

Conclusion: The information got appear to be adequately dependable for normal clinical practice, however it must be considered that successive and significant varieties may happen.

Keywords: Surgical anatomy; Superficial peroneal nerve; Anatomical variations.

Introduction

Superficial perineal nerve (SPN): It is the nerve of lateral compartment of leg. It is one of the two terminal branches of the common perineal nerve given at the neck of the fibula, it arises in the substance of peroneus longus on the lateral side of the neck of fibula. It begins on the lateral side of the neck of the fibula and descends for a short distance between the peroneus longus and peroneus brevis, and then lies in a groove between the peroneus brevis and extensor digitorum longus. At the junction of upper two-third lower one -third of the leg, appears as a division, and soon divides into lateral terminal branches which reach the dorsum of the foot. It gives muscular branch to peroneus

longus and peroneus brevis. It also gives cutaneous branches supply the skin of the lower one third of the lateral side of the leg and dorsum of the foot.

The middle terminal branches of the superficial perineal nerve cross the ankle and divides into two dorsal digital nerves, one for the medial side of the big toe and other for the second interdigital cleft. The lateral terminal branch of the superficial peroneal nerve also divides into two dorsal digital nerve for the third and the fourth inter-a digital cleft.[1]

The varieties about the shallow peroneal nerve are the non attendance of either the sidelong cutaneous branch or the average cutaneous branch, which are viewed as 8.6% and 0.8%, individually. It has been demonstrated that without the average cutaneous branch, the saphenous nerve innervates the average aspect of the foot, while without the horizontal cutaneous branch, the sural nerve supplies the sidelong aspect of the dorsum of the foot.[2] Sometimes, the shallow peroneal nerve punctures the intermuscular septum and passes from the horizontal compartment into the foremost compartment, following a shallow course. In different cases, the average and sidelong branches partition before the shallow sash is punctured.[3] The average branch might be missing and might be supplanted by the profound peroneal nerve. The nerve has been portrayed as emerging from the nerve to peroneus brevis.[4] The shallow peroneal nerve may bifurcate in the upper aspect of the leg and both the branches may stay in the horizontal compartment.[5] Very seldom the shallow peroneal nerve might be found in the foremost intermuscular septum.[6–8]

Materials and Methods

A dissection was performed in ten lower limbs (five from men and five from women) that had been preserved in a formalin solution. There were six (58.33%) right legs and four left legs (41.66%), the difference being due to the fact that only in some cases were both lower limbs available. The subcutaneous layer was cautiously dissected in every preparation so thatdistortionof neural structures and their connections would be maintained a strategic distance from quite far. When the nerve structures had been recognized, the accompanying estimations were done: a) distance (in millimeters) from the upper end of the fibular head to the lower prominence of the lateral malleolus; b) number of branches from SPN when it arises through the superficial sural belt; c) distance (in millimeters) from the SPN rise highlight to the lateral malleolus; d) distance (in millimeters) from the SPN division to the lateral malleolus.We likewise had recorded where the SPN pierces the sural fascia from the anterior or lateral muscular compartment (Fig. 1).

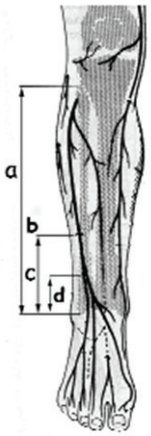

Fig. 1: Measures taken in our examination. a: distance (in mm) from the upper end of the fibular head to the lower distinction of the parallel malleolus; b: number of branches from SPN when it arises through the shallow sural fascia; c: distance (in mm) from the SPN development pointto the lateral malleolus; d: distance (in mm) from the SPN division to the lateral malleolus.

Subsequent to finishing these estimations, a deep and wide dissection was performed, particularly in those cases demonstrating a few varieties, so the anatomy of the SPN could be noticed and properly ordered along its whole course. A computer was utilized to sort out and measure all the information procured.

Results

There were two primary discoveries from these dissections. To begin with, we noticed varieties in the quantity of branches passing through superficialanterior fascia of the leg. A few cases indicated a solitary trunk rise, later partitioning into two branches, while others demonstrated two diverse recently separated cutaneous branches (Table 1). Second, in both anatomical sorts, a checked variety was seen in the relative distance from the lateral malleolus, emerging at a low, medium or elevated level (Fig. 2). Regarding the compartment from which the SPN pierces the superficial fascia, the contrast between single-trunk and two branches ought to be considered. The outcomes for single trunks are appeared in Table 2. Three specimens showed a SPN bifurcation before nerve rise; two of them had a superficial branch piercing the superficialfascia from the anterior compartment

and a second branch emerging from the lateral compartment. In the other specimen, both branches emerged from the anterior compartment (Fig. 2).

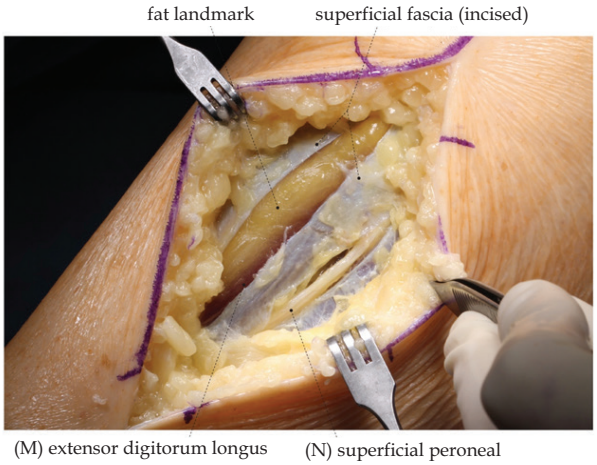

Fig. 2: Case of a superficial peroneal nerve.

Table 1: Frequency of a single-trunk for superficial peroneal nerve and two independent nerve branches when emerging.

	Number of Cases	Percentage
Single-Trunk	8	75%
Two Branches	2	25%

Table 2: Cases with a single trunk for the SPN when emerging.

	Number of Cases	Percentage
Emergence from anterior compartment	2	28.57%
Emergence from lateral compartment	4	57.14%
Emergence on intermuscular septum	1	14.28%

The level at which the SPN pierces the superficial fascia in the leg and enters into the subcutaneous cell tissue is significant. On average, the SPN gets superficial at 117.9 mm proximal to the lateral malleolus; i.e., in 95% of cases SPN emergence is somewhere in the range of 166 and 66 mm over the lateral malleolus, accepting a typical appropriation for this distance (95% confidence interval = mean ± 2 standard deviation).

Table 3: Peroneal length and emergence point of the SPN.

	Mean ±SD	Maximum	Minimum
Lateral malleolus-fibular head distance	354.2±28.3 mm	400 mm	313 mm
Malleolus-emergence distance	117.9±24.22 mm	135 mm	35 mm
Malleolus-emergence distance in percentage of fibular length	34.52%	42.72 %	11 %

This information in millimeters could be deceiving in individuals with extreme morphometric boundaries, and characterizing the development point for the SPN as a level of leg length is substantially more valuable. The level at which the SPN gets superficial was expressed as a percentage of the distance fromthe fibular head to the lateral malleolus, two palpablebone eminences helping clinical application. As per such estimations, SPN development speaks to 34.52% of the lateral malleolus-fibular head distance; in other words, at the upper two-thirds/lower-thirdlimit level of fibular length (Table 3).

The SPN division level may likewise be significant in some clinical cases. Unmistakably, in cases with the SPN piercing the fascia in the wake of having separated into two branches, the division point is proximal to the rise point. In such cases, we saw that nerve division is on normal 227 mm over the lateral malleolus. This distance speaks to some 63.17% of the distance from the lateral malleolus to the fibular head; i.e., at the upper third/lower two-thirds limit level in the leg. Then again, in (more normal) cases with the SPN piercing the superficial fascia as a single trunk, nerve division is 66 mm proximal to the lateral malleolus; i.e., some 18.76% of the distancefrom the lateral malleolus to the fibular head, representing to the upper 4/5/lower 1/5 limit level in the leg (Table 4).

Discussion

Clinical interest in a precise information on SPN courses and connections includes a few strengths. In patients with direct injuries or wounds to the anterolateral part of the distal third of the leg, expected association of this structure must be investigated by methods for delicate assessment in its self-governing innervation region. Neurophysiologists, be that as it may, could be keen on building up the specific point where to put cathodes to acquire an electroneurographic record, and discovering its shallow confinement could be fundamental. There is an actual sign to recognize the SPN just underneath the skin, by methods for a plantar flexion and reversal of the lower leg and foot and, optionally, an aloof flexion of the fourth toe (Fig. 2). Along these lines, the distal subcutaneous

Table 4: Distance between the malleolus and the division of the SPN.

	Mean	Standard Dev.	Maximum	Minimum	Percentage
Division-malleolus distance (all cases)	94.41 mm	68.42 mm	264 mm	35 mm	27.14 %
Single-trunk	65.21 mm	21.53 mm			18.76 %
Two branches	227 mm	54.23 mm			63.17%

course of the SPN can be recognized.[9-13] In the field of muscular medical procedure, a few specialists in fringe nerve a medical procedure have proposed utilizing this nerve as a vascularized join, since it is generally joined by a little course and vein, or utilizing the engine branches to the peroneal muscles for a neurotization of the front tibial muscle in patients with L4 root wounds (polio, spinal wounds, and others). Neither remedial choice is at present being utilized in clinical practice, yet contemplates have been wanted to survey their likely use soon.[14] On the other hand, in plastic medical procedure little corridors and veins going with the SPN are being utilized in vascularized skin unites.[15]

In some very basic circumstances, for example, arthroscopy, there is a cozy connection between lower leg ports and the danger of injury to the SPN terminal branches.[16] Also, when an anterolateral fasciotomy is performed because of a compartment condition of the leg, SPN position infers a danger for the nerve, wounds and delicate sequelae not being extraordinary.[17] One of the careful moves that may include the most noteworthy danger of SPN iatrogenic wounds is the horizontal methodology normally utilized in fibular osteosynthesis to treat lower leg cracks. The careful doctrine requiring an immediate cut from the skin to the fibula, evading a layer dismemberment, may cause accidental segment of the nerve if the specific position has not been distinguished or an anatomical variation exists. Percutaneous emplacement of pins or screws, for example, those utilized for outside fixing gadgets, or fasteners to obstruct tibialendomedullary nails can likewise cause nerve wounds.[18]

SPN compressive conditions have additionally been portrayed where the nerve penetrates the shallow belt of the leg, causing a clinical picture like a L5 root sickness (dysesthesia on the dorsal surface of the foot and particular toes). This circumstance requires a precise differential analysis.[19]

In all the above circumstances, exact ID of the course of the SPN is critical. Our information is promptly material and are adequately solid for common clinical practice. It ought to be reviewed, nonetheless, that specific significant varieties may happen. In some 75% of cases, the SPN shows up as a solitary nerve trunk, however in the excess 25% of patients it shows up as two separate branches. This must be considered so the second SPN branch isn't harmed in the wake of finding a branch when playing out a surgery.

As indicated by traditional portrayals, the SPN penetrates the shallow belt of the leg from the parallel compartment (55%), despite the fact that in a not immaterial level of cases (35%) it comes from the foremost compartment. Besides, SPN rise can be found at the upper 66%/lower third cutoff level of the fibular length. Albeit all this information will assist with finding the SPN with a serious level of dependability, it will never be conceivable to arrive at the 100% level. This is the reason a profound information on nerve SPN life structures and great careful abilities to discover the nerve and try not to cause nerve wounds are required.

Conclusion

The data obtained seem to be sufficiently reliable for usual clinical practice, but it must be taken into account that frequent and important variations may occur.

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Differential Pattern in Course and Origin of Pancreatico Duodenal Arteries

Suresh Babu K[1], Mithil Potuganti[2]

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Author's Affiliation: [1]Assistant Professor, Department of Anatomy, Rohilkand Medical College and Hospital, Bareilly, Uttar Pradesh 243006, India, [2]Assistant Professor, Department of Anatomy, Ayaan Institute of Medical Sciences, Moinabad (M) 501504, Rangareddy (D), Telangana, India,.

Corresponding Author: Mithil Potuganti, Assistant Professor, Department of Anatomy, Ayaan Institute of Medical Sciences, Moinabad (M) 501504, Rangareddy (D), Telangana, India.

E-mail: pmithil@gmail.com

Abstract

Introduction: Pancreas is a mixed gland with exocrine and endocrine components. Arterial supply comprises of branches from coeliac trunk and from Superior Mesenteric Artery [SMA]. The frequency of complications can be reduced by refining the surgical techniques and detailed knowledge of the regional anatomy of pancreas, including collateral circulation which formed the basis for our study.

Materials and methods: After obtaining ethical committee approval, we observed the pattern of blood supply of pancreas in 40 cadavers, kept for undergraduate dissection in Ayaan Institute of Medical Sciences and Rohilkand Medical College and Hospital during 2018-2020.

Results: Of the 40 cadavers, we observed following variations in 2 different cadavers.

1. Inferior pancreatico-duodenal artery took origin from a common stem along with first jejunal artery from SMA.

2. Double inferior pancreatico-duodenal artery. One took origin from superior mesenteric artery [usual] and the other from second jejunal artery.

Keywords: Pancreas; Duodenum; Superior mesenteric artery.

Introduction

Pancreas is a mixed gland with exocrine and endocrine components. Arterial supply comprises of branches from coeliac trunk and from Superior Mesenteric Artery [SMA]. Arteries, after entering parenchyma, of specific parts of gland, forms anastomosis for a dense vascular network which is essential for pancreas function.[1]

Pancreas develops at the junction between foregut and midgut. The area derived from foregut is supplied by coeliac trunk and superior mesenteric artery supplies the midgut derivatives.[2]

The branch of Pancreatology has greatly evolved in the last few decades. Various novel surgical and interventional approaches were implemented for treating diseases like tumours, acute pancreatitis and others. In spite of many great evolutionary inventions regarding treatment of pancreatic disorders, vascularisation demands further attention. The frequency of complications can be reduced by refining the surgical techniques and detailed knowledge of the regional anatomy of pancreas, including collateral circulation which formed the basis for our study.[3]

Materials and Methods

After obtaining ethical committee approval, we observed the pattern of blood supply of pancreas

in 40 cadavers which are kept for undergraduate dissection in Ayaan Institute of Medical Sciences and Rohilkand Medical College and Hospital during the time period of 2018-2020.

Cadavers of age group 25–50 were included in the study and cadavers with death due to past history of pancreatic diseases were excluded from study.

We observed the branching pattern of arteries supplying pancreas, with dissection done according to standard procedures.

Results

Of the 40 cadavers, we observed following variations in 2 different cadavers.

1. Inferior pancreatico-duodenal artery took origin from a common stem along with first jejunal artery from SMA (Fig. 1).

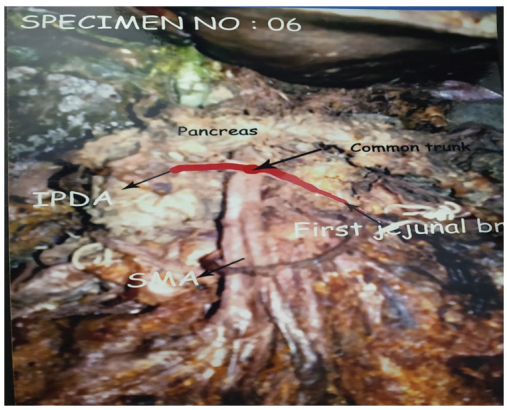

Fig. 1: Inferior pancreatico-duodenal artery took origin from a common stem along with first jejunal artery from SMA.

2. In another specimen, we observed double inferior pancreatico-duodenal artery. One took origin from superior mesenteric artery (usual) and the other from second jejunal artery (Fig. 2).

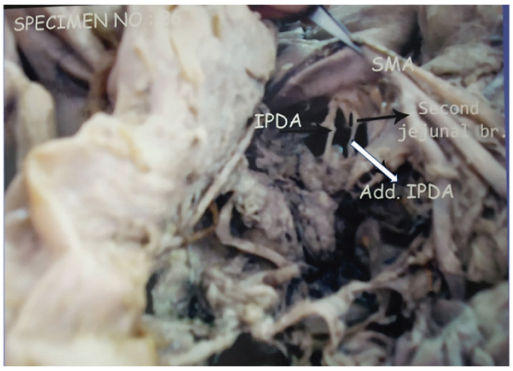

Fig. 2: Double inferior pancreatico-duodenal artery. One took origin from superior mesenteric artery [usual] and the other from second jejunal artery.

Discussion

Pancreas has a rich arterial supply via branches from coeliac trunk and superior mesenteric artery. Head and adjoining duodenum are supplied mainly by 4 arteries – 2 from coeliac trunk via Gastro Duodenal Artery (Anterior and Posterior Superior Pancreatico-Duodenal arteries SPDA) and 2 from Inferior Mesenteric Artery [IMA] (Anterior and Posterior Inferior Pancreatico Duodenal arteries IPDA). IPDA is present in most individuals. Usually arises either directly from SMA at the inferior border of pancreas. As a common branch with first jejunal artery (Pancreaticoduodenojejunal trunk) from posterior or left aspect of SMA (Horiguchi et al 2008, Bertelli etal 1996). When arising as Pancreaticoduodenojejunal trunk, the artery gives off a jejunal branch and then runs posterior to both SMA and vein before dividing into terminal branches. Occasionally IPDA is absent.[4]

Maneesh Joleya[5] (2016) reported Gastro duodenal artery (GDA) from coeliac trunk rather than common hepatic artery. In our study, we couldn't find this type of variation.

Chavan[2] (2015) reported in 2% of cases, anterior arterial arcade of pancreas is by dorsal pancreatic artery. In our study, in one case, double IPDA is found, where, one which took origin from SMA, completed anterior arterial arcade.

Covantev[3] (2019) observed in 24% of cases, majority of pancreas blood supply is by splenic artery. However, in our study, we didn't observe any such arterial dominant pattern.

Okahara[6] (2009), found in one case, a common trunk of coeliac trunk and superior mesenteric artery. However, we didn't observe such. They also reported IPDA arising as a common trunk with first jejunal artery in 14 cases. However, we found such a variant in only one case.

Ranjeeta[7] (2013), reported an anomalous dorsal pancreatic branch from coeliac trunk with absence of SPDA. However, in our study, we didn't find such variation.

Patil VR[8] (2017), observed an anomalous GDA from SMA, giving rise to SPDA and right gastro-epiploic artery. We didn't observe such variant.

Silva Jr (2014)[1], found and reported a variation in which IPDA and middle colic artery took origin from SMA as common trunk. In our study, IPDA and first jejunal artery took a common origin from a stem which originated from SMA.

Conclusion

Having Knowledge about variations in arterial supply to pancreas will be always an advantage during various abdominal surgeries involving pancreas or not.

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One Minute Preceptor Method for Teaching Histology: An Efficient Tool

Poorwa Baburao Kardile[1], Jaideo Manohar Ughade[2], Mohammad Aneesur Rahman[3],

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Author's Affiliation: [1]Assistant Professor, [3]Associate Professor, Department of Anatomy, Dr Shankarrao Chavan Government Medical College, Nanded, Maharashtra 431601, India, [2]Professor, Department of Anatomy, Government Medical College, Nizamabad, Telangana 503001, India.

Corresponding Author: Jaideo Manohar Ughade, Professor, Department of Anatomy, Government Medical College, Nizamabad, Telangana 503001, India.

E-mail: drjaideoughade@gmail.com

Abstract

Introduction: One-Minute Preceptor (OMP) model was first introduced by Neher et al1 in 1999 as "Five Step Micro skill Model" of teaching learning for the clinicians having busy ambulatory clinics. Although the model is conceptually sound, it remains largely untested. So present study have been conducted in identification and interpretation of routine histology slides of Anatomy among undergraduate students to assess the effectiveness of this model over that of the traditional model.

Aim: To study the utility of One minute preceptor as a teaching method for the students of Anatomy.

Objectives: 1. To sensitize the students for One minute perception. 2. To sensitize the faculty members to one minute perception. 3. To assess the perception of the faculty and the students for this new teaching method.

Methodology: With prior approval of ethical committee, Orientation Programme on OMP teaching learning was conducted to introduce the concept of "One Minute Preceptor" for the faculty as well as the students. Perception evaluation of the students to OMP method was done from checklist of pre-test and post-test performance through observation (Likert scale). Effectiveness of the OMP method was judged by comparing the performance of the students in Pre-test and Post-test. Wilcoxon sign rank test was used. P value <0.05 was taken as statistically significant. Perception evaluation of the faculty was done by taking their feedback.

Results: OMP proved to be useful for the students in improving presentation skills, involvement in decision making, reasoning behind decision making, providing positive feedback as 'p' value was highly significant for it. Most of the faculty members strongly agreed that OMP is feasible, useful and an effective tool which improves teaching skills, helps identify specific lacunae in learner's understanding. However, they also felt that it was more time consuming but not cumbersome. It must also be mentioned that all the faculty members were willing to adopt OMP as a teaching methodology.

Conclusion: All the 30 students showed satisfaction at the end of OMP session. OMP proved to be useful in improving presentation skills, involvement in decision making, reasoning behind decision making, providing positive feedback as p value was highly significant for it. .As it was a new teaching methodology students were apprehensive initially but with repeated sessions students showed enthusiasm and were eager to continue OMP sessions in future for histology and other subheads of Anatomy. It must also be mentioned that all the faculty members were willing to adopt OMP as a teaching methodology.

Keywords: One-Minute Preceptor; Teaching learning methods; Anatomy; Histology.

Introduction

As a responsible teacher it is our duty to get equipped and updated with appropriate teaching - learning tools that will impart knowledge, built confidence and improve the presentation skills of the medical graduates. Due to shortage of staff, time and ever expanding knowledge, conventional method of teaching medical graduates rarely spare time for giving constructive feedback. One Minute Preceptor method of teaching aims to overcome this shortcoming. One-Minute Preceptor (OMP) model was first introduced by Neher et al[1] in 1999 as "Five Step Micro skill Model" of teaching learning for the clinicians having busy ambulatory clinics. One Minute Preceptor includes five micro-skills for teaching: Get a commitment, Probe for supporting evidence, Teach general rules, Reinforce what was right and Correct the mistakes.[1]

Preceptorship lies in the domain between teaching (delivering knowledge in a group or individually) and mentoring (providing individual support). In this teaching technique a commitment is made by the student. The act of commitment pushes the learner to move beyond their level of comfort and makes the teaching encounter more active and more individualized. This also shows respect for the learner and fosters an adult learning style. Initially it enables the preceptor to have adequate information on learner's knowledge and thus teaching does not become misdirected and disobliging. Preceptor evaluates the thought process of the student and identifies the gaps. Certain general rules are taught by the teacher to improve the concepts of the student as the given information is both memorable and more transferable. Learner's knowledge, skill and attitude are not well established unless reinforced. So a positive feedback[2] is given and his deficiencies are pointed out using constructive feedback. Mistakes, if left unattended often get repeated, therefore suggestions are given in the end to the student on how to make improvements in the future. Thus the preceptor bridges the needs and expectations of students with varied learning skills and preferences.

In the OMP method, inquiry and discussion phases are learner focused.[2] In the inquiry phase, OMP elicits the learner's understanding of the topic by asking open-ended "what" type of questions. For this we followed following procedure.

1. Commitment:
    a. What do you think is the slide of?
    b. What other information would you like to add?
2. Evidence to support the finding:
    a. What lead you to that conclusion?
    b. What else did you consider, what kept you from that choice?
    c. What are the key features of identifying this slide?
    d. What questions are arising in your mind?
3. Whatever the learner revealed (some teaching values) he needs to know certain general concepts, considerations according to learner's level of understanding. Information given at this stage is more memorable and transferable.
4. Feedback:
    a. Positive feedback for good work.
    b. Constructive feedback for mistakes, omission, distortion and misunderstanding.

Based upon this information, the preceptor better comes to know about the learner's understanding of the topic, make a targeted teaching point, and provide positive and corrective feedback. Although the model is conceptually sound, it remains largely untested. No studies have been conducted in identification and interpretation of routine histology slides of Anatomy among undergraduate students. The effectiveness of this model over that of the traditional method, is yet to be assessed.

One Minute Preceptor is a learner-centered, one to one teaching model focused on the learner's thinking and reasoning behind decision making ability which provides standard checklists and criticism.[3] In the present study both faculty and under graduate students were sensitized to OMP method of teaching. Perception evaluation of this teaching method was done by taking feedback from the faculty and conducting pre-test and post-test for the students.

Aim

To study the utility of One minute preceptor as a teaching method for the students of Anatomy.

Objectives

1. To sensitize the students for One minute perception.
2. To sensitize the faculty members to one minute perception.
3. To assess the perception of the faculty and the students for this new teaching method.

4. To evaluate the use of OMP method on performance of the students.

Methodology

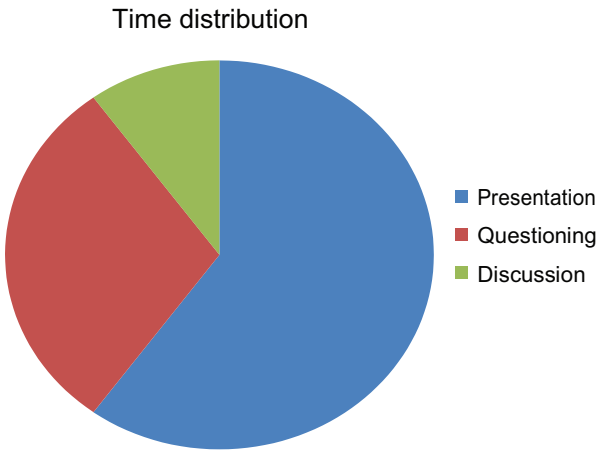
Research type: Educational research.

Study Design: Prospective, Interventional.

Intervention: Use of one minute preceptor method of teaching-learning.

Setting: Department of Anatomy, at Dr Shankarrao Chavan Government Medical College, Nanded. Ethical issues: Prior approval of ethical committee has been taken as per letter no 87/2018.

Duration: 6 months October 2017 to March 2018. The time frame for various steps in study is shown by graphically representation. (Graph 1).



Graph 1: Pie diagram showing time distribution.

Participants: Subjects for the research project were first year MBBS students from batch 2017-2018. All the students were already exposed to the traditional/conventional teaching methods for routine Histology slide identification and interpretation.

Inclusion: Low achievers. Exclusion: Not ready for consent.

Sampling and Randomization: A pre-test was conducted and then the students with low performance in the terminal exams were sensitized to One minute preception test.

Sample size: 30 students, 3 undergraduate teachers. Sampling method: Cross sectional interventional method.

Study design:

1. Low achiever students were sorted.
2. Pre-test was been conducted by giving a Questionnaire to the participants who were already exposed to the conventional method of teaching.
3. Orientation Programme on OMP teaching learning was conducted to introduce the concept of "One Minute Preceptor" for the faculty as well as the students.
   a Sensitization of the students to OMP by Small workshop on OMP, Role play, Interactive lecture.
   b. Sensitization of the faculty members by Small workshop on OMP, Role play, Interactive lecture.
4. Histology slides for OMP teaching were chosen and evaluated for the level of difficulty by senior faculty members. These slides were given to the students for a period of five minutes.

   Histology slide → OMP → Get commitment → Probe for support evidence → Reinforce what was done well → Guidance about errors and omissions → Teach a general principle → Feedback.

   10 sessions were carried out by three faculty members on each student in rotation. Duration of each session was approximately 5–8 minutes depending on the interpretation and knowledge of the learner and the time availability.
6. Post test Questionnaire form was filled and submitted by the students.
7. Effectiveness of the OMP method was judged by comparing the performance of the students in Pre-test and Post-test.
8. Perception evaluation of the faculty was done by taking their feedback.

The survey form was filled in anonymously to protect the identity of the students. The questions included the efficacy of OMP in identification and differentiation of slides, reasoning behind decision making, evaluating knowledge and skills and encourage to read more.

Data collection method: Quantitative data was collected from checklist of pre-test and post-test performance through observation (Likert scale).

Qualitative data was collected by feedback form having open ended Questionnaire. Wilcoxon sign rank test was used for statistical analysis. 'p' value < 0.05 was taken as statistically significant.

Result

OMP is a vibrant learning activity unlike passive traditional teaching methods. The primary aim

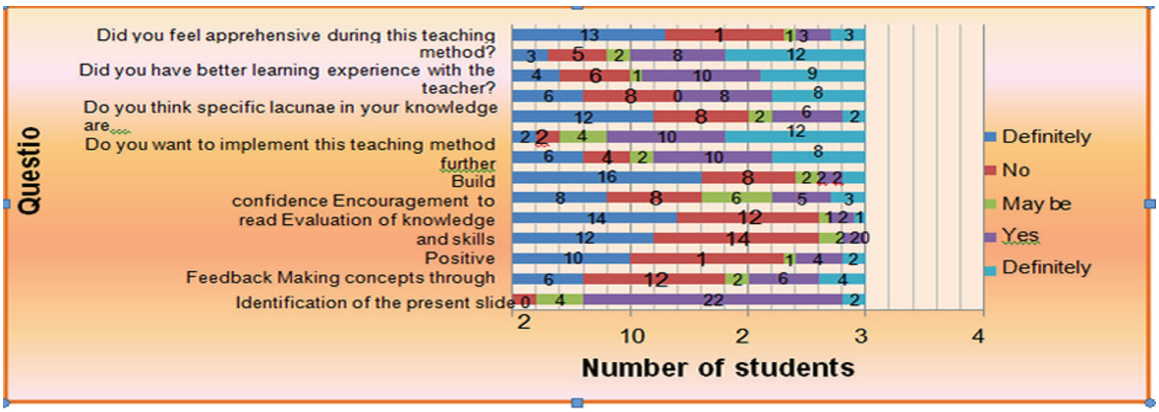
of this educational research project was to know if one minute preceptor (OMP) is an effective teaching method for the first year medical graduate students. Secondary aim was to sensitize the other faculty members so that OMP teaching method can become a routine practice in the histology practicals if proved to be effective.

The total number of participants was 33 which included 30 medical Graduate students and 3 Under Graduate Teachers. The attendance of the participants was 100%. Survey response rate was 100% for both pretest as well as post-test questionnaires. All the 30 students showed satisfaction at the end of OMP session. The learners were given pre-test questionnaires to answered, response of pretest is shown in Table no 1.

After which an orientation programme was conducted on One Minute Preceptor (OMP).

Table 1: Students pre test questionnaire with their response representing conventional Method.

Q.No	Statement	Likert's scale					Total	Mode
	The conventional method technique helped in	Definitely No (1)	No (2)	May be (3)	Yes (4)	Definitely Yes (5)		(Column No)
1	Identification of the present slide	0	2	4	22	2	30	4
2	Differentiate present slide from other slides	6	12	6	2	4	30	2
3	Improve Presentation skills	10	13	1	4	2	30	2
4	Involvement in decision making process	12	14	2	2	0	30	2
5	Reasoning behind decision making	14	12	1	2	1	30	1
6	Making concepts through general rules	8	8	6	5	3	30	1 and 2
7	Positive Feedback	16	8	2	2	2	30	1
8	Evaluation of knowledge and skills	6	4	2	10	8	30	4
9	Encouragement to read	2	2	4	10	12	30	5
10	Build confidence	12	8	2	6	2	30	1
11	Do you want to implement this teaching method Further	6	8	0	8	8	30	2, 4 and 5
12	Do you think specific lacunae in your knowledge are identified?	4	6	1	10	9	30	4
13	Did you have better learning experience with the teacher?	3	5	2	8	12	30	5
14	Did you feel apprehensive during this teaching method?	13	10	1	3	3	30	1



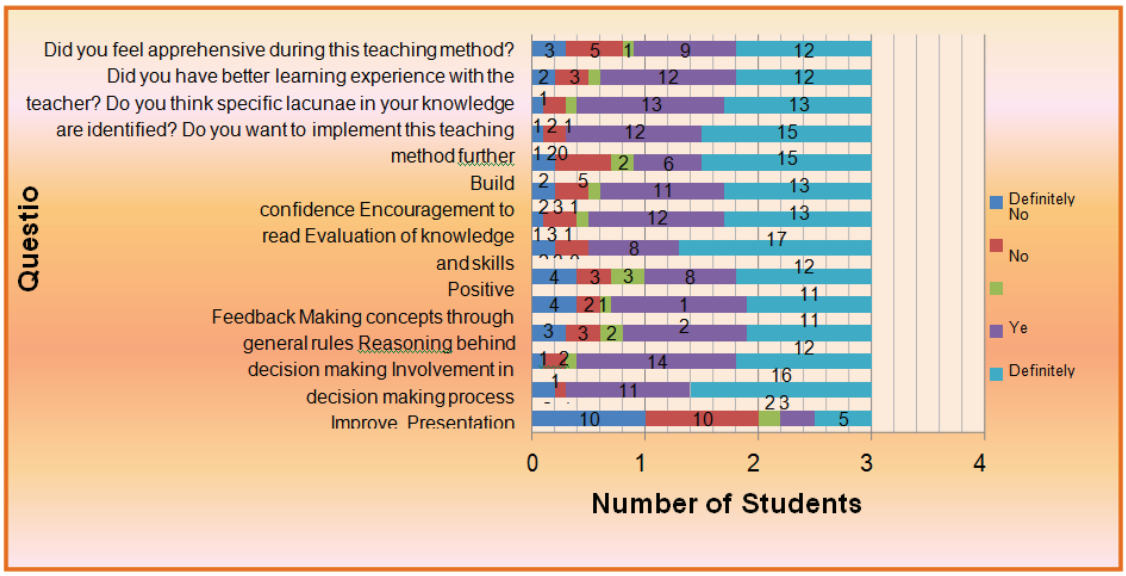
Graph 2: Student participants Pretest.

Table 2: Students post test questionnaire with their response representing OMP Method.

Q. No	Statement	Likert's scale					Total	Mode
	The OMP technique helped in	Definitely No (1)	No (2)	May be (3)	Yes (4)	Definitely yes (5)		(Column No)
1	Identification of the present slide	10	10	2	3	5	30	1 and 2
2	Differentiate present slide from other slides	2	1	0	11	16	30	5
3	Improve Presentation skills	1	2	1	14	12	30	4
4	Involvement in decision making process	3	3	2	11	11	30	4 and 5
5	Reasoning behind decision making	4	2	1	12	11	30	4
6	Making concepts through general rules	4	3	3	8	12	30	5
7	Positive Feedback	2	3	0	8	17	30	5
8	Evaluation of knowledge and skills	1	3	1	12	13	30	5
9	Encouragement to read	2	3	1	11	13	30	5
10	Build confidence	2	5	2	6	15	30	5
11	Do you want to implement this teaching method Further	1	2	0	12	15	30	5
12	Do you think specific lacunae in your knowledge are identified?	1	2	1	13	13	30	4 and 5
13	Did you have better learning experience with the teacher?	2	3	1	12	12	30	4 and 5
14	Did you feel apprehensive during this teaching method?	3	5	1	9	12	30	5

Table 3: Showing the statistical significance of OMP method.

Q. No	Statement	p value	Interpretation
1	Identification of the present slide	0.0003	Significant
2	Differentiate present slide from other slides	0.0002	Significant
3	Improve Presentation skills	<0.0001	Highly Significant
4	Involvement in decision making process	<0.0001	Highly Significant
5	Reasoning behind decision making	<0.0001	Highly Significant
6	Making concepts through general rules	0.0073	Significant
7	Positive Feedback	<0.0001	Highly Significant
8	Evaluation of knowledge and skills	0.0500	Significant
9	Encouragement to read	0.7683	Non Significant
10	Build confidence	0.0005	Significant
11	Do you want to implement this teaching method Further	0.0014	Significant
12	Do you think specific lacunae in your knowledge are identified?	0.0359	Significant
13	Did you have better learning experience with the teacher?	0.5043	Significant
14	Did you feel apprehensive during this teaching method?	0.0002	Significant



Graph 3: Student participants Post test.

Table 4: Response of teachers.

	T1	T2	T3
Question1 :	Were you aware of OMP method of teaching with its five microskills before attending role-play and the interactive lecture on it?		
Response	No.	No, I was not aware of this teaching technique.	No, It was completely new method for me.
Question2 :	Whether OMP method of teaching is feasible? If yes why? Or If no why?		
Response	Yes, it is simple method without use of audiovisual aids. Can be used routinely	Yes, initially we have to take efforts to train the faculty and do proper orientation of the students.	Yes, as it does not require much resource, it is easily feasible.
Question 3:	Whether OMP method has improved your teaching skills?		
Response	Yes, definitely.	Yes, it has improved my teaching skill, but as it is one to one teaching method, it is time consuming.	Yes.
Question 4:	Whether traditional method of teaching is more effective than OMP method?		
Response	OMP is more effective in giving positive feedback which is often not given in traditional method.	OMP is more effective than traditional methods in certain areas like Involvement in decision making process, Positive Feedback, Evaluation of knowledge and skills. But it is more time consuming.	Personally, I didn't feel there was a difference with or without the OMP
Question 5:	Did you come across any difficulties with the OMP teaching?		
Response	No, Students were equally enthusiastic in learning with new teaching technique.	Yes, As it is one to one teaching learning method, it is time consuming, it is difficult to keep other students engaged.	Yes, As I am used to traditional teaching method, teaching at times was not spontaneous.
Question 6:	Would you adopt the OMP teaching technique in future sessions? If so, why?		
Response	Yes, I would use this teaching technique. Commitment to enhance thought process and feedback to overcome mistakes are two essential steps of this technique not covered in traditional teaching.	Yes, I would adopt some portions of it as it is difficult and time consuming to cover all the steps of the OMP. Even in routine teaching I correct errors made by students.	Yes, it is useful for students.

The important observation of the present study was that none of the participant was aware of OMP. None of the participants could correctly note the order of the steps involved in the OMP process. After 10 sessions of OMP conducted by three faculty members with the thirty learners in rotation; post-test questionnaires were answered by the participants. Comparison of the change in behaviour of the learner after intervention is given in Table 2. Results were shown after the analysis of

pre- and post project questionnaire which depicts their learning experience with traditional and OMP teaching method, respectively.

The finding of table 1 and table 2 are also summarized by graphical representation (graph 2 and 3).

Table 3 depicts the statistical significance of OMP method. OMP proved to be useful in improving presentation skills, involvement in decision making, reasoning behind decision making, providing positive feedback as p value was highly significant for it. The p value was significant for differentiating present slide from other slides, evaluation of knowledge and skills, encouragement to read, build confidence and identify specific lacunae in their knowledge. As it was a new teaching methodology students were apprehensive initially but with repeated sessions students showed enthusiasm and were eager to continue OMP sessions in future for histology and other subheads of Anatomy.

After the sessions of OMP, the three faculty members involved in the teaching were requested to fill a feedback questionnaire regarding their experiences with OMP. The findings were summarized in Table 4. Most of the faculty members strongly agreed that OMP is feasible, useful and an effective tool which improves teaching skills, helps identify specific lacunae in learner's understanding. However, they also felt that it was more time consuming but not cumbersome. It must also be mentioned that all the faculty members were willing to adopt OMP as a teaching methodology.

Discussion

The OMP begins with open-ended questions that enhance the learner to reveal their thinking process rather than the more superficial information received spontaneously in the traditional model.[4] The OMP may help to disclose the students reasoning behind decision making. The preceptors in our study rated the OMP to be more efficient and effective teaching method. As it is one to one teaching, students got the opportunity to interact with the teacher individually so that they could ask their difficulties more frankly and the teacher could then adjust the level of questions and teaching according to the student's needs.[2,5] This reduces distance between the teacher and the student.[6] Early exposure to OMP in medical education in Anatomy may help students prepare for clinical settings, where the OMP is commonly used.[7] Positive feedback to the students is important step in their learning.[8]

In the present study teachers had already inculcated some microskills of OMP into their routine teaching. However, this finding doesn't match so well with the finding in the study by Chan LK et al,[9] which states that training experienced anatomy teachers in the use of OMP did not improve the student learning perception in the gross anatomy laboratory. They stated, confining the teachers teaching behavior to OMP structure could limit their performance. According to script theory altering the teaching behaviors of experienced teachers by asking them to adopt new teaching technique, may have decreased their teaching performance until the new scripts could be equally automated.[10]

In order to make best use of OMP the teacher should have stronger knowledge base. The lack of such knowledge and pedagogical skills may be the reason for more students saying that the post OMP was worse than the pre OMP.[9] In the present study students were eager and enthusiastic to adapt new teaching method but commented that some students were shy and it was difficult to get commitment from such students.[11] These students did not prefer the OMP since it was stressful for them. The OMP offered the teachers greater insight into student knowledge and reasoning. This result is similar to the improved self-evaluation after OMP training as reported by Kertis M.[5] The Preceptors in present study mentioned a few difficulties in using the OMP. It was more time- consuming; and it required the teachers to be more confident and have stronger knowledge base. Kachewar SG[12] implemented one minute preceptor for effective learning among radiology residents by a systematic approach toward performing the ultrasound scan for acute abdomen. In the post interventional phase he found that resident's level of learning had significantly improved. In a study conducted by Gulati HK[13] on routine teaching of histopathology slides to pathology postgraduates, found OMP to be effective in improving exam skills, communicating the findings, thinking of logical differentials, and in motivating the learner to do self-study. Most of the faculty agreed that OMP was an effective teaching tool which helped identify specific lacunae in learner's understanding and were willing to adopt OMP as a teaching methodology. However, they also felt that it was more time consuming. In another study by Harkare et al[14] use of OMP for effective clinical teaching in ear, nose and throat (ENT) for final year MBBS students found implementing OMP as effective teaching and learning method for undergraduate's students in ENT. Chan et al[9] in his study on novice teachers in the gross anatomy

laboratory found OMP to be very useful in faculty development as anatomy teacher. Aagaard et al[2] in his study on OMP found that they had greater self-confidence in rating students. Similar to present study Furney et al[15] found OMP model as a easy to administer intervention that provides modest improvements in resident's teaching skill. Teherani A et al[16] stated that students rate the OMP as a more effective model of teaching than traditional model.

OMP makes the preceptors aware of the learner's strong and weak areas of need so that they can focus on those areas and bring out the best in the learners. OMP has thus emerged as a satisfactory approach and a problem-solving tool in this scenario.

Limitations of the present study: This study has small sample size (n=30) and was performed over short period. Hence, further study with large number of subjects and conducted for longer duration is needed for drawing statistical inferences. This study mainly takes account of effect of this intervention on students as only three undergraduate teachers were involved in the study.

Conclusion

All the 30 students showed satisfaction at the end of OMP session. OMP proved to be useful in improving presentation skills, involvement in decision making, reasoning behind decision making and providing positive feedback as 'p' value was highly significant for it. As it was a new teaching methodology students were apprehensive initially but with repeated sessions students showed enthusiasm and were eager to continue OMP sessions in future for histology and other subheads of Anatomy. Most of the faculty members strongly agreed that OMP is feasible, useful and an effective tool which improves teaching skills, helps identify specific lacunae in learner's understanding. However, they also felt that it was more time consuming but not cumbersome. It must also be mentioned that all the faculty members were willing to adopt OMP as a teaching methodology.

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Pineal Gland, Some Secrecy Yet to Clear Towards its Metaphysical Connection: A Review Study

Dhanesh Kumar Sharma

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Author's Affiliation: Additional Professor and HOD, Department of Anatomy, All India Institute of Medical Sciences, Raipur 942099, Chhattisgarh, India.

Corresponding Author: Dhanesh Kumar Sharma, Additional Professor and Head, Department of Anatomy, All India Institute of Medical Sciences, Raipur 942099, Chhattisgarh, India.

E-mail: dhyanesh.sharma@gmail.com

Abstract

The pineal gland was remained and unexplained inexplicable and the last of the endocrine gland to be discovered by the scientists, hence long considered odd and mystifying. Still it is not fully understood as some underground secret of the pineal gland is still a bit of mystery and yet to be clear. Although medical science has immeasurably progressed, even then there are a few things and some mysterious facts about this critical organ the pineal gland we might not have known. The evidence from the structural and developmental biology suggests that pinealocytes possess a common evolutionary predecessor with the retinal cells. It collects information related to light through the eye and then the melatonin produced by the pineal gland directs hormonal messages to the various parts of the body. The pineal gland is recognized today as performing a great part in controlling all aspect of human functions as the regulator of regulators. It establishes our sleep-wake cycles and regulates the major physical processes like growth, metabolism and sexual development by releasing and controlling the hormones. Apart from its documented special effects on reproductive system, body growth, body temperature, blood pressure, motor activity, sleep, tumor growth, mood and the immune system, it also seems to be a definitive factor in longevity. The calcified pineal gland by disturbing the circadian rhythm affects the nature and the quality of the sleep; reduces mental performance, sexuality and cognitive functioning; and finally makes the body prone to disease and disturbed overall personality. Decalcification of the pineal gland is an imperative part of our awakening and development; as doing so intensifies our perception and awareness of realism and for higher mental performance. Also it is very important and necessary for all the achievers interested in a state of mastery and blissful life; and seeking to be converted into more evolved humans.

Keywords: Metaphysical; Mysterious; Melatonin; Circadian rhythm; Decalcification.

Introduction

The pineal gland was remained unexplained and inexplicable and the last of the endocrine glands to be discovered by the scientists, hence long considered odd and mystifying. Still it is not fully understood as some underground secret of the pineal gland is still a bit of mystery and yet to be clear. Although medical science has immeasurably progressed, even then there are a few things and some mysterious facts about this critical organ the pineal gland we might not have known. The pineal gland is significant since the ancient time. The pineal gland had been documented as very noteworthy minute organ in the human body since the ancient Greek time. Although anatomically

its location had been described in the writings of Claudius Galen, a Greek doctor and philosopher of Pergamum (an ancient Greek city) during 3rd century, but the pineal gland was not almost fully understood until the 20th century.

The pineal gland or conarium, or epiphysis cerebri, is a small endocrine gland near the center of the brain, between the two hemispheres in most of the vertebrates.[1] Although the pineal gland is located in our brain, it is actually a vital part of the endocrine system and regulates major physical processes like growth, metabolism and sexual development by releasing and controlling the hormones. The scientists have come to know that the pineal gland synthesizes a key hormone melatonin from the neurotransmitter serotonin. We have understood more about the functions of the pineal gland that melatonin production establish our sleep-wake cycles and is entirely determined by the recognition of light and dark. It has also been understood that the retina conveys these signs to the hypothalamus in the brain and from here these signals are sent to the pineal gland. If the retina detects more light then the less melatonin is produced by the pineal gland and vice versa. Melatonin level is maximum or highest in the dark at night that helps one sleep.

The pineal gland is named on the basis of its shape resemblance with the pinecone. The pineal and pinea terms are derived from the French and Latin respectively and meaning a pinecone. The pineal gland is a tiny little gland about one third of an inch in size and situated deep in the centre of the brain. The pineal gland receives a sympathetic innervation from the superior cervical ganglion. A parasympathetic innervation from the pterygopalatine and otic ganglia is also present.[2] The pineal gland connects the endocrine and the nervous system in the body. The pineal gland transforms the neural signals of the sympathetic nervous system into the hormonal signals of the endocrine system. The pineal gland is not affected by the blood-brain barrier, unlike almost rest of the brain and as not cut off from the body by the blood-brain barrier.

Discussion

The old hypothesis related to the pineal gland has not been really replaced. The relationship of the pineal gland with the mind and the soul is still a bit of mystery and yet to be cleared. Rene Descartes a French philosopher and mathematician in the 16th-century had described this pineal gland as the principal seat of the soul. He also considered it the place in which all our thoughts are formed and miniature animal strength of mind is like a very fine wind or an extremely lively and clean glow, providing life into the numerous minute arteries surrounding the pineal gland. This was likely due to his individual understanding about the anatomy and the physiology. On the other side the scientists now credit and acclaim that function to the neocortex of the brain and the academic philosophy amongst his colleagues considered the pineal gland as a neuroanatomical structure with no individual metaphysical qualities; science considered it as one endocrine gland in the body.[3]

The pineal gland was commonly described as the third eye or representing the third eye on the basis of many grounds like its situation deep in the midpoint of the brain and its association to the light and its pathways. Mystic and obscure spiritual tradition and civilization propose that it serves as a metaphysical connection between the bodily and spiritual holy worlds.[4] The pinealocytes in the pineal gland in many non-mammalian vertebrates have an obvious semblance and similarity with the photoreceptor cells of the eye. The evidence from the structural and developmental biology suggests that pinealocytes possess a common evolutionary predecessor with the retinal cells.[5] In the world, there are two religions the Hinduism and the Buddhism which use the third eye as representation for the enlightenment. The third is referred to as the eye of knowledge in Indian custom and belief. The East Asian and the Indian iconography have shown the third eye as a dot, eye or mark on the forehead of deities and other enlightened persons. The mark tilak on the forehead between the eyebrows of Hindus is a representation of the third eye, which is also seen on forehead of Lord Shiva. The Buddhists consider the third eye as the eye of consciousness that represents the vantage point from which enlightenment or Buddhism beyond one's physical sight is obtained.[6]

The tiny pineal gland is like the light measuring device in our body. It collects information related to light through the eye and then directs hormonal messages to the various parts of the body. The messages transmission to the body is finally concluded about the length of daylight and is commonly called the circadian rhythm. In this manner the pineal gland notices the body as to whether any particular duration of time is light or dark, about the climate or season around and longer or shorter days or nights. To understand what does the pineal gland do practically, if we eliminate our self for a short time from the present environment

and assisting objects, clocks and calendars we will appreciate without delay about the fundamental role of pineal gland in maintaining our connection and correlation with the nature and environment. The pineal gland maintains light sensitivity and is responsible for the construction and release of dimethyltryptamine (DMT) an entheogen; which is possibly excreted in the large quantities at the moments of birth and death.[7]

The secretory function of the pineal gland is only understood to some extent. Its situation deep central in the brain suggested to philosophers all over history that it possesses particular scrupulous importance. This grouping and combination led to its being regarded as an anonymous or mystery gland with mystical, metaphysical and occult theories and assumptions surrounding its perceived functions. The pineal gland was formerly believed to be a vestigial remnant or residue of a larger organ. The melatonin produced by the pineal gland is also significantly involved in regulating the reproduction and sex organs. The melatonin reduces the release of reproductive hormones gonadotropins from the pituitary gland and so affects growth and activities of male and female reproductive organs. In this manner, the melatonin and therefore the pineal gland control the sexual development and reproduction in the body. The melatonin also Regulates the bone mass in the body.[8] (Fig. 1).

Jacob Liberman has explained in 'Light: Medicine of the Future' reviewed in the United States and United Kingdom in 2017 and 2019, that in animals the pineal gland's size differs on the basis of the animal's location. He claimed, the animals habitual of living nearer to the equator possess a smaller pineal gland than the animals living towards progressively North or South poles. The more the animal needs to be adapted to a changing climate and environment, the larger the pineal gland in size. In Journal of Pineal Research, the pineal gland has been claimed to play a significant role in maintaining body temperature. Liberman has commented even in extensive manner that the pineal is recognized today as performing a great part in all aspect of human functions as the regulator of regulators. Apart from its documented special effects on reproductive system, body growth, body temperature, blood pressure, motor activity, sleep, tumor growth, mood and the immune system, it also seems to be a definitive factor in longevity. Researchers have found evidence that melatonin produced by the pineal gland can have a positive impact on our heart and blood pressure and may be used to treat cardiovascular disease. Lower pineal gland volume and its secretion may increase our risk of developing schizophrenia and other mood disorders. Some researchers suggest that there is a connection between impaired pineal gland function and cancer risk. A recent study evidenced that lowering pineal gland function through overexposure to light led to cellular damage and increased risk for colon cancer.

The pineal gland synthesizes a hormone called melatonin and being endocrine gland secretes or pours it directly into the blood. The melatonin being a serotonin-derived hormone modulates the sleep patterns. Melatonin production is stimulated by darkness and inhibited by light.[9] The melatonin by inhibiting the release of some reproductive hormones from the hypophysis cerebri affects the reproductive system both in males and females. The pineal gland synthesizes more melatonin in children than the adults to inhibit premature sexual development or excessive growth of reproductive organs. The pineal gland gradually shrinks and gets smaller after puberty and so releases less amount of melatonin. How much melatonin the pineal gland synthesizes and secretes is determined mainly by the amount of light exposed to the eyes. Less melatonin is secreted in the daylight hours and an increased secretion occurs during the night.

Although the pineal gland is a part of the brain but the blood-brain barrier doesn't separate the pineal gland from the body and it has a tremendous amount of blood supply, second to the kidneys only. The calcification of the pineal gland is typical and characteristic in young adults and has been found in the children as young as two years of age period. The calcified gland is frequently noticed in the skull x-rays. The calcification rates differ commonly by country to country and correlate with the increasing age.[10] The calcification of the pineal gland is connected with corpora arenacea or brain sand. Pineal gland calcification is detrimental and disadvantageous to its capability to produce melatonin[11] and associated with the gradual decrease in the mental performance. British scientist Jennifer Luke in 1990s discovered a high concentration of fluoride in the pineal gland of her subjects. Later it is found that the fluoride accumulates in the pineal gland more than any other part of the body. This accumulated fluoride commonly forms the phosphate crystals and creates a hard shell or shield around the pineal, this process is called calcification.

Human and animal are exposed to fluoride mainly via water, food and air. The natural fluoride

concentration varies strongly and depends on the local geological structure and also on local/ regional human activity. In many regions of the world, including Europe, fluoride in ground and surface water sometimes exceeds the level considered to be safe for humans.[12] The exposure of fluoride chemicals in fluoridated water, tooth pastes, pesticides, herbicides, air conditioners, freezers, computer screens, fluorescent light bulbs, plastics is the main cause of calcification of the pineal gland. The research studies have shown that as pineal gland calcification advances there is gradual decrease in synthesis of melatonin which disturbs the sleep-wake cycle and maintenance of the circadian rhythm and the children achieve pubertal age earlier. The animal studies have found the decreased melatonin secretion on fluoride exposure resulted into their accelerated sexual development. Numerous studies have claimed the hazards of fluoride exposure like calcification of the pineal gland, arthritis by calcification of cartilages, kidney diseases, low IQ and brain damage, male and female infertility, weak skeletal health by skeletal fluorosis, cardiovascular inflammation and atherosclerosis and increased lead absorption. Scientist Paul Connett and other scientists in their over 50 studies have claimed that fluoride harms our brain, bone kidney etc. and reduces human intelligence. The Biological Trace Element Research study in November 2019 claimed that feeding male rats with fluoride free diet showed stimulation of their pineal growth.

No doubt, in many ways the pineal gland connects our body with the nature. When the pineal gland is calcified, body loses its balance with nature due to lacking proper biological adjustment and improper brain execution. The calcified pineal gland by disturbing the circadian rhythm affects the nature and the quality of the sleep; reduces mental performance, sexuality and cognitive functioning; and finally makes the body prone to disease and disturbed overall personality. We know that the sleep is a very important vital biological natural function and is designed to repair and restore us every night. With a calcified pineal gland we join a race of beings mostly disconnected from instincts, from planet and from each other. Also with this non-functioning pineal gland the media messages will more easily program us, false belief systems will shape us, poor decisions will plague us and so will limit our marvelous excellent potential. Decalcification of the pineal gland is an imperative part of our awakening and development; as doing so intensifies our perception and awareness of realism and for higher mental performance. Also it is very important and necessary for all the achievers interested in a state of mastery and seeking to be converted into more evolved humans.

So, to avoid these consequences of a calcified pineal gland there is need to avoid our pineal gland to be calcified, then only we can prevent the decreased production of melatonin, throwing off the circadian rhythm and disordering the reproductive system. We require three things, the elimination of some foods and ecological reasons to avoid additional calcification; the removal of the already existing calcification of the pineal gland and the creation of a surrounding environment to support normal pineal gland execution. The three primary causes of calcification are the chlorine, the synthetic calcium and the fluoride. The chlorine in virtually all of our public water supply is also with calcifying special effects on the pineal gland. The calcium is necessary for the strong bones, but a Harvard study has linked the calcium supplements in their synthetic forms with the calcifying effect on the pineal gland and some other sites in the body, also with dementia and more risk of heart attack in aged women. For the additional calcium in diet, some organic foods like spinach, kale, broccoli, sesame seeds, chia seeds and quinoa can be consumed. Also mega food calcium, magnesium and potassium can be considered as pairing magnesium with calcium directs the calcium into the bones in place of the brain.

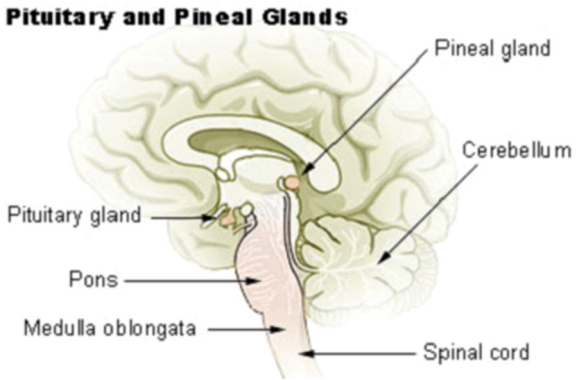

Fig. 1: Pituitary and Pineal Glands.

A research has shown that the young tea leaves have more antioxidants and less fluoride levels than older leaves; and it is the reverse with the older tea leaves. White teas have the least fluoride and most antioxidants. Mostly non-stick cookware coatings contain perfluorooctanoic acid (PFOA) and perfluorinated compounds (PFCs) which are fluoride-based substances so they are toxic and calcifying. This cookware can be replaced with stainless steel, ceramic, glass or cast iron cookware.

All processed foods possess some form of synthetic calcium like calcium phosphate, dicalcium phosphate and calcium carbonate and lead to calcification of pineal gland. The sulfuryl fluoride used in food processing to fumigate the processing tools as well as the food contaminates the food with fluoride. So for decalcifying pineal gland, processed foods should be replaced by the unprocessed organic foods. Careful consumption and exposure to these things will stop auxiliary calcification in the pineal gland. The main steps to be followed for decalcifying the pineal gland include using fluoride filters to minimize fluoride intake from the water; using alternative, fluoride-free toothpaste; stopping taking calcium supplements with synthetic calcium; avoiding using nonstick cookware with PFOA and PFC; eating organic whole foods; and avoiding processed foods and foods sprayed with pesticides.

The pineal gland is believed to be the organ of supreme universal connection and its importance come into sight in every ethnicity all over the world. The chakra system is the means the spirit moves within the physical body. The chakras are like the wheels of energy to remain balanced and experienced active only for the optimal finest human function. In standstill situation in the chakra system, the physical body gets affected with unease, depression and various illnesses.[13] The chakras are energy centers in the organs but not confined to a specific location. For instance, the heart chakra lies in the center of the chest while the physical heart is on the left side of the midline of body. Similarly, the third eye chakra lies in the center of the head at the pineal gland. The pineal gland is well-known as the third eye or Ajna chakra in the Hinduism and a closed Ajna chakra is said to develop confusion, uncertainty, cynicism and pessimism. Every esoteric and mysterious tradition heralds the third eye as our association to spirit and as a space between human and God. With an effervescent and vibrant third eye, we find our highest source of ethereal energy experiencing a blissful and joyful life called the condition of Enlightenment or Buddhism.

Conclusion

The pineal gland collects information related to light through the eye and then the melatonin produced by the pineal gland directs hormonal messages to the various parts of the body. It establishes our sleep-wake cycles and regulates the major physical processes like growth, metabolism and sexual development by releasing and controlling the hormones. Apart from its documented special effects on reproductive system, body growth, body temperature, blood pressure, motor activity, sleep, tumor growth, mood and the immune system, it also seems to be a definitive factor in longevity. The pineal gland is recognized today as performing a great part in controlling all aspect of human functions as the regulator of regulators. The calcified pineal gland by disturbing the circadian rhythm affects the nature and the quality of the sleep; reduces mental performance, sexuality and cognitive functioning; and finally makes the body prone to disease and disturbed overall personality. Decalcification of the pineal gland is an imperative part of our awakening and development; as doing so intensifies our perception and awareness of realism and for higher mental performance. The pineal gland is believed to be the organ of supreme universal connection and its importance come into sight in every ethnicity all over the world. The pineal gland is well-known as the third eye or Ajna chakra. With an effervescent and vibrant third eye, we find our highest source of ethereal energy experiencing a blissful and joyful life called the condition of Enlightenment or Buddhism. Also it is very important and necessary for all the achievers interested in a state of mastery and seeking to be converted into more evolved humans.

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Subject Index

Author Index

Guidelines for Authors

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors

Types of Manuscripts and Limits

Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

Review articles: Up to 2500 words excluding references and abstract and up to 10 references.

Case reports: Up to 1000 words excluding references and abstract and up to 10 references.

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Articles can also be submitted online from http://rfppl.co.in/customer_index.php.

I) First Page File: Prepare the title page, covering letter, acknowledgement, etc. using a word processor program. All information which can reveal your identity should be here. use text/rtf/doc/PDF files. Do not zip the files.

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The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

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State the background of the study and purpose of the study and summarize the rationale for the study or observation.

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The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http:// www. consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma. net/e/policy/l7-c_e.html).

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Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

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Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

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List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_ requirements.html) for more examples.

Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, *et al.* Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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- Conflicts of interest disclosed

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