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Journal of the Anatomical Society of India (ISSN: Print 0003-2778) is peer-reviewed journal. The journal is owned and run by Anatomical Society of India. The journal publishes research articles related to all aspects of Anatomy and allied medical/surgical sciences. Pre-Publication Peer Review and Post-Publication Peer Review Online Manuscript Submission System Selection of articles on the basis of MRS system Eminent academicians across the globe as the Editorial board members Electronic Table of Contents alerts Available in both online and print form. The journal is published quarterly in the months of January, April, July and October.

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The aim of the *Journal of the Anatomical Society of India* is to enhance and upgrade the research work in the field of anatomy and allied clinical subjects. It provides an integrative forum for anatomists across the globe to exchange their knowledge and views. It also helps to promote communication among fellow academicians and researchers worldwide. The Journal is devoted to publish recent original research work and recent advances in the field of Anatomical Sciences and allied clinical subjects. It provides an opportunity to academicians to disseminate their knowledge that is directly relevant to all domains of health sciences.

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#### Editorial



## Implementation of Competency Based Medical Education in Anatomy with Poor Teacher-Student Ratio: The Utopia

In recent times, there has been a paradigm shift in medical education due to the implementation of competency-based medical education (CBME) as part of the Government's Regulations on Graduate Medical Education.<sup>[11]</sup> These regulations envisaged that the Indian Medical Graduate (IMG) should develop competencies required to fulfill the patient's basic medical needs in society, i.e., they should possess the requisite knowledge, skills, attitudes, values, and responsiveness, so that he or she be able to solve basic medical problems in the community with skill and efficiency as physicians of the first contact. Simultaneously, he or she should be globally relevant.<sup>[2]</sup>

In addition to the first contact physician, he or she should also be a leader of the healthcare team, a good communicator, lifelong learner, and committed to the profession.<sup>[3]</sup>

The competencies were prepared accordingly, and the medical institutions all over India were asked to incorporate the same in their curriculum. This is a welcome step of the Medical Council of India (MCI)/National Medical Commission (NMC) and needs an appreciation. From these set objectives and goals we can draw that on passing out, a medical graduate should be a combo of (i) physician of the first contact, (ii) ability to solve basic medical problems, (iii) globally relevant, and (iv) have leadership capabilities of the healthcare team. For sure, all this sounds very rosy but needs a strong foundation of basic medical practice besides many other skills.

As we all know that anatomy is the backbone of medicine and provides anatomical basis of clinical practice.<sup>[4]</sup> Therefore, to produce competent IMGs it should be taught thoroughly with a blend of clinical application. Facts which are not clinically relevant for medical graduate should not be taught as a passing reference only because relevance of such anatomical facts comes later when a doctor specializes or super specializes and if he/she go for advanced training in India and abroad. The ideal way to teach anatomy is as under:

 Teaching and discussion in small groups of not >20 students per teacher in demonstrations, seminars, group discussions, and not >5 or 6 per teacher in dissection, laboratory work, etc., for quick and clear understanding along with fair and free interaction between students and teacher and among students of that group. Since the knowledge and skills developed are to be applied on people in real time, very promptly and accurately in emergency management, hence shortcuts to small group teaching should be avoided

- 2. Introduce the topic with a story and tell them about desired outcomes
- 3. Students should conduct thorough dissection of cadavers by themselves under the supervision of a teacher
- 4. Surface marking (living anatomy) should be taught thoroughly. In clinical practice this forms the anatomical basis of likely hood of the structures involved at the site of patient's complaints/trauma, etc
- 5. Expose the students to the patients from time to time so that they can have real-life experience
- 6. Frequent and short assessments of desired outcomes throughout the course of study. To achieve this percentage of marks allotted to the patient should be 50% or more
- 7. Teaching staff should also have some clinical experience.

Keeping the above facts in mind the patient of anatomy was allotted maximum teaching hours as compared to physiology and biochemistry.

All this is possible only if there is a proper teacher-student ratio.

For undergraduate medical education, initially, the MCI maintained the teacher-student ratio of 1:10 up to 2009<sup>[5]</sup> though the Mudaliar Committee (1959) had recommended a teacher-student ratio of 1:5.<sup>[6]</sup> The MCI maintained teacher-student ratio of 1:10 even when there was a shortage of qualified medical teachers by allowing the appointment of nonmedical teachers with MSc., PhD, and DSc. qualification.<sup>[7]</sup>

Thereafter, the MCI gradually reduced the ratio step by step to 1:15 in 2019,<sup>[8]</sup> 1:20 in 2010<sup>[9]</sup> to present low of 1:25 in 2015<sup>[10]</sup> which has adversely affected the anatomy teaching.

As recommended by Mudaliar Committee as early as 1959 and subsequent committees later the MCI had increased the PG seats in almost all the medical colleges across the country. As a result 2013 onwards more and more medical PGs became available for faculty positions, thus there was silver lining to ease out the teacher-student ratio. However, suddenly in 2015 as a bruit-shock to the medical fraternity, the MCI took a quick "U-turn" and reduced the teachersstudents ratio. Since medical teaching has practical, skill oriented teaching modules, CBME has been implemented. The MCI/NMC should restore the teacher-student ratio to at least 1:10 if not 1:5 immediately. At present, besides MD in anatomy, Diplomate of National Board (DNB), Fellowship of National Board (FNB) in anatomy, postgraduate medical specialists with equivalent degrees, fellowships, etc., obtained from the USA, UK, Europe, and Australia are available and can be attracted to join medical institutions in India. Once we start getting the dividends the teacher-student ratio should be further reduced to 1:5 as per Mudaliar committee and to be globally competitive which is one of the aims and objectives of the health education in the country. Furthermore what already exists in AIIMS, most of the USA, UK, European, and Australian medical institutions, providing for adjunct faculty is another feasible and good option.<sup>[5]</sup> The adjunct faculty can be drawn from the retired teachers in anatomy and specialists of various disciplines in government and private setup who can give clinically relevant lectures on clinical anatomy. This will ease out the problem of teacher-student ratio not only in anatomy but in other subjects too.

So far the CBME has been experimented only in few medical institutions that too in postgraduation with a good result because teacher-student ratio is good in postgraduation, i.e., 1:1 to a maximum of 1:3 but to have a near similar teaching comfort for undergraduates (IMGs) a systemic planning and changes in the "UG regulations" and "Recommendations on appointment of teachers in the medical colleges," as envisaged, is necessary and should be urgently taken.

#### Conclusion

In our opinion, the following steps should be taken as early as possible to produce good IMGs.

- 1. Teacher-student ratio should be increased to 1:5 as recommended by the Mudaliar committee.<sup>[6]</sup> If not as an emergency measure, it should be restored to 1:10 with immediate effect
- 2. As per the existing rule book of MCI,<sup>[7]</sup> since the demonstrators or postgraduates are not recognized as teachers they should not be included while calculating the teacher-student ratio
- 3. The present overlap of 2 months (August and September) needs to be addressed immediately. As the session should begin on <sup>t</sup> August, 1 of every year but last admissions are allowed up to September, 30 in accordance with the Hon'ble apex court. Therefore, practically the session starts from October 1<sup>st</sup> every year
- 4. The anatomy faculty for MBBS students should not be used to take anatomy classes for other courses such as dental, paramedical (viz. Occupational Therapy (OT), Bachelor in Medical Laboratory Technology (BMLT), Bachelor of Medical Radio Diagnosis and Imaging Technology (BMRIT), etc.), and nursing students. For these courses, extra faculty should be appointed as per requirement of respective councils. This adversely affects the research work which is expected of them as per "Recommendations on appointment of teachers in the medical colleges"
- 5. It is the sacrosanct for any government or management (private medical institution) to provide

good quality infrastructure and research laboratory in the anatomy and other departments for quality, meaningful, and globally competitive research. In the existing scenario, the teachers are being forced to do research without proper research facilities. This is unnecessarily killing their time in producing false-positive results which are published in predatory journals. As a corollary, a large number of International Journals have come into existence abroad publishing research papers without peer review in shortest possible time after charging money. The selection committee members should be very careful about it

- 6. Publication of research article should not be the only criteria for promotion. According to Sahai's Dogma<sup>[5]</sup> for teachers, to impart knowledge is at a higher pedestal than creation of knowledge. Therefore during the selection process for appointment and promotion, how much time did a person spent in teaching (prepared with a properly structured format duly certified by the institution) should be given more weightage than research publication. Similarly his/her depth of knowledge and the way it is expressed should be assessed
- 7. A good teacher-student ratio will also be essential to start new postgraduate/super-specialty courses in anatomy such as clinical embryology and reproductive anatomy, imaging anatomy, musculoskeletal anatomy, hepatopancreaticobiliary anatomy, endocrine anatomy, ocular anatomy, neuroanatomy, pediatric anatomy, cardiovascular and pulmonary anatomy, etc., similar to advanced countries<sup>[6]</sup>
- 8. Duration of first professional MBBS course should be restored to 18 months for countless reasons.

#### Vishram Singh<sup>1</sup>, Ashok Sahai<sup>2</sup>

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## A Computed Tomography Angiography Study to Correlate Main Renal Artery Diameter with Presence of Accessory Renal Artery in Healthy Live Kidney Donors

#### Abstract

Introduction: The chosen technique of surgery during nephrectomy can be influenced by the sudden discovery of an aberrant source of blood supply to the kidney. Thus, a prospective study was undertaken to investigate the relationship between the diameter of the main renal artery and the presence of an accessory renal artery by computed tomography (CT)-angiography. Material and Methods: The study was conducted on 115 healthy kidney donors who presented to the department of nephrology and radiology for voluntary kidney donation. All CT examinations were performed on a 64-slice CT scanner in the arterial phase. The number of the renal arteries supplying each kidney was evaluated and their diameters were measured. Results: Eighty-six of the right side and 88 of the left side of the 115 kidneys donors had a single renal artery whereas 29 had one or more accessory renal artery (aRA) on the right side and 27 had one or more aRA on the left side. The mean diameter of mRA was  $5.4 \pm 1.0$  mm in kidneys without aRA and  $4.6 \pm 10$  mm in kidneys with aRA on the right side and on the left side the mean diameter of mRA was  $5.59 \pm 1.12$ mm in kidneys without aRA and  $4.7 \pm 1.2$  mm in kidneys with aRA. Discussion and Conclusion: The presence of additional renal arteries is very probable when the main renal artery has a diameter of < 4.15 mm. Kidneys presenting a main renal artery > 5.5 mm very probably do not present additional renal arteries. Hence, the renal artery diameter is a factor which should be considered for predicting the presence of additional renal arteries.

**Keywords:** Accessory renal artery, renal artery, renal artery diameter

#### Introduction

Renal artery variations are common in the general population and the frequency of variations shows social and racial differences.<sup>[1,2]</sup> Variations are more common in Africans and Caucasians and seen less common in Indians. The frequency of extrarenal arteries shows variability from 9% to 76% and is generally between 28% and 30% in anatomic and cadaveric studies.<sup>[1,3,4]</sup> The renal arteries have been conventionally described as the lateral branches of the abdominal aorta arising inferior to the origin of the superior mesenteric artery.

Variation in the pattern of renal arteries has been reported more frequently than other large vessels in the literature and alternative nomenclatures have been used to describe the same, these include aberrant artery, supernumerary artery, etc., Graves (1956) states that any artery arising from the aorta in addition to the main renal artery should be named accessory and the arteries arising from sources other than the aorta should be called "aberrant." According to the law of poiseuille, the most important factor determining the volume of blood flow in a vessel is the diameter of the vessel and the flow is directly proportional to the fourth power of vessel diameter It is imperative that artery supplying the kidney should have a sufficient caliber to supply the organ with the amount of blood it needs.<sup>[5]</sup> The diameter of a renal artery in a kidney supplied by >1 artery can be expected to be smaller than that of the renal artery in a kidney supplied by that single artery. Thus, it can be safely derived that the blood supply to a kidney is dependent on both the number of vessels supplying the kidney and their diameters.

Since renal transplantation is the treatment of choice for patients with end-stage renal

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failure and kidney transplantation with accessory renal arteries pose a challenge to the surgeon due to higher risks of complications, both vascular and urological it is of utmost importance that the surgeon enters the operative field armed with the most accurate information about the vascular supply of the kidney.

In the past, extensive research has been undertaken on the renal arteries, both cadaveric and using imaging techniques such as ultrasonography, angiography, computed tomography (CT), and magnetic resonance imaging.<sup>[6-9]</sup> Digital angiography is considered to be the gold standard for studying the renal arterial anatomy, but CT angiography carries fewer risks and is more accurate with better detailing of the vessel wall and lumen.<sup>[10,11]</sup> Thus, whenever surgical procedures such as partial nephrectomies, pyeloplasty for uretropelvic junction stenosis, and kidney transplantation are planned, the correct anatomical information, especially regarding to vascular supply can affect the chosen surgical technique.[11-13] Thus, a prospective study was undertaken to investigate the relationship between the diameter of the main renal artery and the presence of an accessory renal artery by CT-angiography.

#### Aims and objectives

To find out the relationship between diameter of the main renal artery with the presence of an accessory renal artery.

#### **Material and Methods**

The study was conceptualized in the department of anatomy and was done in collaboration with the department of nephrology and radiology of an institute running a regular kidney transplant program. Prior approval from the Institutional Ethical Committee of the institute was obtained.

The study group consisted of 115 healthy kidney donors (92 women and 23 men with mean age 45.46 years) who presented to the nephrology and radiology department for voluntary kidney donation. All CT examinations were performed on a 64-slice CT scanner in the arterial phase. The number of renal arteries supplying each kidney was evaluated and their diameters were measured.

A total of 80–100 ml of 300 mg/ml intravenous contrast material plus a 20-ml chaser bolus was injected through an 18-gauge cannula positioned in an antecubital vein at a flow rate of 4 ml/s by a dual-head power injector. An automatic triggering system provided the scanning delay time to obtain an arterial phase image. For this purpose, a single unenhanced low-dose scan was first obtained. Based on this image, a region of interest (ROI) with an area of 1 mm<sup>2</sup> was then established by CT technologist in the lumen of the aorta at the level of the superior mesenteric artery. The patient was instructed to hold his breath as soon as the signal density in this ROI reached a threshold of 180 Hounsfield units, and the scan was initiated. The

scanning delay time was 18–24 s after the start of the injection.

In our study, the main renal artery was taken as the artery with the largest caliber and originating at the expected level in case of >1 artery supplying the kidney.

The measurement of the RA diameter was performed in the proximal segment of the renal artery at a distance of 1-1.5 cm from the ostium, where the renal artery reaches a uniform width. The data of each patient with the number of arteries supplying the kidney and their diameter were tabulated.

#### Statistical analysis

The results were expressed as the  $\pm$  standard deviation. The mean differences among the groups with and without accessory renal artery (aRA) were evaluated using the Student's *t*-test, and the correlations with mRA diameter were evaluated by Pearson's correlation analysis.

#### Results

The data for 115 healthy kidney donors were analyzed, 23 (20%) of whom were male and 92 (82%) were female. The mean age of the female kidney donors was 45.52 years and the mean age of the male kidney donors was 44.26 years (total mean age of male and female was 45.26 years) [Table 1].

Eighty-six (75%) of the 115 kidneys of the right side and 88 (76.52%) of the 115 kidneys of the left side had a single RA (with no aRA) [Figures 1 and 2], whereas 29 (25%) had one or more aRA on the right side [Figures 3 and 4] and 27 (23.48%) had one or more aRA on the

Table 1 : Distribution of Subjects according to theGender & Age						
Gender	No. (%)	Ag	ge			
		Mean	SD			
Female	88 (79)	45.52	10.46			
Male	23 (21)	44.26	13.15			
Total	111 (100)	45.26	11.02			

Table 2 : Comparison	of mRA between the Subjects
having/not hav	ing aRA in Right Side

aRA (Rt)	п	Diameter o	f mRA (Rt)	t	р
		Mean SD			
absent	86	5.40	1.05	3.579	0.001
present	29	4.60	1.00		

Table 3 : Comparison of mRA between the Subjects							
aRA (Lt)	n	Diameter o	f mRA (Lt)	t	р		
		Mean	SD				
absent	88	5.60	1.12	3.639	< 0.001		

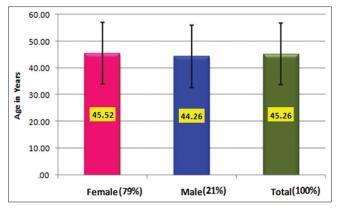


Figure 1: Distribution of subjects according to gender and age

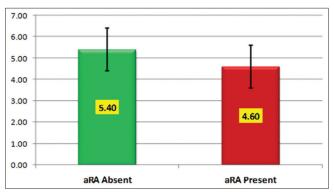


Figure 3: The mRA diameter of a kidney without an aRA and with aRA in the right kidney

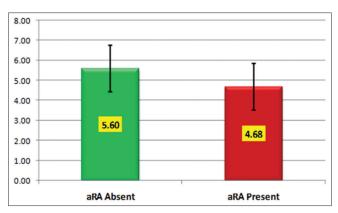


Figure 5: Comparison of mRA between the subjects having/not having aRA in left side

left side [Figures 5 and 6]. Fifty-six of the 230 kidneys had aRA. No aberrant renal artery was found.

The mean diameter of mRA was  $5.4 \pm 1.0$  mm in kidneys without aRA and  $4.6 \pm 1.0$  mm in kidneys with aRA on the right side and on the left side the mean diameter of mRA was  $5.59 \pm 1.12$  mm in kidneys without aRA and  $4.7 \pm 1.2$  mm in kidneys with aRA [Tables 2 and 3].

The mRA diameter was smaller in kidneys with aRA than in those without aRAs (P < 0.001 on the right side and P < 0.0001 on the left side).



figure 2: CT Angiography showing single renal artery on both sides

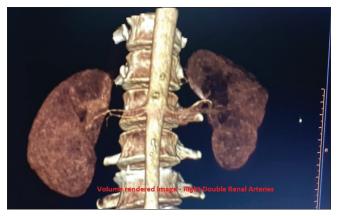


Figure 4: CT Angiography showing double renal arteries on right side



Figure 6: CT Angiography showing double renal arteries on left side

The absence or presence of an aRA was predicted by applying a Binary Regression Analysis [Table 4]. After including the parameter Side and Diameter of mRA in the Binary Logistic Regression Analysis to Predict Absence/Presence of aRA the regression equation of prediction was found to be:

 $Pr = 2.717 - 0.007 \times Side - 0.760 \times mRA$ 

In the above equation Side = 1 if there is right side else Side = 0

mRA represents the diameter of mRA.

The presence of aRA predicted if Pr > 0.5.

The above model has predictive accuracy of 77.4%.

Binary Logistic Regression Analysis to Predict Absence/ Presence of aRA by the Side and Diameter of mRA.

Validity Parameter	<b>Estimated Value</b>	95% Confidence Interval		
		Lower	Upper	
Sensitivity (Presence of aRA accurately Predicted)	14.3	9.8	18.8	
Specificity (absence of aRA accurately Predicted)	97.7	95.8	99.6	
PPV (Prob. Of aRA presence)	66.7	60.6	72.8	
NPV (Prob. Of aRA absence)	78.0	72.6	83.3	
Accuracy	77.4	72.0	82.8	
Parameter <i>B</i> S.E.	p			

.982

< 0.001

.002

A poor sensitivity (only 14.3%) was found for the fitted logistic regression model. However, the model has a good specificity of 97.7% and accuracy (77.4%). To predict the presence of aRA on the basis of side and diameter of mRA then a positive value of Predictive value (Pr) meant aRA present, but in that case, only among 14.3% cases we actually found the presence of aRA. On the other hand, a negative value of Pr meant aRA is absent, and in that case, prediction will be accurate in 97.7% cases that is we actually found the absence of aRA. Hence, the overall accuracy of model is 77.4%.

-.007

-.760

2.717

.327

.167

.856

#### Discussion

Side Rt

Constant

Dia. of mRA

Embryologically kidney develops from pronephros, mesonephros, and metanephros. The former two regresses but the arterial network to these segments may remain and lead to supernumerary renal arteries. Knowledge of the arterial anatomy of the kidney is important in partial and complete nephrectomies. If there is a single renal artery it is considered as favorable factor with a lower incidence of complications. Bleeding due to an invasive procedure performed on an aRA may result in open laparotomy. Missed case of an aRA may cause infarct in a transplanted kidney and associated hypertension in the recipient. It has been documented that the presence of variations increases the likelihood of warm ischemia time, vascular thrombosis, hemorrhage, difficulty in carrying out anastomosis, and greater risk of urinary fistulas and urethral lesions.<sup>[14,15]</sup>

Accessory renal arteries constitute the most common, clinically important renal vascular variant. The prevalence of an aRA has been generally accepted to be approximately 25%–30% in different study series.<sup>[1-4,7]</sup> The prevalence of aRA in our study group (24%) was similar to that described in the literature. Cadaveric studies have also found >1 aRA in different studies with the incidence of 2%–3%<sup>[16,17]</sup> but we could not find a  $3^{rd}$  renal artery supplying the kidney in our study.

Not only the number of arteries supplying the kidney but also the orthogonal diameter of each artery is important information for the radiologists and surgeons before any intended instrumentation. According to a study carried out by Aytac *et al.* using sonography and digital subtraction angiography,<sup>[6]</sup> it was derived that if the renal arterial diameter is 4.65 mm or less there is quite probability of an accessory renal artery, and if it is <4.15 mm the probability of an accessory increases with 98% specificity. They also concluded that if they encountered mRA diameter 5.5 mm or more, they could not find an accessory renal artery. In our study too we found out that the mRA diameter was smaller in kidneys with aRA than in those without aRAs. In our study, the mean renal artery diameter in case of absent aRA was 5.40 mm (right side) and 5.59 mm (left) which was significantly more as compared to cases with present aRA, 4.6 mm (right), and 4.7 mm (left) (P < 0.001in the right side and P < 0.0001 in left side).

Our study suggests that the diameter of the main renal artery supplying the kidney is a key factor to be investigated and documented before proceeding with any interventions such as transplant or nephrectomies.

#### Conclusion

In light of the fact that there is limited surgical visibility and exposure in laparoscopic nephrectomies and complications associate with accessory renal arteries, it is imperative to have a thorough knowledge about renal vasculature before any intervention is planned. The statistically significant difference in diameter of mRA in kidneys without aRA and in kidneys with aRA shows that the renal artery diameter is a factor which should be considered preoperatively for predicting the presence of additional renal arteries.

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Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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#### **Original Article**



## Morphological Side Differences of the Hemipelvis

#### Abstract

Introduction: Differences of anatomical characteristics regarding side and gender have been the topic of interest in various recent studies. Studies have reported either significant or insignificant differences of the bony pelvis. The aim of this study was to evaluate possible gender and side differences of the pelvis in a cadaveric model. Material and Methods: Fifty human cadaver pelves, preserved by the use of Thiel's method, underwent measurement during this study. Diverse parameters were measured on both hemipelves by three surgeons. Analysis of the morphology of the acetabular cavity was performed by measuring its longitudinal, horizontal, and maximal diameters. Results: The distance between the anterior superior iliac spine and the posterior superior iliac spine (females: mean of 15.9 cm; males: mean of 16.9 cm) and the horizontal diameter of the acetabular cavity (females: mean of 4.5 cm; males: mean of 4.9 cm) were statistically significantly shorter in females than in males. The subpubic angle was significantly (P < 0.001) larger in females (mean 61.4°; standard deviation [SD] 11.02°; range 37°-82°) when compared to males (mean 45.5°; SD 7.48°; range 35°-60°). The vertical diameter of the obturator foramen was significantly (P = 0.002) smaller for the right (mean 3.1; SD 0.56; range 1.9-4.6) in comparison to the left side (mean 3.4; SD 0.57; range 2.5-5.2). Discussion and Conclusion: Overall, a clear gender difference was observed for typical gender-specific parameters, whereas the anatomy of the hemipelves showed no relevant side differences.

Keywords: Gender differences, hemipelvis, morphologic differences, pelvis, side differences

#### Introduction

Differences of anatomical characteristics regarding side and gender have been the topic of interest in various recent studies.

Magee,<sup>[1]</sup> Macedo and as well as Moromizato et al.<sup>[2]</sup> found statistically differences regarding significant the passive range of motion between dominant and nondominant sides in several joints. Bonneau et al.<sup>[3]</sup> examined 91 adult femora and evaluated an increased anteversion of the femoral neck in women compared to men. In Rouleau et al.'s<sup>[4]</sup> radiologic study concerning the proximal ulna dorsal angulation, this was significantly decreased on left female extremities compared to male elbow joints. Chanplakorn et al.[5] conducted a computed tomographic (CT) analysis of 740 pedicles concerning the cervical vertebral segments three to seven. The authors did not find significant differences during side comparison; however, male cervical pedicles showed larger dimensions of some studied parameters compared to females in their sample. Yu *et al.*<sup>[6]</sup> found an increased pedicle height and width in the thoracic vertebral segments 1-6in 503 specimens, whereas Chawla *et al.*<sup>[7]</sup> stated no significant side differences regarding the third lumbar vertebra.

Some studies have also reported either significant or insignificant differences of the bony pelvis.<sup>[8-10]</sup> The aim of this study was to evaluate possible gender and side differences of the pelvis in a cadaveric model.

#### **Materials and Methods**

#### **Dissection and measurement**

A total of 50 pelves from adult human cadavers donated to science, embalmed with Thielxs method,<sup>[11]</sup> were evaluated. All investigated cadavers were donated to the Department of Macroscopic and Clinical Anatomy of the Medical University of Graz under the approval of the Anatomical Donation Program of the University of Graz and according to the Austrian law for donations. Twenty-eight were from male and 22 from female body donors. None of the specimens had signs of

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previous fractures or malformations. After removal of the surrounding soft tissues and bilateral enucleation of the hip joints, measurements in centimeters were conducted on both hemipelves using a digital caliper. Three surgeons individually measured several parameters [Figures 1 and 2], and the mean of their results was assessed.

Detailed analysis of the morphology of the acetabular cavity was performed by measuring its long, horizontal, and maximal diameters [Figure 2].

In addition, the thickness of the acetabular fossa, was measured (in millimeters), and the anterior acetabular ridge shape was morphologically classified as either straight, curved, or irregular [Figure 3]. The acetabular depth was evaluated by the placement of a ruler across its diameter and measurement of the distance between the deepest part of the acetabular cavity and the ruler.

#### Statistical analysis

All measurements were exported into Microsoft Excel sheets (Microsoft Excel 2010; Microsoft, Redmond, WA, USA). Statistical analysis was performed using SPSS

- 1 Anterior superior iliac spine-posterior superior iliac spine
- 2 Anterior inferior iliac spine-posterior inferior iliac spine
- 3 Anterior superior iliac spine–proximal part of ischial tuberosity
- 4 Anterior superior iliac spine-iliopectineal eminence
- 5 Anterior inferior iliac spine-iliopectineal eminence
- 6 Anterior superior iliac spine-anterior inferior iliac spine
- 7 Posterior superior iliac spine-posterior inferior iliac spine
- 8 Iliopectineal eminence-pubic tubercle
- 9 Pubic tubercle-pubic symphysis
- 10 Height of pubic symphysis
- 11 Anterior superior iliac spine-iliac eminence
- 12 Anterior superior iliac spine-thinning out of iliac eminence
- 13 Posterior wall of acetabulum proximal
- 14 Posterior wall of acetabulum distal
- 15 Distance between 13 and 14
- 16 Subpubic angle
- 17 Obturator foramen oblique diameter
- 18 Obturator foramen vertical diameter
- 19 Ischial tuberosity height
- 20 Ischial tuberosity breadth

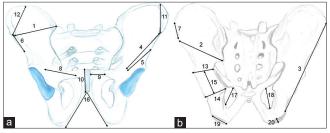


Figure 1: Measurement pattern. The pelvis is depicted from the ventral (a) and dorsal sides (b)

statistical software (version 22.0; IBM Corp, Armonk, NY, USA). For descriptive statistic mean  $\pm$  standard deviation (SD), median, minimum, and maximum were calculated. For side comparison, dependent *t*-tests were conducted and for gender comparison, a repeated ANOVA measurement was conducted, including gender as within-subject effect. By the use of Bonferroni correction, a value of P < 0.002 for side and gender differences was regarded as statistically significant.

#### Results

The distance between the anterior superior iliac spine (ASIS) and the posterior superior iliac spine (PSIS) showed a mean of 16.9 cm in males (SD 1.10; range 14.3-19.4) and 15.9 cm in females (SD 0.99; range 13.50-17.40, respectively. Repeated measurement ANOVA revealed a statistically significant difference between sexes (P = 0.001). The horizontal diameter of the acetabular cavity was significantly (P = 0.002) shorter in females (mean 4.5; SD 0.38; range 3.1-5.3) than in males (mean 4.9; SD 0.42; range 4.1-5.9). In addition, the subpubic angle was significantly larger in females (mean  $61.4^\circ$ ; SD  $11.02^\circ$ ; range  $37^\circ - 82^\circ$ ) compared to males (mean 45.5°; SD 7.48°; range 35°–60°), (P < 0.001), [Figure 4].

Regarding side differences, the vertical diameter of the obturator foramen was significantly (P = 0.002) smaller for the right (mean 3.1; SD 0.56; range 1.9–4.6) in comparison to the left side (mean 3.4; SD 0.57; range 2.5–5.2).

Statistically insignificant differences regarding gender and sides are presented in Tables 1 and 2.

The anterior acetabular ridge's shape was straight in 57 hemipelves, curved in 36 and irregular in seven specimens. In 14 pelves, their hemipelves showed different patterns,

1	Longitudinal diameter	
2	Horizontal diameter	
3	Maximal diameter	

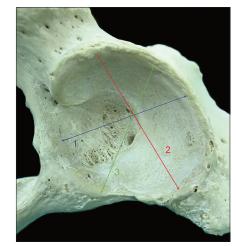


Figure 2: Evaluation of the acetabulum

n	Sex	n	Mean	SD	Median	Minimum	Maximum	Р
1	Male	56	16.87	1.10	17.05	14.30	19.40	0.001
	Female	44	15.87	0.99	16.00	13.50	17.40	0.001
2	Male	56	15.26	1.67	15.30	10.10	17.70	0.096
-	Female	44	14.45	1.77	15.01	10.30	17.00	0.070
3	Male	56	14.29	1.11	14.26	12.36	16.54	0.025
5	Female	44	13.48	1.41	13.30	9.70	17.29	0.025
4	Male	56	8.20	1.22	8.38	3.90	10.30	0.705
т	Female	44	8.31	1.17	8.53	3.90	10.40	0.705
5	Male	56	5.34	1.17	5.11	3.68	10.40	0.030
5	Female	50 44	4.79	0.96	4.60	3.50	8.80	0.030
6	Male	56	4.79	0.90	4.50	2.95	7.90	0.900
0	Female	30 44	4.56	0.74	4.50	3.53	5.62	0.900
-							5.62 6.54	0.022
7	Male	56	4.07	0.93	3.92	2.23		0.932
0	Female	44	4.09	0.97	3.93	2.10	6.87	0.051
8	Male	56	5.92	0.97	6.00	3.65	7.89	0.951
~	Female	44	5.90	1.07	5.98	3.10	7.90	0.400
9	Male	56	7.56	1.12	7.74	2.51	9.60	0.490
	Female	44	7.73	1.09	7.90	5.20	9.60	
10	Male	56	5.19	0.49	5.20	4.02	6.46	0.142
	Female	44	4.93	0.78	4.89	3.32	7.40	
11	Male	56	7.40	1.63	7.77	3.54	10.00	0.537
	Female	44	7.14	1.33	7.46	2.93	8.80	
12	Male	56	5.55	1.64	5.33	2.60	8.81	0.330
	Female	44	5.15	1.51	4.91	2.30	8.17	
13	Male	56	5.33	0.61	5.26	3.94	7.43	0.091
	Female	44	5.07	0.59	5.04	3.87	6.42	
14	Male	56	5.07	0.63	5.10	3.10	7.32	0.064
	Female	44	4.78	0.57	4.73	3.40	6.35	
15	Male	56	4.41	0.78	4.25	2.80	6.40	0.399
	Female	44	4.24	0.83	4.08	2.80	6.40	
16	Male	56	45.50	7.48	45.00	35	60	< 0.001
	Female	44	61.41	11.02	59.00	37	82	
17	Male	56	4.92	0.68	5.07	3.00	6.26	0.039
	Female	44	4.56	0.83	4.64	2.60	6.00	
18	Male	56	3.19	0.53	3.09	2.00	4.60	0.570
-	Female	44	3.27	0.64	3.25	1.90	5.15	
19	Male	56	6.02	1.02	5.89	4.23	9.20	0.504
- /	Female	44	5.79	1.44	5.41	3.80	9.50	0.001
20	Male	56	3.23	0.45	3.24	2.25	4.31	0.180
-0	Female	44	3.07	0.45	3.07	1.80	3.90	0.100

SD: Standard deviation



Figure 4: Difference of subpubic angle in a female (a) and male (b) pelvis

whereas eleven of these had a combination of a curved and straight ridge and further three pelves had an irregular and a curved form.

Figure 3: Variations of the anterior acetabular ridge's shape (a) straight, (b) curved, (c) irregular

n	Side	п	Mean	SD	Median	Minimum	Maximum	Р
1	Left	50	16.47	1.15	16.70	13.50	19.00	0.346
	Right	50	16.38	1.18	16.55	13.80	19.40	
2	Left	50	14.92	1.77	15.30	10.10	17.50	0.798
	Right	50	14.89	1.76	15.06	10.30	17.70	
3	Left	50	14.00	1.35	13.85	9.70	17.29	0.085
	Right	50	13.87	1.28	13.90	10.20	16.79	
4	Left	50	8.20	1.09	8.40	3.90	10.30	0.586
	Right	50	8.30	1.30	8.38	3.90	10.40	
5	Left	50	4.85	0.78	4.81	3.50	7.80	0.020
	Right	50	5.34	1.43	5.02	3.60	10.50	
6	Left	50	4.52	0.56	4.51	2.95	5.72	0.518
-	Right	50	4.57	0.68	4.47	3.53	7.90	
7	Left	50	4.19	1.07	4.03	2.10	6.87	0.085
	Right	50	3.97	0.80	3.85	2.23	6.06	
8	Left	50	5.97	0.95	6.00	3.65	7.90	0.496
-	Right	50	5.85	1.07	5.98	3.10	7.89	
)	Left	50	7.66	1.22	7.81	2.51	9.60	0.76
	Right	50	7.60	0.99	7.82	5.20	9.60	
10	Left	50	5.07	0.61	5.10	3.39	6.90	0.902
	Right	50	5.08	0.69	5.10	3.32	7.40	
1	Left	50	7.39	1.38	7.80	3.54	9.80	0.119
	Right	50	7.18	1.63	7.61	2.93	10.00	01112
12	Left	50	5.17	1.48	5.14	2.60	8.69	0.023
-	Right	50	5.58	1.68	5.41	2.30	8.81	0.02
13	Left	50	5.20	0.66	5.20	3.87	7.43	0.687
	Right	50	5.24	0.57	5.19	3.96	6.70	0.00
14	Left	50	4.97	0.55	4.95	3.89	6.35	0.410
	Right	50	4.91	0.68	4.85	3.10	7.32	0111
15	Left	50	4.32	0.78	4.20	2.80	6.40	0.834
	Right	50	4.35	0.83	4.09	3.13	6.40	0.05
7	Left	50	4.79	0.69	4.80	3.00	5.81	0.66
	Right	50	4.73	0.84	4.80	2.60	6.26	01000
8	Left	50	3.37	0.57	3.35	2.50	5.15	0.00
	Right	50	3.09	0.56	3.03	1.90	4.60	0.002
9	Left	50	5.95	1.21	5.80	4.18	9.50	0.44
.,	Right	50	5.88	1.21	5.69	3.80	9.20	0.77
20	Left	50	3.15	0.51	3.13	1.80	4.30	0.74
_0	Right	50 50	3.17	0.45	3.19	1.90	4.31	0.740

SD: Standard deviation

Table 2a: Eva	aluated char	acterist	ics of the ac	etabulum	concerning	gender differe	ences	
	Sex	п	Mean	SD	Median	Minimum	Maximum	Р
1	Male	56	5.11	0.44	5.10	4.20	6.05	0.050
	Female	44	4.86	0.49	4.79	4.10	5.81	
2	Male	56	4.88	0.42	4.90	4.12	5.93	0.002
	Female	44	4.54	0.38	4.50	3.12	5.34	
3	Male	56	5.12	0.37	5.09	4.30	6.08	0.010
	Female	44	4.86	0.36	4.80	4.24	5.63	
Depth acetabular cavity	Male	56	3.39	0.51	3.34	2.40	5.10	0.038
	Female	44	3.11	0.55	3.09	1.80	4.80	
Thickness acetabular fossa (mm)	Male	56	6.64	4.33	6.75	0.60	16.00	0.104
	Female	44	4.79	3.41	5.00	0.50	13.00	

SD: Standard deviation

Table 2b	: Evaluat	ed chai	racteristics	of the acc	etabulum wit	h sides differer	ices	
	Side	n	Mean	SD	Median	Minimum	Maximum	Р
1	Left	50	5.00	0.48	4.96	4.16	6.03	0.836
	Right	50	5.00	0.48	5.00	4.10	6.05	
2	Left	50	4.71	0.48	4.67	3.12	5.68	0.414
	Right	50	4.75	0.40	4.73	4.01	5.93	
3	Left	50	4.98	0.38	4.94	4.24	6.00	0.272
	Right	50	5.03	0.40	5.00	4.30	6.08	
Depth acetabular cavity	Left	50	3.27	0.49	3.29	1.90	4.40	0.924
	Right	50	3.27	0.60	3.20	1.80	5.10	
Thickness acetabular fossa(mm)	Left	50	5.74	3.91	5.81	0.70	16.00	0.448
	Right	50	5.91	4.21	5.61	0.50	16.00	

SD: Standard deviation

#### Discussion

Possible side and gender differences have been under discussion in various articles.

Boulay *et al.*<sup>[8]</sup> performed measurements by the use of an electromagnetic device which provided three-dimensional spatial coordinate measurements in twelve anatomical specimens. During this analysis, 349 different targets, including angles and distances, were checked in detail for side differences. On the right side, the main axis of the obturator foramen was significantly larger compared to the left side. This was comparable to the presented sample with a significantly smaller vertical diameter (P = 0.002) on the right (mean 3.1 cm) compared to the left side (mean 3.4 cm).

The distance between the ASIS and the PSIS showed no side differences which was also observed by Boulay *et al.*<sup>[8]</sup> However, a statistically significant difference (P = 0.001) between the sexes was observed in the present study between ASIS and PSIS (males: 16.9 cm; females: 15.9 cm).

Among other characteristics, Ma *et al.*<sup>[12]</sup> evaluated the acetabular depth in 100 patients by routine CT scans. They found a significant difference between male and female specimens in both coronal (2.1 vs. 1.8 cm) and axial (2.5/2.6 vs. 2.3 cm) planes but no differences regarding side.

Zeng *et al.*<sup>[10]</sup> in a CT analysis reported that the acetabular depth was significantly smaller in women (left: 1.74 cm; right: 1.73 cm) than in men (left: 1.94 cm; right: 1.93 cm). However, further statistical tests did not reveal significant differences when the acetabular depth was adjusted for individual body height.

Chauhan *et al.*<sup>[13]</sup> found a significant gender difference between both acetabular depths (males: left: 28.18 mm, right: 27.49 mm; females: left: 25.70 mm, right: 24.68 mm). In contrast, our results only showed a tendency of a larger acetabular depth compared to previous studies. No gender difference (female: 3.11 cm; male: 3.39 cm) or side difference (left: 3.27 cm; right: 3.27 cm) was observed. However, the horizontal diameter of the acetabular cavity was significantly shorter in females (mean 4.5 cm) than in males (mean 4.9 cm). Chauhan *et al.*<sup>[13]</sup> observed a slight significant difference between sexes on the right side (males: right: 4.71 cm, left: 4.75 cm; females: right: 4.44 cm, left: 4.60 cm). These results were comparable to the presented values.

In a noncomparable analysis of the morphology of the anterior acetabular ridge's shape in 154 hip joints by Taştekın Aksu *et al.*,<sup>[14]</sup> the shape was found to be curved in 46.1%, straight in 23.3%, angular in 16.8%, and irregular in 13.6%. Thoudam and Chandra.<sup>[15]</sup> and Vyas *et al.*<sup>[16]</sup> found the curved form to be most common with 60%. In our sample, the straight form was most present in even 57%, although the occurrence of this form has been reported in a low range of 4%–4.5%<sup>[15]</sup> up to 31.6%.<sup>[16]</sup> However, these highly varying results may be due to interobserver variability.

Not surprisingly, we also observed a significantly larger subpubic angle in females (mean  $61.4^{\circ}$ ; SD  $11.02^{\circ}$ ; range  $37^{\circ}-82^{\circ}$ ) in comparison to males (mean  $45.5^{\circ}$ ; SD  $7.48^{\circ}$ ; range  $35^{\circ}-60^{\circ}$ ). Igbigbi and Nanono-Igbigbi<sup>[17]</sup> observed higher values for their Ugandan collective with a mean of  $116.1^{\circ}$  (range: 75-155) for females and  $93.9^{\circ}$  (range: 50-140) in males in a radiologic study. Small *et al.*<sup>[18]</sup> reported an average angle of  $84.1^{\circ}$  (range: 52.5-100.2) in females and of  $63.9^{\circ}$  in males (45.3-93.2) black South Africans. Since our sample consisted of Caucasian specimens, varying values may be the result of racial differences.

#### Conclusion

Overall, a clear gender difference was observed for typical gender-specific parameters, whereas the anatomy of the hemipelves showed no relevant side differences. In contrast, Boulay *et al.*<sup>[8]</sup> found some differences, which were clinically unimportant due to their large sample size. The results in the present study indicate that gender and side differences may be neglected during preoperative planning of the acetabular component for total hip arthroplasty

or during the use of prebended plates during acetabular osteosynthesis.

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#### **Conflicts of interest**

There are no conflicts of interest.

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#### **Original Article**



## The Morphological Variants of Dural Venous Sinuses

#### Abstract

**Introduction:** In this study, we aimed to analyze the dural venous system variations in Turkey by magnetic resonance imaging examinations. **Material and Methods:** Images of a total of 200 patients (65 males, 135 females M/F: 0.48) who underwent a magnetic resonance venography examination were retrospectively screened. **Results:** Variation was detected in 101 patients (53.85% of males [35/65] and 48.89% of females [66/135]). In 16.5% of the patients, only one variation of dural venous system was detected, while the most common variation was left transverse hypoplasia in this group. Twenty-six percent of the patients had two variations of the dural venous system since the most common dual variations were left transverse hypoplasia + left sigmoid hypoplasia in this group. In 8% of the patients, three or more variations of the dural venous system were observed as the most common variations were right transverse hypoplasia + right sigmoid hypoplasia + presence of occipital sinus in this group. **Discussion and Conclusion:** It is essential to know the anatomical variations of the dural venous system for the discrimination between pathological processes such as thrombosis and physiologic conditions. Furthermore, the association of these variations with each other must be kept in mind for the explanation of the presence of multiple variations in the same individuals.

Keywords: Anatomy, dural venous sinuses, magnetic resonance venography, variation

#### Introduction

The dural venous system, comprised including widespread venous structures superior sagittal (unpaired), inferior sagittal (unpaired), transverse, sigmoid, straight (unpaired), superior-inferior tentorial, occipital (unpaired), petrosal, cavernous, sphenoparietal, petrosquamous, anterior-posterior intercavernous sinuses (unpaired), torcular herophili (unpaired), basilar and falcine venous plexuses. Because of this complex texture, various variations may be seen in the dural venous system.<sup>[1]</sup>

Thrombosis and septic thrombophlebitis are the main pathological conditions of the dural venous system, which leads to severe neurological consequences and even death.<sup>[2]</sup> For the differentiation of anatomic variations and pathological conditions such as dural venous thrombosis-thrombophlebitis, anatomy and the variations of the dural venous system must be well-known to prevent misdiagnosis.<sup>[1]</sup> Advances in imaging techniques provide a better examination of main dural venous structures. Especially

Computed Tomography Venography and magnetic resonance venography (MRV) are very useful for the discrimination of variations and thrombosis.<sup>[3]</sup>

In this study, we examined the frequency and associations of anatomical variations of the dural venous system in our patients by MRV.

#### **Material and Methods**

Medical records of 206 patients  $(41 \pm 19 \text{ years old } [18-78])$ who underwent MRV with complaints of headache, nausea, vomiting, vertigo, seizure or neurological deficits between June 2017 and June 2019 were screened in the hospital database. Six patients were excluded from the study in whom a dural venous sinus thrombosis was detected. Among these 6 ones, thrombosis was seen in only right transverse sinus in two patients, in only superior sagittal sinus (SSS) in one patient. In two patients, left transverse + left sigmoid sinus thrombosis was detected. SSS + left transverse sinus thrombosis was observed in remained one patient. Finally, 200 patients were included in this study.

MRV examinations were performed by using a two-dimensional TOF MRV

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technique on GE Signa Excite 1.5 Tesla Magnetic Resonance Imaging system (Signa Excite HD; GE Medical Systems, Milwaukee, WI, USA) with a standard head coil. Time of repetition/time of echo (TR/TE) was 28/10.8, and a Flip angle of  $60^{\circ}$  was used. The field of view was 240 mm, with a matrix size of  $256 \times 160$ . Images were acquired in coronal planes with a slice thickness of 1.5 mm. Maximum pixel intensity projection images were obtained, and the images were interpreted by two radiologists. The sinuses, except occipital and falcine sinuses, were reported as normal, hypoplastic, or agenetic. Occipital and falcine sinuses were reported as absent or present.

Patients were classified into four groups as having no dural venous variation (Group C), only one variation (Group O), two variations (Group D), and three or more variations (Group T+).

The study was approved by a local ethics committee on August 06, 2019, with a number of 2019-14/142

#### Results

Among these 200 patients, variation was observed in 101 ones. The overall variation rate was 50.5%. The incidence of dural venous system variation was found to be as 53.85% (35/65) in males and 48.89% (66/135) in females. The variation rate was found higher in males than in females [Table 1]. These variations are believed to be occurring as a consequence of a congenital condition with no association with aging and other pathological processes as they may be seen in childhood and do not exhibit an alteration at the subsequent imaging examinations.<sup>[4]</sup>

#### Groups

In 99 patients (49.5%) (control Group 1 [C]) there was no dural venous system variation. Only one variation was detected in 33 patients (16.5%) (Group 2 [O]) [Table 2]. Two variations were observed in 52 patients (26%) (Group 3 [D]) [Table 3]. Three or more variations were seen in 16 patients (8%) (Group 4 [T+]) [Table 4].

The most common single variation was left transverse hypoplasia, which was detected in 17 patients (8.5%) [Figure 1]. Left transverse hypoplasia + left sigmoid hypoplasia was the most common dual variation that was detected in 25 patients (12.5%) [Figure 2]. The most common triple or more variations was right transverse hypoplasia + right sigmoid hypoplasia + presence of occipital sinus, which was observed in 4 patients (2%) [Figure 3].

There was no significant difference for the presence of complaints between the groups as the symptoms were observed with similar rates in these four groups.

#### **Transverse sinuses**

A total of 33 variations were detected in the right transverse sinus. The frequency of variation of the right transverse sinus was found to be 16.5%. Hypoplasia was observed in 30 patients while aplasia in remained three ones.

A total of 59 variations were detected in the left transverse sinus. The frequency of variation of the left transverse sinus was found to be 29.5%. Hypoplasia was observed in 56 patients, while aplasia remained three ones [Figure 4].

#### Sigmoid sinuses

A total of 23 variations were detected in the right sigmoid sinus. The frequency of variation of the right sigmoid sinus was found to be 11.5%. Twenty-two of the variations of right sigmoid sinus were hypoplasia while one aplasia detected.

A total of 37 variations were detected in the left sigmoid sinus. The frequency of variation of the left sigmoid sinus was found to be 18.5%. Thirty-six of the variations of left sigmoid sinus were hypoplasia, while aplasia was detected in one patient.

#### Superior sagittal sinus

A total of two variations of the SSS were observed, while all of them were hypoplasia. No aplasia of SSS was detected. The frequency of variation of SSS was found to be 1%.

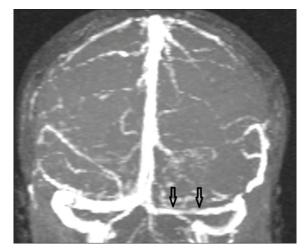


Figure 1: Left transverse sinus hypoplasia (arrows)

	Table 1: Distribution of the	ne patients to the groups acc	ording to the number of vari	ations		
	n (rate%)					
	Group 1 (C)	Group 2 (O)	Group 3 (D)	Group 4 (T+)		
	No variation	One variation	Two variations	≥3 variations		
Female	69 (51.1)	22 (16.3)	34 (25.2)	10 (7.4)		
Male	30 (46.2)	11 (16.9)	18 (27.7)	6 (9.2)		
Total	99 (49.5)	33 (16.5)	52 (26)	16 (8)		

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#### Straight sinus

A total of four variations were detected in the straight sinus. The frequency of variation of the straight sinus



Figure 2: Left transverse sinus hypoplasia (long arrows) +left sigmoid sinus hypoplasia (short arrows)



Figure 3: Right transverse sinus hypoplasia (long arrows) + right sigmoid sinus hypoplasia (short arrows) + occipital sinus (arrowheads)

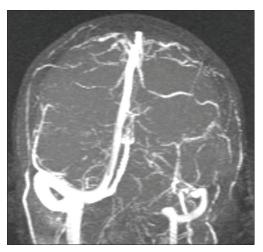


Figure 4: Left transverse sinus aplasia

was found to be 2%. Three hypoplasia and one aplasia of straight sinus were observed.

#### Inferior sagittal sinus

A total of 11 variations were detected in the inferior sagittal sinus. The frequency of variation of the inferior sagittal sinus was found to be 5.5%. Six of the variations of inferior sagittal sinus were hypoplasia, while 5 ones were aplasia.

#### Occipital and falcine sinus

In 19 patients (9.5%), occipital sinus was detected. The most common variations which accompanied the presence of occipital sinus were right transverse hypoplasia + right sigmoid hypoplasia [Table 5].

We did not observe falcine sinus in this study.

#### Discussion

As the dural venous system includes various superficial and deep structures, various pathological conditions and anatomical variations can affect this system. For accurate differentiation of these physiological and pathological conditions, the variations and their association must be well known.<sup>[5]</sup> In this study, we examined the frequency and types of variations. Furthermore, we investigated the associations of these variations with each other.

We identified thirty hypoplasia and three aplasias in the right transverse sinus while the overall variation frequency of right transverse sinus was 16.5%. In 56 patients, hypoplasia and in 3 ones aplasia of the left transverse sinus was detected with a total variation frequency of 29.5%. In a study by Surendrababu *et al.*<sup>[6]</sup> they detected right transverse sinus hypoplasia in 13%, left transverse sinus hypoplasia in 35%, and left transverse sinus aplasia in 1% of their patients. Our results were compatible with this study regarding variation rates of right and left transverse sinuses. Goyal *et al.*<sup>[7]</sup> observed variation of the right transverse sinus in 0.7%). They detected hypoplasia of the left transverse sinus in 21.3% and aplasia of

		n (rate%)	
	Female	Male	Total
Left transverse hypoplasia	11 (8.1)	6 (9.2)	17 (8.5)
Inferior sagittal hypoplasia	1 (0.7)	2 (3.1)	3 (1.5)
Right transverse hypoplasia	2 (1.5)	-	2 (1.0)
Straight sinus hypoplasia	2 (1.5)	-	2 (1.0)
Inferior sagittal aplasia	1 (0.7)	2 (3.1)	3 (1.5)
Occipital sinus	2 (1.5)	1 (1.5)	3 (1.5)
Left sigmoid hypoplasia	2 (1.5)	-	2 (1.0)
Straight sinus aplasia	1 (0.7)	-	1 (0.5)
Total	22 (16.3)	11 (16.9)	33 (16.5

Table 3: Distribution of the patients with two va	ariations (Group 3) acco	ording to their variation	n types		
Double variations	<i>n</i> (rate%)				
	Female	Male	Total		
Left transverse hypoplasia + left sigmoid hypoplasia	18 (13.3)	7 (10.8)	25 (12.5)		
Right transverse hypoplasia + right sigmoid hypoplasia	5 (3.7)	8 (12.3)	13 (6.5)		
Left sigmoid hypoplasia + left transverse aplasia	1 (0.7)	1 (1.5)	2 (1.0)		
Right transverse hypoplasia + left transverse hypoplasia	2 (1.5)	-	2 (1.0)		
Right transverse hypoplasia + inferior sagittal aplasia	-	1 (1.5)	1 (0.5)		
Left transverse hypoplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Right transverse hypoplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Left sigmoid hypoplasia + inferior sagittal aplasia	1 (0.7)	-	1 (0.5)		
Right sigmoid hypoplasia+ occipital sinus	1 (0.7)	-	1 (0.5)		
Right transverse hypoplasia + superior sagittal hypoplasia	-	1 (1.5)	1 (0.5)		
Left transverse hypoplasia + inferior sagittal hypoplasia	1 (0.7)	-	1 (0.5)		
Left transverse hypoplasia + left sigmoid aplasia	1 (0.7)	-	1 (0.5)		
Right transverse aplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Right tranverse aplasia + right sigmoid hypoplasia	1 (0.7)	-	1 (0.5)		
Total	34 (25.2)	18 (27.7)	52 (26.0)		

## Table 4: Distribution of the patients with three or more variations (Group 4) according to their variation types

Three or more variations	<i>n</i> (rate%)			
	Female	Male	Total	
Right transverse hypoplasia + right sigmoid hypoplasia + occipital sinus	3 (2.2)	1 (1.5)	4 (2)	
Left transverse hypoplasia + left sigmoid hypoplasia + occipital sinus	-	2 (3.1)	2(1)	
Right transverse hypoplasia + Left transverse hypoplasia + occipital sinus	2 (1.5)	-	2(1)	
Right transverse aplasia + Left transverse hypoplasia + right sigmoid hypoplasia	-	1 (1.5)	1 (0.5)	
Right transverse hypoplasia + left transverse hypoplasia + inferior sagittal hypoplasia	1 (0.7)	-	1 (0.5)	
Right transverse hypoplasia + right sigmoid hypoplasia + straight hypoplasia	-	1 (1.5)	1 (0.5)	
Left transverse aplasia + left sigmoid hypoplasia + occipital sinus	1 (0.7)	-	1 (0.5)	
Left transverse hypoplasia + Left sigmoid hypoplasia + superior sagittal hypoplasia	1 (0.7)	-	1 (0.5)	
Left transverse hypoplasia + left sigmoid hypoplasia + occipital sinus + inferior sagittal hypoplasia	-	1 (1.5)	1 (0.5)	
Right transverse hypoplasia + left transverse hypoplasia + right sigmoid hypoplasia + right	1 (0.7)	-	1 (0.5)	
sigmoid hypoplasia + occipital sinus				
Right transverse hypoplasia + right sigmoid aplasia + occipital sinus	1 (0.7)	-	1 (0.5)	
Total	10 (7.4)	6 (9.2)	16 (8)	

Variations	<i>n</i> (rate%)				
	Female	Male	Total		
Occipital sinus (one variation)	2 (1.5)	1 (1.5)	3 (1.5)		
Left transverse hypoplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Right transverse hypoplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Right sigmoid hypoplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Right transverse aplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Right transverse hypoplasia + right sigmoid hypoplasia + occipital sinus	3 (2.2)	1 (1.5)	4 (2.0)		
Left transverse hypoplasia + left sigmoid hypoplasia + occipital sinus	-	2 (3.1)	2 (1.0)		
Right transverse hypoplasia + left transverse hypoplasia + occipital sinus	2 (1.5)	-	2 (1.0)		
Left transverse aplasia + left sigmoid hypoplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Left transverse hypoplasia + left sigmoid hypoplasia + occipital sinus + inferior sagittal hypoplasia	-	1 (1.5)	1 (0.5)		
Right transverse hypoplasia + left transverse hypoplasia + right sigmoid hypoplasia + right sigmoid hypoplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Right transverse hypoplasia + right sigmoid aplasia + occipital sinus	1 (0.7)	-	1 (0.5)		
Total	14 (10.4)	5 (7.7)	19 (9.5		

the left transverse sinus in 4.1% of their patients (total variation rate was 25.4%). While our left transverse sinus variation rate was similar to their results, but our right transverse sinus variation rate was markedly higher. Their bilateral transverse sinus hypoplasia rate was 1.5%. We found bilateral transverse sinus hypoplasia in our 6 patients (3%). Our bilateral transverse sinus hypoplasia rate was compatible with their study. Alper et al.[8] detected left transverse sinus hypoplasia in 39%, aplasia in 20%, right transverse sinus hypoplasia in 6%, and aplasia in 4% of their patients. When compared with their study, our left transverse sinus variation rate was lower and right transverse sinus variation rate was higher. Tantawy *et al.*<sup>[9]</sup> reported left transverse sinus hypoplasia in 22%, aplasia in 3.6%, right transverse sinus hypoplasia in 8%, and aplasia in 1.7% of their 363 patients. When compared with their results, our transverse sinus variation rate was higher on both sides (right and left).

We observed 23 variations in the right sigmoid sinus, while 22 of them were hypoplasia and one was aplasia. The variation rate of the right sigmoid sinus was 11.5%. There were 36 hypoplasia and one aplasia in the left sigmoid sinus with a variation rate of 18.5%. The variation rate of the left sigmoid sinus was higher than the right sigmoid sinus variation rate. In a study by Ahmed et al.[5] they detected right sigmoid sinus hypoplasia in 2.9% (n = 6) of their patients, but they did not observe right sigmoid sinus aplasia. The variation rate of left sigmoid sinus was 23.3% while they found left transverse sinus hypoplasia in 22.05% (n = 45) and aplasia in 0.98% (n = 2) of their patients. Our variation rate of the left transverse sinus was mildly lower than their results (18.5% vs. 23.3%), but we observed higher variation rates of the right sigmoid sinus (11.5% vs. 2.9%). Shirodkar et al.[10] reported right transverse sinus variations in 6% (hypoplasia in 5% and aplasia in 1%) and left transverse sinus variations in 13% (hypoplasia in 7% and aplasia in 6%) of their patients. Our results were similar to their results, but the rate of aplasia of the left transverse sinus was lower in our patients (1.5% vs. 6%).

A total of 2 variations of the SSS were observed in our study, while all of them were hypoplasia of  $1/3^{rd}$  of the SSS. No aplasia of SSS was detected. The rate of variation of SSS was found to be 1%. Goyal *et al.*<sup>[7]</sup> reported a 2.3% variation rate (0.4% (n = 6) was hypoplasia of anterior  $1/3^{rd}$  part) of SSS. Ahmed *et al.*<sup>[5]</sup> did not observe any SSS variations in their 204 patients. Our variation rate results of SSS was compatible with previous studies.

Three hypoplasia and one aplasia (2% n = 4) of straight sinus were detected in our study. Ahmed *et al.*<sup>[5]</sup> reported no variation of the straight sinus in their patients. Goyal *et al.*<sup>[7]</sup> identified 4 hypoplasia (0.2%) in their patients. We found higher variation rates of straight sinus when compared with previous studies. Among 11 variations (5.5%) of inferior sagittal sinus variations, 6 ones were hypoplasia, and 5 ones were aplasia in our study. Ahmed *et al.*<sup>[5]</sup> reported a 13.95% variation rate of the inferior sigmoid sinus. Ayanzen *et al.*<sup>[11]</sup> detected inferior sagittal sinus in only 52% of their patients. The variation rate of the inferior sagittal sinus in our study was lower than the results of previous studies.

We detected occipital sinus in 19 patients (9.5%). Goyal *et al.*<sup>[7]</sup> reported occipital sinus in 1.4% of their patients. However, in literature, some studies suggested that occipital sinus was found in 4%–10%<sup>[11,12]</sup> of individuals. Our occipital sinus results were similar to previous studies.

Falcine sinus is a dural venous sinus variation with an incidence of 5.3%, which may be associated with straight sinus variations.<sup>[13]</sup> We did not observe any falcine sinus in especially our patients with hypoplastic/agenetic straight sinus.

We detected dual venous sinus variations in 51 patients (25.5%). The most common dual variations were left transverse hypoplasia + left sigmoid hypoplasia (n = 25, 12.5%), right transverse hypoplasia + right sigmoid hypoplasia (n = 13, 6.5%), left transverse aplasia + left sigmoid hypoplasia (n = 2, 1%), and right transverse hypoplasia + left transverse hypoplasia (n = 2, 1%).

We observed three or more variations in 16 patients (8%). The most common three or more variations were right transverse hypoplasia + right sigmoid hypoplasia + occipital sinus (n = 4 2%), left transverse hypoplasia + left sigmoid hypoplasia + occipital sinus (n = 2 1%) and right transverse hypoplasia + left transverse hypoplasia + left transverse hypoplasia + occipital sinus (n = 2 1%).

We observed that in the patients with multiple dural venous variations, ipsilateral transverse and sigmoid variations usually accompany each other, and also frequently an occipital sinus presents with such conditions. This is probably due to provide the drainage via the occipital sinus.

#### Conclusion

Multiple dural venous sinus variations may be misdiagnosed as thrombosis especially in the patients with neurological complaints as dural venous thrombosis usually involves two or more different dural sinuses. The frequency and the association of multiple variations must be well known because accurate discrimination between these variations and pathological conditions such as thrombosis is essential to prevent misdiagnosis.

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#### **Conflicts of interest**

There are no conflicts of interest.

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## An Evaluation on the Morphology of the Nasal Bone, Piriform Aperture, and Choana on Dry Skulls

#### Abstract

Introduction: Piriform aperture (PA) and nasal bone (NBs) are important structures that contribute to the formation of the nose. Both anatomic structures show differences based on ethnicity, gender, and age. Hence, it is widely used to determine sex in science branches such as anthropology and forensic medicine. Furthermore, morphometry of the PA and choana is an important criterion for physiological nasal respiration of individuals. Recognition of structural differences of PA, NB, and choana along with all this information becomes important during maxillofacial procedures to be performed especially in plastic and reconstructive surgery. The aim of the present study is to determine the individual differences in NBs, PA, and choana. Material and Methods: This study was conducted on 83 Turkish dried skulls and PA, NBs, and choana was examined as morphometrically. PA was classified into seven types and NB was classified into eight subtypes. Results: The most common type of PA was found type 5 (20%-24.1%) and the least most common type of PA was type 7 (4%-4.8%). Type 1 was determined as the most common observed shape of the NB. The mean width of the choana on the right and left sides was found  $13.21 \pm 1.4$  and  $13.98 \pm 1.81$ mm, respectively. Moreover, the mean height of the choana on the right and left sides was found  $25.56 \pm 3.06$  and  $26.1 \pm 2.5$  mm, respectively. Discussion and Conclusion: We believe that obtained data from our study will constitute a morphometric data set and will be useful in a wide range of fields from forensic science to reconstructive surgery.

**Keywords:** Choana, morphometry, nasal bone, piriform aperture, variation

#### Introduction

The piriform aperture (PA) and nasal bones (NBs) are important structures that contribute to the formation of facial structures. Morphological features of the PA and NBs are widely used in different areas, such as nasal reconstruction, rhinoplasty, and for surgical or aesthetic purposes. Further, they can be used in forensic facial reconstruction, which is a method used to identify the dead and determine sex for anthropological purposes.<sup>[1]</sup> Furthermore, the structure of the NB and the PA for physiological respiration is as important as the morphological structure of the choana and the length of the airway.<sup>[2]</sup>

The NB is an important structure in the center of the face. It is surrounded by the frontal bone on the superior. The superior margin of the NB is narrow, thick, and wavy, and articulated with the nasal notch of the frontal bone. The inferior margin

of the NB is thin. This inferior margin clings to the lateral cartilages of the nose. The lateral margin of the NB is redented for articulation with the frontal process of the maxilla. The medial margin articulates with NB on the other side and superior this margin is thicker. The vertical part of the medial margin extending posteriorly is articulated with the spine of the frontal, the perpendicular plate of the ethmoid bone, and the septal cartilage of the nose.<sup>[2-4]</sup> The nose has three vaults, including the bony, upper cartilaginous, and lower cartilaginous vaults. The shape of the bony vault (comprising of paired NBs and the frontal process of the maxilla) is usually pyramidal and forms one-third of the external nose.[3-6]

The PA is located at the most anterior side of the bony nasal airway<sup>[7]</sup> and it is generally pear-shaped and surrounded by the NBs on the top, the frontal process of the maxilla on the left and right sides, and the palatine process of the maxilla below.<sup>[1]</sup> The region of the PA is the narrowest portion of the

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bony nasal airway, and this region constitutes 2/3 of the total nasal resistance in the bony cavum.  $^{[8]}$ 

All surgical procedures in this region should be performed on the basis of NBs and PA differences. Rhinoplasty is a surgical procedure that should have as little margin of error as possible. Anatomical structural differences should be taken into account in surgeries performed in this area. Similarly, osteotomies are widely used for narrowing or widening the nasal base, improving bone deformities, and repairing open-roof deformities. NB osteotomies (especially lateral osteotomy) may be performed safely at the transition zone that exists along the frontal process of the maxilla from the PA to the radix, along the lateral nasal wall. Because of the fragmentation risk, osteotomies may be more difficult in patients who have short NBs.<sup>[3-6]</sup> A detailed understanding of the structure and function of the nasal region is critical for providing proper treatment. Besides knowing where the anatomical differences are, it is important to know how they contribute to clinical procedures. Thus, we aimed to determine if there is any difference in the NB, PA, and choana that may affect structurally the nasal respiration.

### **Material and Methods**

Our study was performed with 83 Turkish dried skulls obtained from the bone collection at Necmettin Erbakan University and Karatay University. Permissions were obtained from Karatay University's, Pharmaceuticals, and Non-Medical Devices Research Ethics Board (2016/012). A digital caliper was used for skull measurements including the height of the NBs at the midpoint (HNB), the lateral edge height of the right NB (HNB-R), the lateral edge height of the left NB (HNB-L), the width of the NB at the top (WNBT), the width of the NB at the bottom (WNBB), the width of the right NB (RNB) and left NB (LNB) at the midpoint (WRNB and WLNB, respectively), the apex width of the PA (AWPA), the width of the PA at the level of the lower ends of the NBs (WPAT), the width of the PA at the bottom (WPAB), the height of the PA (HPA), the distance between the nasion and anterior nasal spine (N-ANS). the width of the right and left choana (CW1, CW2) at the level of the midpoint of the posterior bony aperture of the choana and the height of the right and left choana (CH1, CH2). The shape of the NBs and PA were classified.

A new classification derived from the Lang and Baumeister<sup>[9]</sup> classification was used for NB typing. NBs were classified into eight subtypes.

- Type 1 and Type 3: There is an angulation which becomes narrower from the edges toward the midline at the bottom. If the transverse thickness of the bone on the midline is greater than 4 mm, it is called type 1. If the thickness is lower than 4 mm, it is called type 3
- Type 2 and Type 6: There is an angulation which becomes narrower from the edges toward the midline

on the top of the NB. If the transverse thickness of the bone is greater than 4 mm, it is called type 2. If the thickness is lower than 4 mm, it is called type 6

- Type 4 and Type 8: There is no angulation. Nasomaxillary sutures are parallel to each other on the midline. If the transverse thickness of the bone is greater than 8 mm, it is called type 8. If the thickness is lower than 4 mm, it is called type 4
- Type 5: There was an angulation at the midpoint of the NB
- Type 7: There was no angulation. The NB is wider at the bottom and narrower on the top [Figures 1-3].

The PA was classified into seven types, including 1-Pear shape, 2-Reverse heart shape, 3-Rhomboid shape, 4-Drop shape, 5-Ellipsoid shape, 6-Trapezoid shape, and 7-Round shape [Figures 4 and 5].

#### Statistical analysis

All data were evaluated using SPSS 21.0 (Statistical Package for the Social Science; IBM, Chicago, IL, USA). The mean values, standard deviations, maximum and minimum values, and percentages were determined for descriptive analyses. Furthermore, the relationship between RNB and LNB shape and PA shape was analyzed with multiple corresponding analyses.

#### Results

This study was conducted on 83 dried skulls (unknown gender). The mean values and standard deviations of the parameters of the NBs, PA, and choana were identified and are shown in Table 1. NB forms were categorized into eight subtypes by revising the classification of Lang and Baumeister [Figures 1-3].<sup>[9]</sup> Type 1 (narrowing from the edges to the midline, angulation at the lower part of the bone, bone mid thickness greater than 4 mm) was the most commonly observed shape of NB [Table 2, and Figures 1, 2a]. Type 3 was the least common type of NB [Table 2, Figure 1 and 2b]. The most common type of PA was Type 5 (ellipsoid shape, 20%–24.1%) and the least common type of PA was Type 7 (round shape, 4%–4.8%) [Table 3, Figures 4 and 5].

The association between shapes of the NB and PA was evaluated through multiple corresponding analyses (SPSS 21.0). The statistical analysis revealed that the shape of the PA tended to be in the form of a reverse heart, ellipsoid, or drop if the left NB was Types 2 or 7. The shape of the PA tended to be in the form of a trapezoid if the left NB was Type 3 or 6. Similarly, it was found that the shape of the PA tended to be in the form of a pear, round, or rhomboid if the left NB was Type 1, 4, or 8. Besides, it was observed that NBs that had an angulation at the midpoint, namely Type 5, did not contribute to the formation of the PA shape [Figure 6].

When the relationship between the RNB and the PA was examined, it was seen that pear and round shaped PA

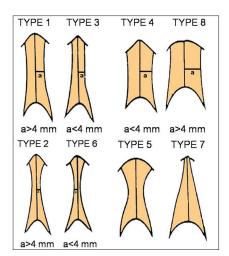


Figure 1: Schmeatic drawing of nasal bone types (Lang and Baumeister<sup>(9)</sup> classification was revised, a: midline transverse thickness)



Figure 3: (a) Type 6 NB, (b) Type 7 NB, (c) Type 4 and 8 NB (NB: Nasal bone, RNB: Right nasal bone, LNB: Left nasal bone, yellow arrows show angulation level in nasal bones, \*angulation is in the upper part of type 6)



Figure 2: (a) Type 1 NB, (b) Type 2 and 3 NB, (c) Type 5 NB (NB: *Nasal bone*, RNB: Right nasal bone, LNB: Left nasal bone, yellow arrows show angulation level in nasal bones, angulation is in the lower part of the nasal bone in type 1 and type 3, in the upper part of type 2, and in the middle part in type 5)

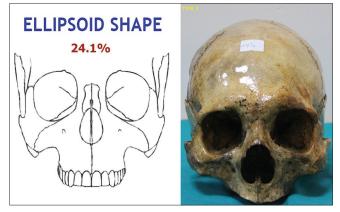


Figure 4: The most common type of piriform aperture (Type 5-Ellipsoid shape)



Figure 5: Other types of piriform aperture (pear shape, drop shape, reverse heart shape, trapezoid shape, rhomboid and round shape of piriform aperture)

were present with Type 4 and 7 RNBs. If the RNBs were Type 1 or 8 the PA tended to be in the form of a reverse heart, ellipsoid, or rhomboid. Similarly, if the RNB was in the form of Type 2, the PA tended to be in the form of a drop. In addition, Type 5 and 6 did not contribute to the formation of PA shapes [Figure 7].

#### Discussion

The NB, PA and choana were evaluated on 83 dried skulls of Turkish origin in this study. The size and morphology of the NB, PA, and choana are variables between different races, ethnic groups, genders, and ages.<sup>[6,7,10-14]</sup> The differences in these parameters revealed the importance of measurements of NB, PA, and choana in a wide range of fields from forensic medicine to maxillofacial surgery. There are studies that present the bone structure of the nasal through computed tomography (CT) and bone studies in the literature.<sup>[1,4,10,15,16-21]</sup> Although recent CT studies have gained momentum due to clear gender determination and high resolution, the shapes of the NB due to the angulation with the maxillary bone may be detected.<sup>[1]</sup>

The nose is located in the central part of the face and is physiologically and esthetically important. Esthetic and functional nasal surgeries are performed in plastic

Table 1: Maximum, minimum, mean values and							
standard deviations of the nasal bone, piriform aperture,							
a para	ameters (n: s	skull number	; mm)				
п	Minimum	Maximum	Mean±SD				
83	12.55	32.87	21.5±3.76				
83	11.73	32.67	$24.05 \pm 3.74$				
83	13.72	31.11	$23.66 \pm 3.83$				
83	6.52	18.08	$12.65 \pm 2.68$				
83	11.34	23.51	16.74±2.63				
83	3.69	12.18	7.73±1.69				
83	3.77	10.90	7.58±1.7				
83	7.65	20.50	$11.99 \pm 2.6$				
83	10.70	27.41	20.24±2.97				
83	20.23	31.37	24.27±2.13				
83	19.10	42.90	28.63±4.58				
83	24.27	60.31	50.81±4.91				
	tions of a part	n      Minimum        83      12.55        83      11.73        83      13.72        83      6.52        83      11.34        83      3.69        83      7.65        83      10.70        83      20.23        83      19.10	tions of the nasal bone, piriformnMinimumMaximum8312.5532.878311.7332.678313.7231.11836.5218.088311.3423.51833.6912.18833.7710.90837.6520.508310.7027.418320.2331.378319.1042.90				

77 CH2 19.88 31.38 26.1±2.5 PA: Piriform aperture, HNB: The height of nasal bone at the midpoint, HNB-R: The lateral edge height of right nasal bone, HNB-L: The lateral edge height of left nasal bone, WNBT: The width of nasal bone at the top, WNBB: The width of nasal bone at the bottom, WRNB: The width of the right nasal bone at the midpoint, WLNB: The width of the left nasal bone at the midpoint, AWPA: The apex width of PA, WPAT: The width of the PA at the level of the lower ends of the nasal bones, WPAB: The width of PA at the bottom, HPA: The height of PA, N-ANS: The distance between nasion and anterior nasal spine, CW1. CW2: The width of the right and left choana. CH1. CH2: The height of right and left choana, SD: Standard deviation.

10.11

10.08

15.31

77

77

77

16.67

21.13

34.65

13.21±1.4

13.89±1.81

25.56±3.06

	Table 2: Nasal bone types (%)					
Туре	Left, <i>n</i> (%)	Right, <i>n</i> (%)				
Type 1	32 (38.6)	32 (38.6)				
Type 2	22 (26.5)	19 (22.9)				
Type 3	2 (2.4)	0 (0)				
Type 4	6 (7.2)	6 (7.2)				
Type 5	1 (1.2)	3 (3.6)				
Type 6	2 (2.4)	2 (2.4)				
Type 7	6 (7.2)	4 (4.8)				
Type 8	12 (14.5)	17 (20.5)				
Total	83 (100)	83 (100)				

Table 3: Piriform aperture types (%)				
PA	n (%)			
Pear shape (Type 1)	17 (20.5)			
Reverse heart shape (Type 2)	11 (13.3)			
Rhomboid shape (Type 3)	5 (6)			
Drop shape (Type 4)	16 (19.3)			
Ellipsoid shape (Type 5)	20 (24.1)			
Trapezoid shape (Type 6)	10 (12)			
Round shape (Type 7)	4 (4.8)			
Total	83 (100)			
PA: Piriform aperture				

216

CW1

CW2

CH1

surgery procedures worldwide.<sup>[4]</sup> Two-thirds of the nose isstructurallyimmobile and is comprised two separate anatomic parts, including the bony (upper) vault and the cartilaginous (middle) vault. The anatomical structure of the nasal region may be deformed after rhinoplasty procedures. Surgeons should be aware of the anatomical structure of the nasal region and the possible conditions of the nasal region to avoid potential risks (nasal valve obstructions-middle vault collapse). The incidence of collapse on the middle vault was higher in patients with shorter NBs, longer, weaker or upper lateral cartilages, and thinner skin compared to patients with longer NBs. The knowledge of the morphometric measures in the nasal region is of relevance for surgical procedures such as rhinoplasty, osteotomies, and aesthetic reconstructions.<sup>[22]</sup>

The most remarkable findings in NB measurements of this study that the mean NB width was 12.65 mm at the proximal location and the lateral side edges of the NBs stretched down, becoming concave at the midpoint (approximately 7.66 mm) [Table 1]. In comparison to other studies conducted on Turkish populations, it was observed that the NB height in our study was higher in the data obtained from the studies of Kaplanoglu et al.<sup>[15]</sup> and Yuzbasioglu et al.,<sup>[1]</sup> but lower than the data obtained from Karadag et al.[20] Our findings were consisted with the data of Uygur et al.<sup>[24]</sup> In this study, the NB height has been found lower than German, Austrian, Black American, Korean, and Iranian populations. Especially, this height was found to be higher than the Indian population [Table 4]. The NB width on the midpoint was lower than German, Korean, Indian population and also than in the Turkish population in the study of Yuzbasioglu et al.<sup>[1]</sup> [Table 4]. This finding confirms that the NB has a more concave structure toward the midline in our population.

The NB shape was categorized into eight types in the German population (79 dried skulls) by Lang and Baumeister.<sup>[9]</sup> Although the Lang and Baumeister<sup>[9]</sup>'s classification is quite wide, it is considered complex by some authors. Thus, other researchers have revised the classification into five types.<sup>[4,16-18]</sup> Type B and Type A were most common (52.3% and 43.2%, respectively-88 dried skulls from Korean adults) according to Hwang et al.[4] Similarly, Prado et al.[16] found that the most common type of NB was A (28.6% for female, 20.6% for male), while D (1% for female, 1% for male) was the least common type in both genders. They also emphasized that there was a significant difference between sexes. Asghar et al.[17] stated that the most common types of NB were Type A (45%) and Type C (20%), followed by Type B (15%), Type E (15%), and Type D (5%), without any significant sexual variation (P = 0.1443). It was emphasized that the Type A and Type B were the most common types of NBs in the most comprehensive studies about Turkish NB classifications which were performed by Yuzbasioglu et al.<sup>[1]</sup> and Kaplanoglu et al.<sup>[15]</sup> According to this classification, the most common NB types were Types

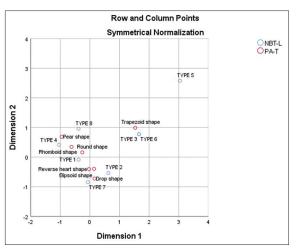


Figure 6: The multiple corresponding analysis graphic of the left nasal bone and piriform aperture

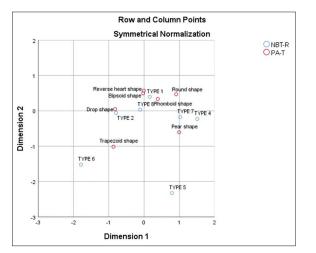


Figure 7: The multiple corresponding analysis graphic of the right nasal bone and piriform aperture

A (39.76%), B (27.11%), and E (24.7%), respectively. Unlike other researchers, type E has a significant value with a percentage of 24.7 [Table 5] [The types corresponding to type A, B, C, D, and E are given as an explanation at the bottom of Table 5]. When compared with other studies conducted on Turkish populations, type A was higher in this study than the data obtained by Kaplanoglu *et al.*<sup>[15]</sup> and Yuzbasioglu *et al.*<sup>[11]</sup> but lower than the data obtained by Uygur *et al.*<sup>[24]</sup>

Anthropological studies have emphasized that nasal structures of individuals belonging to races living in different geographies are adapted for warming and moistening of inspired air. The length of the nasal airway is increased and the base is narrowed in colder and drier climates. Depending on the increase on the surface area, the period for warming and moistening of the inspired air is longer.<sup>[19]</sup> Hwang *et al.*<sup>[4]</sup> has been reported that the shape and size of the PA may be affected by environmental conditions.<sup>[4,15,16]</sup> Furthermore, morphological differences and dimensions of the PA have been reported as an

important sex indicator. PA size and shape may be used as an anatomical landmark for determination of ethnic differentiation.<sup>[1,16]</sup> PA is a variant opening in terms of shape. These differences also affect the width and height of the opening. Therefore, the width measurement was performed on three different regions, including the apex, upper and lower part in this study.

Our study showed that the width of the PA ranged from 20–32 mm (the mean PA width was 24.27 mm) [Tables 1 and 4]. When compared with other studies conducted on the Turkish populations, it was observed that the PA width in our study was found consistent with the data of Aksu *et al.*,<sup>[2]</sup> Yuzbasioglu *et al.*,<sup>[1]</sup> and Uygur *et al.*,<sup>[24]</sup> but also higher than the data obtained by Karadag *et al.*<sup>[20]</sup> In addition, the PA width data was similar to other races; however, it was found significantly larger than the study of Lopez *et al.*<sup>[25]</sup> [Table 4]. The mean PA height was found 28.63 mm in our study.

This data were lower than the findings of other researchers. This finding supports the ellipsoid shape, which was the most common PA shape observed in our study [Table 4].

Ofodile<sup>[19]</sup> emphasized that the PA was oval in the Ashanti population, triangular in White and Indian populations, and varied from oval to triangular in American individuals. Prescher et al.[30] evaluated 84 human skulls anthropologically. They declared that the PA area was larger in males and the shape of the PA was pear-shaped in both genders. Yuzbasioglu et al.[1] found the most common type of PA was Type 1 (pear-shape). Asghar et al.[17] and Durga et al.<sup>[18]</sup> classified the PA into four types (1-long and narrow, 2-triangular, 3-triangular to oval, and 4-tending to roundness), based on the shape and the PA index. Asghar et al.[17] and Durga et al.[18] found the most common PA type to be Type 3 (83.5% for Asghare et al.<sup>[17]</sup> and 45.09% for Durga et al.[18]). De Araújo et al.[21] stated that the most common PA shape was pear-shape (39.1%). Yuzbasioglu et al.<sup>[1]</sup> classification system was used in our study and the ellipsoid-shape (Type 5, 24.1%) was the most common PA aperture type [Table 3 and Figure 4].

Moreover, choana is the convex structure where the airflow passes from the posterior cavum to the nasopharynx. Therefore, choana width and length measurements are important parameters for NB and PA to warm and moisten the inspired air. The choana width at the level of the midpoint of the posterior bony aperture of the choana and choana height was found to be  $13.21 \pm 1.4$  mm,  $13.89 \pm 1.81$  mm, and  $25.56 \pm 3.06$  mm,  $26.1 \pm 2.5$  mm for the right and left side, respectively [Table 1]. Aksu *et al.*<sup>[2]</sup> determined this rate  $13.09 \pm 1.56$  mm,  $13.33 \pm 1.36$  mm and  $24.45 \pm 2.61$  mm,  $23.77 \pm 2.42$  mm, respectively.

#### Conclusion

This study provided information about the morphological structures of the NB, choana, and PA in the Turkish

Table 4: The comparison of the nasal bone and piriform aperture measurements according to the researchers (mm)								
	Sample	The height of nasal	Nasal bone	PA	PA	PA	PA	
		bone at the midpoint	width	height	width 1	width 2	width 3	
Lang and Baumeister <sup>[9]</sup>	German	24.9	13	29.1	-	16.3	23.6	
Ofodile <sup>[19]</sup>	Austrian	30.2	-	-	-	-	21.6	
Ofodile <sup>[19]</sup>	Black American	27.9	-	-	-	-	23.4	
Hommerich and Riegel <sup>[23]</sup>	German	-	-	-	-	15.7	23.1	
Hwang <i>et al</i> . <sup>[4]</sup>	Korean	25.2	9*	29.05	-	16.9	25.55	
Uygur <i>et al</i> . <sup>[24]</sup>	Turkish	21.02	-	35.95	-	15.37	23.99	
Lee <i>et al</i> . <sup>[10]</sup>	Korean	20.95	-	-	-		24.01	
López et al. <sup>[25]</sup>	Brazilian	-	-	49.18	-	17.41	12.64	
Karadag et al. <sup>[20]</sup>	Turkish	29.81	-	-	-	-	18.5	
Prado <i>et al</i> . <sup>[16]</sup>	Brazilian	-	-	47.5	-	-	32.5	
Aksu <i>et al</i> . <sup>[2]</sup>	Turkish	-	-	33.03	-	-	23.24	
Moreddu <i>et al</i> . <sup>[26]</sup>	French	-	-	34.45	-	-	24.66	
Nidugala <i>et al</i> . <sup>[27]</sup>	Indian	19.37	24.03**	-	-	-	-	
Zamani Naser and Panahi Boroujeni <sup>[28]</sup>	Iranian	24.6	-	-	-	-	24.47	
Yüzbaşioğlu et al. <sup>[1]</sup>	Turkish	18	11.9*	31.7	-	-	23.9	
Roy <i>et al</i> . <sup>[7]</sup>	Caucasian	-	-	-	-	-	21.56	
Abdelaleem et al. <sup>[29]</sup>	Egyptian	-	-	36.68	-	-	28.81	
Asghar <i>et al</i> . <sup>[17]</sup>	Indian	17.58	12.2***	30.37	-	-	23.84	
Kaplanoglu <i>et al</i> . <sup>[15]</sup>	Turkish	17.5	-	36.76	-	-	23.41	
De Araújo et al. <sup>[21]</sup>	Brazilian	-	-	30.4	-	-	25.7	
Durga <i>et al</i> . <sup>[18]</sup>	Indian	16.85	11.68***	29.2	-	16.1	24.22	
Our study	Turkish	21.5	7.66***	28.63	11.99	20.24	24.27	

\*This measurement was taken from the top point of each side of nasal bones, \*\*This width is a total breadth of each nasal bone at the midpoint, \*\*\*This width is a breadth that was taken from the midpoint of nasal bone. PA: Piriform aperture

Table 5: Nasal bone types according to some researchers (%)								
Researchers	Sample	Type A (%)	<b>Type B (%)</b>	Туре С (%)	Type D (%)	Туре Е (%)		
Lang and Baumeister <sup>[4]</sup>	German population	68.3	10.1	1.3	10.1	10.1		
Hwang et al. <sup>[3]</sup>	88 dried skulls (Korean population)	43.2	52.3	4.5	0	0		
Uygur et al. <sup>[24]</sup>	38 cranium skeleton (Turkish population)	63.16	7.89	5.26	5.26	18.43		
Lee <i>et al</i> . <sup>[10]</sup>	75 patients' CT images (Korean population)	53.41	14.77	17.05	0	0		
Prado <i>et al.</i> <sup>[16]</sup>	97 individual's radiographs (Brazilian population)	49.48	27.84	13.40	2.06	7.22		
Yüzbaşioğlu et al. <sup>[1]</sup>	120 MDCT images (Turkish population)	38.33	30	3.33	9.17	19.17		
Asghar et al. <sup>[17]</sup>	40 dried skulls (Indian population)	45	15	20	5	15		
Kaplanoglu et al.[15]	363 patients' CT images (Turkish population)	26.7	27.8	10.5	19.6	15.4		
Durga et al. <sup>[18]</sup>	51 dried skulls (South Indian population)	35.2	19.6	17.6	15.6	11.7		
Our study	83 dried skulls (Turkish population)	39.76	27.11	6.02	2.41	24.7		

Type 1 and 3: Type A, Type 2 and 6: Type B, Type 7: Type C, Type 5: Type D, Type 4 and 8: Type E, CT: Computed tomography, MDCT: Multidetector CT

population. The anatomic data presented in this study on the width of the PA correlated with other studies. Compared to other investigators, we observed significant changes in the form of NBs. The data obtained from this study show a data series belonging to the Turkish population. Anatomical structures of races should be well-known to reduce postoperative complications and plan appropriate surgical techniques. We believe that results obtained from this study may provide information for selection of osteotomes in adequate sizes, procedures of corrective rhinoplasty, PA augmentation and resection.

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This study conformed to the Helsinki Declaration. Permissions were obtained from Karatay University's, Pharmaceuticals and Non-Medical Devices Research Ethics Board (2016/012).

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Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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#### **Original Article**



## Morphological and Morphometric Study of the Acetabulum of Dry Human Hip Bone and Its Clinical Implication in Hip Arthroplasty

#### Abstract

Introduction: The objective was to study the morphology of the acetabular margin and articular surface and to measure the various dimensions of the acetabulum in dry human hip bones. Material and Methods: A cross-sectional morphological and morphometric study was performed on 92 undamaged acetabulum of adult dry human hip bone of unknown age and gender. The shape of anterior margin of the acetabulum and shape of the anterior and posterior ends of the articular surface of the acetabulum were observed. Morphometry was done using a Vernier caliper of accuracy of 0.01 mm. Vertical diameter (VD), transverse diameter (TD), anteroposterior diameter (APD), internotch distance (ND), and depth of the acetabulum were measured. Surface area (SA) and volume (V) of the acetabulum were calculated by mathematical calculation of dome. Statistical analysis was done using SPSS software version 22.0 (IBM, SPSS statistics, UNICOM GLOBAL, California, United States). The Pearson's correlation test was used. Results: In the present study, the anterior acetabular ridge was curved in 45.7% (42), angulated in 26.17% (24), straight in 13% (12), and irregular in 13% (12) bones. The anterior end of the lunate articular surface was angulated, and the posterior end was lunate in shape in 45.7% (42), whereas in 54.3% (50), bone both the ends were lunate in shape. Morphometric values and mean  $\pm$  standard deviation were as follows: 48.21 mm ± 3.31 mm (VD), 47.81 mm ± 3.37 mm (TD), 48.79 mm ± 4.08 mm (APD), 23.58 mm  $\pm$  2.77 mm (ND), 27.45 mm  $\pm$  3.02 mm (D), 4162.56 mm<sup>2</sup>  $\pm$  755.58 (SA), and 36,563.65  $mm^3 \pm 9408.67$  (V). Discussion and Conclusion: The knowledge of these acetabular parameters is necessary for the creation of acetabular prosthesis and surgical procedures such as acetabular reconstruction in hip joint surgeries.

Keywords: Acetabular prosthesis, acetabulum, lunate articular surface

#### Introduction

The acetabulum is a cup-shaped cavity present on the lateral side of the hip bone. In Latin, the meaning of the acetabulum is a shallow vinegar cup.<sup>[1]</sup> Acetabulum articulates with the head of the femur to form the hip joint which is a ball-and-socket variety of synovial joint. The acetabulum has a lunate articular surface and a nonarticular fossa on the center of the floor of the acetabulum. The lunate articular surface is covered by hyaline cartilage, and the acetabular fossa is filled with fibroelastic fat and covered with the synovial membrane. All the three bones such as ileum, ischium, and pubis contribute to form the acetabulum. The pubis forms the anterior one-fifth, the ileum forms posterosuperior two-fifth, and the ischium forms posteroinferior two-fifth of the acetabulum. The articular

surface is deficient inferiorly, known as the acetabular notch, and is bridged by transverse acetabular ligament. The depth of acetabulum (DH) is increased by the attachment of fibrocartilaginous rim known as acetabulum labrum. It holds the femoral head and maintains the joint stability.<sup>[2]</sup>

Anterior acetabular ridge's morphology is helpful in the diagnosis of congenital acetabular dysplasia and during acetabular surgeries. Previous studies evaluate the anterior acetabular ridge morphology.<sup>[3-5]</sup>

The diameters and DH are valuable for surgical treatments like total hip arthroplasty. It will also helpful to the anthropologist in determining the gender, and it is also enlightening to the radiologist and prosthetist. According to Kulkarni,<sup>[6]</sup> the acetabulum is divided into three zones to find the degree of slacking of the acetabulum.

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Because of variation in the shape of the acetabulum, the joint congruencies are much frequent even with minor anatomical variations.<sup>[7]</sup> Incongruencies of joint may be prone to the degenerative changes in comparison to the normal joint anatomy.<sup>[8]</sup>

#### Aims and objectives

#### Aim

The aim was to know the morphological features of the acetabulum and to measure various dimensions of the acetabulum in dry human hip bone.

#### **Objectives**

To observe the shape of anterior margin and lunate articular surface of the acetabulum, to measure the various parameters and to calculate the surface area (SA) and volume (V) of acetabulum. To find the correlation between depth and V and depth and SA of acetabulum.

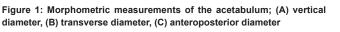
#### **Material and Methods**

This cross-sectional study was conducted after getting the permission from the institutional ethics committee. A total of 92 dry adult human hip bones of unknown gender were taken from the Department of Anatomy of Medical College of Uttar Pradesh. The following morphological and morphometric parameters were recorded.

Morphological features were as follows:

- 1. Anterior acetabular ridge: <sup>[9]</sup> the shape of ridge was evaluated and classified as curved, straight, angular, and irregular
- 2. Ends of lunate surface: <sup>[9]</sup> the shape of anterior and posterior ends of the lunate surface was noted as curved or angular (pointed).

Morphometric parameters were as follows: measured by a digital Vernier caliper in millimeters [Figures 1 and 2].



- 1. Vertical diameters (VDs): farthest distance on the acetabular margin in vertical plane (at the line from the anterior superior iliac spine to the most prominent point on the ischial tuberosity)<sup>[10]</sup>
- 2. Transverse diameters (TD): farthest distance on acetabular rim in horizontal plane<sup>[10]</sup>
- 3. Anteroposterior diameter (APD): farthest distance on acetabular rim in anteroposterior axis<sup>[10]</sup>
- 4. DH: thin metallic scale was placed across the acetabulum. With the help of sliding Vernier caliper, the depth was measured<sup>[10]</sup>
- 5. Width of acetabular notch distance (ND): distance between the two ends of lunate articular surface.
- 6. SA and V: measurement of SA and V of acetabular cavity was challenging. SA and V were calculated by mathematical formulas for dome.

Mathematical calculations of a dome (the hemispherical concavity of the radial head) are as follows: [Figure 2].

Formulas:<sup>[11]</sup>  $SA = \pi (h^2 + r^2)$ 

 $v=1/6\pi h~(3r^2+h^2)$ 

#### Results

Out of the total 92 hip bones, 42 were of the right side and 50 were of the left side. The acetabular anterior margin was found to be curved in 45.7% (57.1% in the right side and 36% in the left side), angulated in 26.1% (23.8% in the right side and 28% in the left side), straight in 15.2% bones (14.3% in the right side and 16% in the left side), and irregular in 13% acetabulum (4.8% in the right side and 20% in the left side) [Figure 3]. The anterior end of the articular margin was angulated in 45.7% of acetabulum and lunate in 54.3% of bones. The posterior end of the articular margin of the acetabulum was lunate in shape in all the acetabulum [Table 1 and Figure 4].

On morphometry of the acetabulum, the mean VD was 48.21 mm  $\pm$  3.31, TD was 47.81 mm  $\pm$  3.37 mm, APD

Table 1: Different shapes of the anterior margin of the

of the acetabulum in the present study					
	Right ( <i>n</i> =42),	Left ( <i>n</i> =50),	Total ( <i>n</i> =92),		
	% (n)	%( <i>n</i> )	% ( <i>n</i> )		
Anterior margin of					
acetabulum					
Curved	57.1 (24)	36 (18)	45.7 (42)		
Angulated	23.8 (10)	28 (14)	26.1 (24)		
Straight	14.3 (6)	16 (8)	15.2 (14)		
Irregular	4.8 (2)	20 (10)	13 (12)		
Anterior end					
Angulated	38.1 (16)	52 (26)	45.7 (42)		
Lunate	61.9 (26)	48 (24)	54.3 (50)		
Posterior end					
Angulated					
Lunate	100 (42)	100 (50)	100 (992)		

	Table 2: Morphometric values of the acetabulum of the right side, left side, and total						
	Right (n=42)		Left	Left ( <i>n</i> =50)		l (n=92)	
	Mean±SD	Minimum-maximum	Mean±SD	Minimum-maximum	Mean±SD	Minimum-maximum	
VD (mm)	48.00±3.56	40.36-53.87	48.38±3.12	43.92-53.99	48.21±3.31	40.36-53.99	
TD (mm)	47.43±3.43	41.97-53.54	48.13±3.33	42.12-54.69	47.81±3.37	41.97-54.69	
APD (mm)	48.70±4.54	40.79-57.19	48.86±3.68	42.73-57.86	48.79±4.08	40.79-57.86	
ND (mm)	23.11±2.70	17.35-29.5	23.98±2.79	19.55-29.86	23.58±2.77	17.35-29.86	
DH (mm)	26.73±3.06	22.69-33.21	28.04±2.88	21.68-34.65	27.45±3.02	21.68-34.65	
SA (mm <sup>2</sup> )	4044.68±801.07	3084.5-5811.42	4261.58±708.08	2926.1-6191.08	4162.56±755.58	2926.1-6191.08	
V (mm <sup>3</sup> )	55,098.26±9950.5	22,760.72-58142.48	37,794.58±8841.16	21,048.1-63,695.5	36,563.65±9408.67	21,048.1-63,695.5	

VD: Vertical diameter, TD: Transverse diameter, APD: Anteroposterior diameter, ND: Notch distance, DH: Depth of acetabulum, SA: Surface area, V: Volume of acetabulum, SD: Standard deviation

Table 3: Comparison of morphometric values of the right and left sides of the acetabulum						
	Right	Left	t	Р		
Mean diameter (mm±SD)	48.04±3.67	48.23±2.93	0.244	0.809		
ND (mm±SD)	23.11±2.7	23.67±2.81	0.876	0.386		
Depth (mm±SD)	26.73±3.06	27.96±2.5	1.83	0.073		
SA (mm <sup>2</sup> ±SD)	4044.67±801.07	4208.47±638.18	0.95	0.346		
V (mm <sup>3</sup> ±SD)	35,098.25±9950	37,313.76±7833.98	1.01	0.316		

SA: Surface area, V: Volume of acetabulum, SD: Standard deviation

Table 4: Correlation between depth, surface area, and volume of the acetabulum of the hip bone				
	Pearson's value	Р	Significance	
Depth versus surface area	0.913	<0.001	Yes	
Depth versus volume	0.955	< 0.001	Yes	
Surface area versus volume	0.945	< 0.001	Yes	

was 48.79 mm  $\pm$  4.08 mm, ND was 23.58 mm  $\pm$  2.77 mm, depth was 27.45 mm  $\pm$  3.02 mm, SA was 4162.56 mm<sup>2</sup>  $\pm$  755.58, and V was 36,563.65 mm<sup>3</sup>  $\pm$  9408.67 [Table 2].

On paired *t*-test, there was no significant difference found between the right and left side values [Table 3]. Statistical analysis using Pearson's correlation test proved an existing correlation between depth versus SA, depth versus V, and SA versus V [Table 4].

#### Discussion

Knowledge of morphology of the anterior acetabular ridge is very important for total hip arthroplasty. Posterior acetabular ridge almost always forms a simple semicircle. However, the anterior acetabular ridge shows variations, and because of these variations, the amount of anteversion is affected by the point of measurement along the anterior

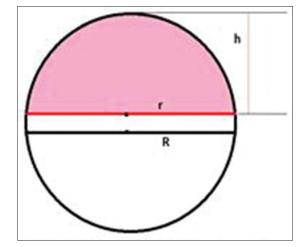


Figure 2: Measurements related to the surface area and volume of the acetabulum; h = depth of articular surface = radius of articular surface, R = radius of hemisphere

ridge.<sup>[3]</sup> In the present study, the most common shape of the anterior margin of the acetabulum was curved, followed by angular, straight, and irregular; similar finding was reported by Thoudam and Chandra,<sup>[8]</sup> whereas Maruyama *et al.*<sup>[3]</sup> and Govsa *et al.*<sup>[5]</sup> reported most common shape as curved, followed by angular, irregular than straight [Table 5].

Anterior and posterior ends of the articular surface were angulated and lunate. In the present study, all posterior

Table 5: Different shapes of the anterior acetabular margin reported by different studies							
Shape of anterior margin	Maruyama <i>et al.</i> (2001) <sup>[3]</sup>	Govsa <i>et al.</i> (2005) <sup>[5]</sup>	Aksu <i>et al</i> . 2006 <sup>[12]</sup>	Gaurang <i>et al.</i> 2014 <sup>[13]</sup>	Thoudam and Chandra 2014 <sup>[8]</sup>	Gangavarapu and Muralidhar 2017 <sup>[9]</sup>	Present study 2019
Curved, $\%$ ( <i>n</i> )	60.50 (121)	43.36 (98)	46.1 (71)	61 (61)	61 (60)	43.75 (35)	45.7 (42)
Angulated, $\%$ ( <i>n</i> )	25.50 (51)	28.33 (64)	16.88 (26)	0	27 (27)	22.56 (18)	26.17 (24)
Straight, $\%(n)$	4.50 (09)	11.94 (27)	23.37 (36)	20 (20)	4 (4)	27.5 (220	15.2 (14)
Irregular, $\%$ ( <i>n</i> )	9.50 (19)	16.37 (37)	13.63 (21)	19 (19)	9 (9)	6.2 (5)	13 (12)
Total	200	226	154	100	100	80	92

Table 6: Comparison of shape of the anterior and posterior ends of the lunate surface of the acetabulum					
Anterior and posterior ends of articular surface	Yugesh and Kumar 2016 <sup>[14]</sup>	Gangavarapu and Muralidhar 2017 <sup>[9]</sup>	Present study 2019		
Anterior end (%)					
Angulated	95.00	85	45.70		
Lunate	5	15	54.30		
Posterior end (%)					
Angulated	1.70	5	0		
Lunate	98.30	95	100		

Table 7: Mean diameter and depth of the acetabulum        reported by various studies					
	Mean diameter (mm±SD)	Mean depth (mm±SD)			
Aksu et al. <sup>[12]</sup> (n=71)	54.29±3.8	29.49±4.2			
Gaurang et al.[13]	42.54±3.6	$19.07 \pm 2.47$			
Thoudam and Chandra <sup>[8]</sup>	50.99±1.99	28.32±1.32			
Yugesh and Kumar <sup>[14]</sup>	Right: 47.4±0.23	Right: 29.9±0.21			
	Left: 48.0±0.37	Left: 29.7±0.23			
Gangavarapu and	Right: 49.40±3.5	Right: 24.09±2.69			
Muralidhar <sup>[9]</sup>	Left: 48.06±5.65	Left: 25.16±2.84			
Khobragade and	-	26.1±2.83			
Vatsalaswamy <sup>[18]</sup> ( <i>n</i> =110)					
Salamon <i>et al.</i> <sup>[16]</sup> ( <i>n</i> =30)	-	30±3.2			
Saikia <i>et al</i> . <sup>[17]</sup> ( <i>n</i> =92)	-	25±8			
Present study ( <i>n</i> =92)	48.27±3.34	27.45±3.02			

Figure 3: Shape of the anterior acetabular margin; (a) curved, (b) angular, (c) straight, (d) irregular

end was lunate in shape which differed from other findings [Table 6].

The mean diameter of the acetabulum of the present study was 48.27 mm  $\pm$  3.34 mm, which was similar with the findings reported by Yugesh and Kumar<sup>[14]</sup> and Gangavarapu and Muralidhar,<sup>[9]</sup> whereas Aksu *et al.*<sup>[12]</sup> and Thoudam and Chandra<sup>[8]</sup> reported higher values and Gaurang *et al.*<sup>[13]</sup> reported less value of the mean diameter of the acetabulum [Table 7].

The mean VD, TD, and AP diameter of the acetabulum were measured as 48.21 mm  $\pm$  3.31 mm, 47.81 mm  $\pm$  3.37 mm, and 48.79 mm  $\pm$  4.08 mm, respectively, in the present study, which was almost similar with the findings of the Jadhav *et al.*<sup>[15]</sup> [Table 8].

DH measured by Yugesh and Kumar<sup>[14]</sup> and Salamon *et al.*<sup>[16]</sup> was higher than that of the present study. In the

higher than the findings of Gaurang *et al.*<sup>[13]</sup> and Saikia *et al.*,<sup>[17]</sup> whereas findings of Thoudam and Chandra<sup>[8]</sup> and Khobragade and Vatsalaswamy<sup>[18]</sup> have similar values of DH as the present study [Table 7].

present study, DH was 27.45 mm ± 3.02 mm, which was

SA in the present study was calculated by mathematical formulas whereas other studies used different methods to calculate the SAs. Jadhav *et al.*<sup>[15]</sup> recorded the SA in female as 1900 mm<sup>2</sup>, in male as 2300 mm<sup>2</sup>, and Salamon *et al.*<sup>[16]</sup> reported 2294 mm<sup>2</sup>  $\pm$ 329.5. In the present study, SA was 4162.56 mm<sup>2</sup>  $\pm$ 755.58, which was higher; it may be because of different methods used for measuring the SAs [Table 8].

In the present study, V of the acetabulum was calculated by mathematical formulas, and it was  $36,563.65 \text{ mm}^3$  (36.56 ml). Other studies used different other methods to measure the V of the acetabulum. Tan

Table 8: Various diameters and surface area of the acetabulum measured by different studies							
	Vertical diameter	Transverse diameter	Anteroposterior diameter	Surface area (mm <sup>2</sup> )			
Jadhav <i>et al.</i> , 2017 <sup>[15]</sup> ( <i>n</i> =72)	Female: 48±3.9	Female: 46.8±3.2	Female: 48±3.4	Female: 1900			
	Male: 52±3	Male: 51.6±3.2	Male: 52±3.6	Male: 2300			
Salamon <i>et al.</i> <sup>[16]</sup> ( <i>n</i> =30)				2294±329.5			
Present study (n=92)	48.21±3.31	47.81±3.37	48.79±4.08	4162.56±755.58			

Table 9: Volume of acetabular cavity measured by						
different authors						
	Volume of acetabulum					
Tan <i>et al.</i> $(n=55)$ , 2001 <sup>[19]</sup>	31.5 cm <sup>3</sup>					
Kordelle <i>et al.</i> ( <i>n</i> =32), 2001 <sup>[20]</sup>	26.3 mm <sup>3</sup>					
Chung et al. (n=17), 2008 <sup>[21]</sup>	14.2 ml					
Khobragade and Vatsalaswamy ( <i>n</i> =100), 2014 <sup>[18]</sup>	20.74 ml					
Vivekbabu et al. (n=40), 2018[22]	Male: 23.13 ml					
	Female: 17.88 ml					
Present study (n=92), 2019	36,563.65 mm <sup>3</sup>					

# Table 10: Internotch distance of the acetabulummeasured by various authors

	Width of acetabular notch		
	Right side (mm)	Left side (mm)	
Yugesh et al.[14]	30.8±0.42	31.1±0.72	
Gangavarapu et al.[9]	22.25±2.97	22.52±2.46	
Present study	23.11±2.7	23.67±2.81	

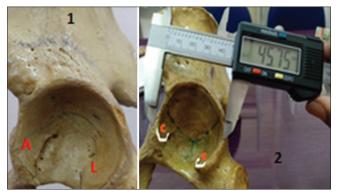


Figure 4: Shape of ends of lunate articular surface. 1 – Anterior end angulated; posterior end lunate 2 – Both ends lunate

*et al.*<sup>[19]</sup> measured V which was almost same as the present study (31.5 cm<sup>3</sup>), whereas other authors such as Kordelle,<sup>[20]</sup> Chung *et al.*,<sup>[21]</sup> Khobragade and Vatsalaswamy,<sup>[18]</sup> and Vivekbabu *et al.*<sup>[22]</sup> reported less value than the present study [Table 9].

The width of acetabular notch (inter ND) in the present study was measured as 23.58 mm  $\pm$  2.77 mm. The finding was similar to the findings of Gangavarapu and Muralidhar.<sup>[9]</sup> Yugesh and Kumar<sup>[14]</sup> reported higher values than that of the present study [Table 10].

#### Conclusion

Clinically, the knowledge of morphology of the anterior acetabular ridge and various dimensions of the acetabulum is very important to orthopedician for hip surgeries and to construct suitable prostheses. The data of the present study may also help the forensic science faculty, orthopedicians, and prosthetists.

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Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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#### **Original Article**



### Polymorphic Study of Ataxin 3 Gene in Eastern Uttar Pradesh Population

#### Abstract

Introduction: Spinocerebellar ataxia type 3 (SCA3), or Machado-Joseph disease (MJD), is a prevalent autosomal dominant-inherited disease that causes progressive problems with movement. Abnormal repetitive expansion of CAG trinucleotide in the ATXN3 gene results in SCA3. This study was done to review the corporation of CAG repeats and polymorphisms in definitive genes with the occurrence of SCA3 in the Indian community, especially in the eastern UP population. Material and Methods: The 40 Ataxia's patient and their parents were listed after obtaining written consent from the participant's attendant/guardians. Out of these, we have identified polymorphism in three patients. **Results:** In one patient, we have found a single base change, g.31483A > T in Exon 10, which changes the nucleotide from Adenine to Thymine (A31483T), while in the second patient, we have identified an intronic change at g.35690A > G in Exon 10, which changes the nucleotide from Adenine to Guanine (A35690G) and in the third patient DNA sequence analysis identified an intronic change at g.35587A > G Exon 10, which changes the nucleotide from Adenine to Guanine (A35587G). Discussion and Conclusion: Although the partial loss of ATXN3 function may also contribute, the disease mechanism in MJD is believed to be a toxic gain-of-function. Several pathogenic cascades have been reported to be triggered by mutant ATXN3, but the critical molecular events driving MJD pathogenesis stay unresolved. While significant developments in studies have enhanced our knowledge of MJD, there is presently a lack of preventive treatment. Results presented here also expand our knowledge about MJD found in the eastern UP population.

**Keywords:** *ATXN3* gene, Machado-Joseph disease, polymerase chain reaction, polymorphism, *Spinocerebellar ataxia type 3* 

#### Introduction

Spinocerebellar ataxia type 3 (Machado-Joseph disease [MJD]/ SCA3), also known as MJD is the prevalent autosomal dominant-inherited cerebellar ataxia.<sup>[1,2]</sup> MJD is diagnosed with molecular genetic testing to detect an abnormal repetitive expansion of CAG trinucleotide in the ATXN3 gene. The gene ATXN3 is found on chromosome 14q32.1 and is suggested in people with progressive cerebellar ataxia and pyramidal signs as well as dystonia, ophthalmoplegia, action-induced facial and lingual fasciculation-like movements, and bulging eyes.<sup>[3,4]</sup> Current therapy is symptomatic without inhibiting neuronal cell death or delaying the age of onset. Identifying molecular pathways of disease is, therefore, essential for unraveling future therapeutic goals. SCA3 is a polyglutamine (polyQ) neurodegenerative disorder. Normal alleles range from 11 to 44 repeats of CAG, while

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pathogenic expansions range from 61 to 87 CAGs.<sup>[5]</sup>

A range of clinical characteristics can be found in subjects with  $\geq$ 52 CAG units.<sup>[6-8]</sup> The identification of founders with Portuguese-Azorean ancestry was noted in the first linkage studies.<sup>[9]</sup>

The trials find the interactions of ataxin-3 with two proteins, HHR23A and HHR23B, both homologs of the DNA repair protein Rad23.[10] Additional research disclosed ataxin-3 as a bona fide deubiquitinating (DUB) enzyme,<sup>[11,12]</sup> giving vital insight into ataxin-3's normal function. More lately, ataxin-3 has been shown to communicate with the transcription factor FOXO4 in the oxidative stress reaction as a transcriptional coactivator.[13] Thus, while some very fundamental ataxin-3 features have been identified, these developments have produced nonintersecting inquiry lines and have not given an underlying mechanism for the pathogenesis of SCA3 disease. To determine the prevalence and

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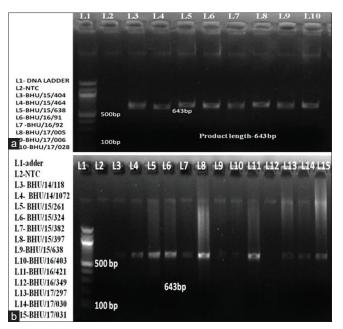


Figure 1: (a and b) Showing gel pictures of amplified genomic DNA (Exon 10 of ATXN 3 Gene) of SCA3 samples

distribution of MJD/SCA3 in the Cuba, a clinical and molecular genetic study of a cohort of SCA3 families was created. Also assessed were the ordinary variation of repeats of CAG and their volatile conduct.

Due to the low prevalence of intermediate alleles at this locus, SCA3 was not validated in the replication cohorts. The major symptoms are as gait ataxia as the disease's initial symptom. Other cerebellar characteristics were dysdiadochokinesia, dysmetria, cerebellar dysarthria, and kinetic tremor. Pathological nystagmus was the most significant oculomotor sign.

#### **Material and Methods**

This study was done to review the corporation of CAG repeats and polymorphisms in definitive genes with the occurrence of SCA 3 in the Indian community, especially in the eastern UP population. The study will also help us in concluding the genetic explanation of these CAG/ polyQ repeat expansion in the SCA3 gene. We looked for Atxn3 polymorphism in a subset of genomic DNA (DNA isolation from blood samples). The 40 Ataxia's patient and their parents were listed after obtaining written consent from the participant's attendant/guardians. A particular admission precedent was based on clinically recognizing cerebellar ataxia. It was determined by the study of medical records. Blood samples from the proband, mother and their various controls were collected from the OPD of the Department of Neurology (Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University [BHU], Varanasi) and evicted to Cytogenetic lab (Institute of Medical Sciences, BHU, Varanasi) for genetic studies in ethylenediaminetetraacetic acid vials and stored at 4°C under

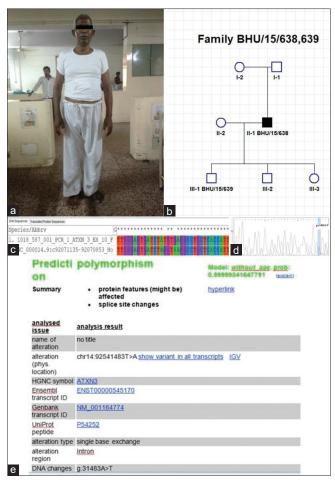


Figure 2: (a) Showing picture of SCA3 Patient, ID- BHU/15/638, (b) Pedigree of Patient BHU/15/638, (c) Sequence analysis of Patient BHU/15/638, (d) Sequence analysis demonstrates single nucleotide change at g.31483A>T position in Exon10 where Adenine was replaced by Thymine (A31483T), (e) MUTATION TASTER result showing polymorphic site

sterile condition till further scrutiny. Genomic DNA was isolated from blood using materials like heparinized blood.

After isolation of genomic DNA, quantification of DNA was measured for the further experiment using the *Nanodrop-Spectrophotometer*. After quantification, primers were designed for the coding region of ATXN3 gene including exon/intron boundaries. The ATXN3 gene contains 10 Exons. The primers were designed using Primer3 software version 0.4.0 (http://frodo.wi.mit.edu/primer3/).

#### Polymerase chain reaction

In molecular biology, the polymerase chain reaction (PCR) was used as a technique to amplify the single or a few copies of a piece of DNA across multiple order of magnitude, generating thousands to millions of copies of particular DNA sequence. The PCR was carried out on an *AppliedBiosystem*, *96 well Veriti Thermal Cycler* (Applied Biosystem, USA).

During the study, molecular analysis was done for *Exon 1-10*. Primers were designed to amplify across each of the exons including *Exon 10*. Sequencing analysis was done to

	<u> </u>			es out of 40 families (3 familial and 3	
BHU ID	Age	Gender	Age of onset	Repeat size (test sample) normal	Repeat size (test sample)
BHU/15/404	35 years	Male	6 years	CAG-28	CAG-22
BHU/15/261	16 years	Male	8 years	CAG-28	CAG-32
BHU/15/297	65 years	Male	5 months	CAG-28	CAG-20
BHU/15/382	36 years	Female	7 years	CAG-28	CAG-20
BHU/15/508	42 years	Male	22 years	CAG-28	CAG-22
BHU/15/533	40 years	Male	5 years	CAG-28	CAG-20
BHU/15/565	19 years	Male	5 years	CAG-28	CAG-21
BHU/15/638	58 Years	Male	6 months	CAG-28	CAG-27 Intronic Changes
BHU/16/91	54 years	Male	5 years	CAG-28	CAG-22
BHU/16/92	40 years	Male	12 years	CAG-28	CAG-24
BHU/16/188	54 years	Male	6 months	CAG-28	CAG-20
BHU/16/334	50 years	Male	5 years	CAG-28	CAG-20
BHU/16/335	39 years	Male	2 years	CAG-28	CAG-22
BHU/16/349	58 years	Male	6 months	CAG-28	CAG-18
BHU/17/05	40 years	Male	1 year	CAG-28	CAG-07
BHU/17/06	45 years	Male	26 years	CAG-28	CAG-27
BHU/17/25	48 years	Female	10 years	CAG-28	CAG-22
BHU/17/28	40 years	Male	2 year	CAG-28	CAG-18 Polymorphism
BHU/17/31	17 year	Male	3 year	CAG-28	CAG-15
BHU/17/34	65 years	Female	15 years	CAG-28	CAG-24
BHU/17/41	40 years	Male	5 years	CAG-28	CAG-10
BHU/17/42	35 years	Male	3 years	CAG-28	CAG-22
BHU/17/43	45 years	Male	2 years	CAG-28	CAG-18
BHU/17/54	75 years	Male	4 years	CAG-28	CAG-15
BHU/17/79	17 years	Male	6 years	CAG-28	CAG-10
BHU/17/145	30 years	Male	2 years	CAG-28	CAG-22
BHU/17/146	32 years	Female	4 years	CAG-28	CAG-11
BHU/17/149	52 years	Female	12 years	CAG-28	CAG-15
BHU/17/152	24 years	Female	5 years	CAG-28	CAG-22
BHU/17/170	18 years	Male	2 years	CAG-28	CAG-22
BHU/18/28	55 years	Male	7 years	CAG-28	CAG-24
BHU/18/29	28 years	Female	2 years	CAG-28	CAG-22
BHU/18/32	40 years	Female	1 year	CAG-28	CAG-22
BHU/18/35	61 years	Male	5 years	CAG-28	CAG-22
BHU/18/36	32 years	Female	2 years	CAG-28	CAG-22
BHU/15/463	24 years	Female	2 years	CAG-28	CAG-21
BHU/15/464	35 years	Male	6 years	CAG-28	CAG-21 Polymorphism
BHU/15/429	40 years	Male	1 year	CAG-28	CAG-18
BHU/15/30	40 years	Male	2 year	CAG-28	CAG-10
BHU/17/28	40 years	Male	5 years	CAG-28	CAG-20

The table shows repeat size found in Ataxin 3 gene of three Cerebellar ataxia patients out of 40 families.

determine polymorphism in all the patients and control for *Exon 1-10*; however, polymorphism and intronic changes were observed only in *Exon 10*.

#### **Gel electrophoresis**

After running PCR, the PCR products were subjected to gel electrophoresis. We have done gel electrophoresis at temperature of 100°C and after that, with the help of the Gel doc system or gel imaging, we have measured and recorded the labeled nucleic acid or DNA. Gel results of amplified genomic DNA of Exon10 can be seen in Figure 1. We run PCR for all collected samples and after that, we had carried out

the gel electrophoresis to see the amplification. In [Figure 1a and b], we can see that the Exon10 of the ATXN3 gene would get amplified at 643bp. After that all the samples were sent for sequencing. In sequencing, mutation was observed in *Exon10*. After that, the analysis was performed in dry lab using Mutation Taster is free web-based application to evaluate DNA sequence variants for their disease causing potential.

The study was performed to determine the impact of the polymorphic site. None of the unaffected members of the family and none of the control individuals had this abnormal conformer. One polymorphism and one intronic changes have been found in the gene ATXN3.

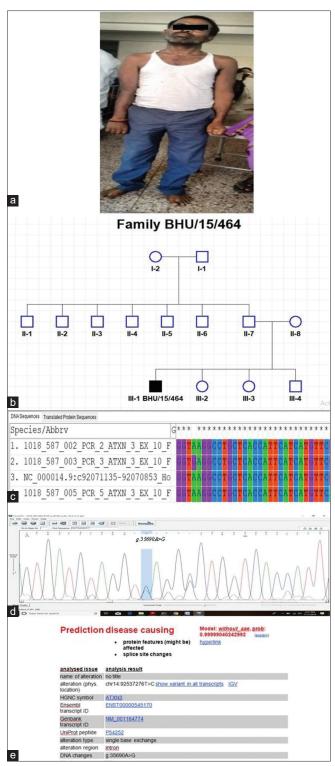


Figure 3: (a) Showing picture of SCA3 Patient, ID- BHU/15/464, (b) Pedigree of Patient BHU/15/464, (c) Sequence analysis of Patient BHU/15/464, (d) Sequence analysis demonstrates single nucleotide change at g.35690A>G in Exon 10, which changes the nucleotide from Adenine to Guanine (A35690G), (e) mutation taster result showing polymorphic site

#### Results

Gel results of amplified genomic DNA of Exon 1 to Exon 10 as shown in Figure 1. We run PCR for all collected samples (40 patients and 20 Controls) as shown in table 1 and had carried out the gel electrophoresis to see the amplification. We can see that the Exon 10 of the Atxn 3 gene would get amplified at 638 bp.

#### **Phenotypic analysis**

Three familial and sporadic patients ranging in age from 16 years to 65 years with clinically apparent ataxia presenting with nystagmus, decreased saccade velocity; amyotrophy fasciculations, sensory loss, and evaluated by history taking and physical examination, magnetic resonance imaging and computed tomography scan for SCA 3 were identified.

#### Molecular analysis

During the study, molecular analysis was done for Exon 1-10. Primers were designed to amplify across each of the Exons, including Exon 10. Sequencing analysis was done to determine polymorphism in all the patients and control for Exon 1-10; however, polymorphism was observed only in Exon 10. During the study, *in silico* analysis was done to determine the impact of the polymorphic site.

In the first case, a male aged 58 years whose ID is BHU/15/638 was detected with no family history (sporadic case) as shown in Figure 2. Chromatogram demonstrates a single base change, g.31483A>T in *Exon 10*, where adenine was replaced by Thymine (A31483T) (Ensembl transcript ID ENST00000545170), these alteration takes place in intron. With the help of *MUTATION TASTER* software, the polymorphic site was detected, wild type gDNA sequence compare to altered gDNA sequence, in these sequences A altered to T, Multiple sequence alignment was done to determine the single base exchange of A>T.

In the second case, also a male aged 35 years whose ID is BHU/15/464 was detected with no familial history (as shown in Figure 3). Multiple sequence alignment was done to determine a single base exchange of A>G. Chromatogram demonstrates single nucleotide change at g.35690A>G in Exon 10, which changes the nucleotide from Adenine to Guanine (A35690G) (Ensembl transcript ID ENST00000398590), these alteration takes place in the intron. MUTATION TASTER result showing the polymorphic site as disease causing. Wild-type gDNA sequence compares to altered gDNA sequence, in this sequence A altered to G.

In the third case, a male aged 40 years whose ID is BHU/17/28 was detected with no family history (sporadic case) as shown in Figure 4. Multiple sequence alignment was done to determine a single base exchange of A>G. (D) Chromatogram demonstrates single nucleotide change at g.35587A>G in *Exon 10*, which changes the nucleotide from Adenine to Guanine (*A35587G*), (Ensembl transcript ID ENST00000545170), *MUTATION TASTER* result showing the polymorphic site as disease causing. Wild type gDNA sequence compared to altered gDNA sequence, in this sequence A altered to G.

Gender	Cerebellar ataxia	Ensembl ID and position of	Polymorphism	Synonymous/nonsynonymou
		nucleotide		(mutation taster prediction)
Male BHU/18/30	Ataxin 3 (Type I)	(ENST00000545170) 31483A	Adenine to	Synonymous Changes
			Thymine (A31483T)	
Male BHU/15/464	Ataxin 3 (Type I)	(ENST00000398590) 35690A	Adenine to	Synonymous Changes
			Guanine (A35690G)	
Male BHU/17/28	Ataxin 3 (Type I)	(ENST00000545170) 35587A	Adenine to	Nonsynonymous Changes
			Guanine (A35587G)	

Variations are analyzed by using Mutation Taster (In silico study)

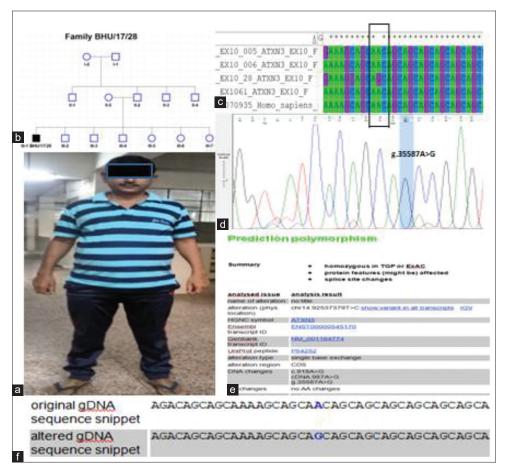


Figure 4: Pedigree of Patient BHU/17/28. (a) Picture of SCA3 patient, ID- BHU/17/28, (b) Pedigree of patient BHU/17/28, (c) Multiple sequence alignment was done to determine single base exchange of A > G. (d) Chromatogram demonstrates single nucleotide change at g. 35587A > G in Exon 10, which changes the nucleotide from adenine to guanine (A35587G), (e) MUTATIONTASTER result showing polymorphic site as disease causing. (f) Wild type gDNA sequence compared to altered gDNA sequence, in this sequence A altered to G

#### Discussion

MJD, the most common dominantly inherited ataxia in the world, is triggered by an expansion of polyQ in the DUB enzyme ataxin-3. The polyQ repeat expansion is thought to give the various polyQ-encoding proteins a toxic gain-of-function, potentially supporting their misfolding and aggregation, but the accurate mechanism of disease pathogenesis remains unknown. The ATXN3 gene offers directions for the production of an enzyme called ataxin3, which is found in cells throughout the body. Ataxin3 is engaged in a mechanism called the ubiquitinproteasome system, which destroys surplus and damaged proteins and gets rid of them. The molecule ubiquitin attaches (binds) to unneeded proteins and marks them within cells to be broken down (degraded). Ataxin3 removes (cleaves) the ubiquitin from these unwanted proteins just before it is degraded to allow the ubiquitin to be reused. Ataxin3 is known as a DUB enzyme because of its function in slicing ubiquitin from proteins.

We observed polymorphism in three patients of SCA3 and those were polymorphism and intronic changes as

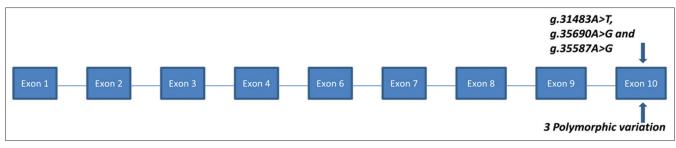


Figure 5: Polymorphism detected in Exon 10 in all the three cases

depicted in Table 2 and Figure 5. SCA3 is a polyglutamine neurodegenerative disorder. Normal alleles range from 11 to 44 repeats of CAG, while pathogenic expansions range from 61 to 87 CAGs. A range of clinical features can be found in subjects carrying  $\geq$ 52 CAG units. This has led us to speculate on the existence of common features in Ataxia type 1 disorder. While well characterized at the molecular level, the encoded protein function is not currently known.

Normally, *ATXN3* helps to control the stability and activity of multiple proteins in various cellular pathways. Mutant (expanded) *ATXN3* is susceptible to form insoluble aggregates and to undergo proteolysis that produces fragments containing polyQ that further encourage aggregation. Although the partial loss of the *ATXN3* function may also contribute, the disease mechanism in MJD is believed to be a toxic gain-of-function. Several pathogenic cascades have been reported to be triggered by mutant *ATXN3*, but the critical molecular events driving MJD pathogenesis stay unresolved. In the pursuit of MJD treatment, several pathways have been targeted, but none have yet progressed to human clinical trials.

The single-nucleotide change in the first patient was at g.31483A>T position in Exon10, where Adenine was replaced by Thymine (A31483T) as shown. The change of nucleotide was in the intronic region. While, in the second patient, a single base change was observed at g.35690A>G in *Exon 10*, which changes the nucleotide from Adenine to Guanine (A35690G). It can be predicted as disease causing by altering the nucleotide variants as shown.

In the third patient, the nucleotide change occurred at site g.35587A>T position in Exon10, where Adenine was replaced by Guanine (A>G), as shown. This nucleotide change represents single base change. A study was done to screen polymorphism through mutation taster.

#### Conclusion

Mutations in the ATXN3 gene mostly affect nerve cells and other types of brain cells. SCA3 is associated with cell death in the portion of the brain attached to the spinal cord (the brainstem), the part of the brain engaged in coordinating motions (the cerebellum), and other brain regions. This condition is also associated with the death of nerve cells in the spinal cord. Over time, the brain and spinal cord cell loss cause distinctive signs and symptoms of SCA3. While significant developments in studies have enhanced our knowledge of MJD, there is presently a lack of preventive treatment. Results presented here also expand our knowledge about MJD found in the eastern UP population. These findings deserve confirmation in a second population and more in-depth research in cellular and animal models.

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#### **Conflicts of interest**

There are no conflicts of interest.

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# An Anatomical Description of the Vermian Fossa: The Reappraisal of an Overlooked Entity

#### Abstract

Introduction: The vermian fossa (VF) is a shallow depression at the inferior end of the internal occipital crest, which lodges the inferior part of the cerebellar vermis. Published literature describes the VF as having a highly variable incidence and morphology. The present study is aimed to investigate the incidence, morphology, and morphometry of the VF within a select South African population and to conduct a review of the literature regarding this structure. Material and Methods: A total of 100 dry, adult skulls of South African origin were analyzed to determine the morphological and morphometric parameters of the VF. Results: The VF was found to be present in 62% of cases. The shape of the VF was classified as triangular (27%), quadrangular (8%), and atypical (27%). The average length of the VF was 13.78 mm, and the average width was 11.62 mm. The morphometric findings of this study correlate with that of previous studies; however, the incidence of atypical shaped VF (27%) is higher in comparison to previous studies (9.7%). Discussion and Conclusion: The detailed anatomical description of the VF may aid in the study of diseases which cause alterations in the size and morphology of the vermis of the cerebellum as well as in transvermian approaches to tumors within the fourth ventricle. Furthermore, due to the paucity of anatomical descriptions of the VF, a reappraisal of this structure is warranted as it is of prime importance to clinicians operating in or interpreting radiological images of the posterior cranial fossa.

Keywords: Cerebellar vermis, posterior cranial fossa, vermian fossa

#### Introduction

The vermian fossa (VF) is a shallow endocranial depression located between the inferior end of the internal occipital crest and the posterior border of the foramen magnum.<sup>[1]</sup> The function of the VF and the reason for its development in the posterior cranial fossa has been a mystery to anatomists and anthropologists for centuries.<sup>[2,3]</sup> Lombroso, known as the father of criminology, who proposed the atavistic tendencies of criminals, had identified the VF as one of the anatomical features of a criminal.<sup>[2]</sup> This author, who referred to the VF as the median occipital fossa, had made this conclusion after identifying an enlarged vermis and VF during the postmortem of one who had committed "atrocious crimes."[2] In 1926, East had attributed an enlarged VF to intracranial pressure as the result of hydrocephaly in a 4-month-old infant.[3] strange These seemingly attributions of the VF are not acknowledged in current times and literature regarding the VF is quite brief in standard anatomical texts. According to Grays Anatomy (41<sup>st</sup> edition), the VF "may exist" which alludes to the variability of this structure. and this may account for the absence of VF literature in other anatomical textbooks.<sup>[1]</sup> However, there is a necessity for anatomists and medical educators to have a thorough knowledge of all structures, which may lead to a higher quality of medical education. Furthermore, in the present era of radiological imaging, structures of the skull may serve as an important role in diagnostic medicine. Berge and Bergman stated that knowledge of the size and incidence of structures and variations of the skull may aid in the diagnostic evaluation of radiologic images and acknowledged that the absence of essential anatomic data on normal variations is a severe deficiency of modern anatomy textbooks.[4] The VF has been identified as one such structure which has been sparsely described from an anatomical perspective.

In addition, the anatomy of the VF and its variations may be of relevance to the surgeon employing a transventricular

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and supracerebellar infratentorial approach to remove midline tumors of the posterior cranial fossa or a transvermian approach to remove tumors within the fourth ventricle.<sup>[5,6]</sup> A detailed description of the VF may also aid in the study of diseases that cause alterations in the size and morphology of the vermis of the cerebellum, as it has been reported that certain cases of cerebellar cortical dysplasia are associated with VF variations.<sup>[5]</sup>

Therefore, the present study is aimed to investigate the anatomical parameters of the VF by determining its incidence, morphology, and morphometry.

#### **Material and Methods**

A total of one hundred adults, dry skulls were obtained from the Department of Clinical Anatomy, University of KwaZulu-Natal, South Africa. Ethical approval was obtained from the Institutional Ethics Committee (BREC Ref No: 256/19). The specimens were of South African origin; however, the gender of each specimen was unknown.

The incidence, shape, length, and width of the VF were recorded. A digital Vernier caliper (Linear Tools, 2012, 0–150 mm, LIN 86500963) was employed to measure the morphometric parameters. The shape of the VF was classified as Type 1 (Triangular), Type 2 (Quadrangular), and Type 3 (Atypical), according to the classification system used by Kale and Öztürk.<sup>[7]</sup> In cases of quadrangular and atypically shaped VF, the length and width were taken at the longest and widest part of the VF, respectively.

Descriptive statistics (incidences) were used to describe the results of this study.

All specimens with macroscopic damage or lesions of the posterior cranial fossa were excluded from this study, and only specimens without macroscopic damage or lesions of the posterior cranial fossa were included.

#### Results

The VF was found to be present in 62% of cases [Table 1]. The shape of the VF was classified as Triangular in 27% of cases, Quadrangular in 8% of cases, and Atypical in 27% of cases [Figure 1 and Table 1]. The average length of the VF was 13.78 mm, and the average width was 11.62 mm [Table 2].

#### Discussion

The term "Vermian" is related to the cerebellar vermis, which is commonly known to be the primary structure found within the VF. The Latin term vermis is translated worm and this may refer to the shape of the cerebellar vermis, which includes the tuber, pyramid, uvula, and nodule.<sup>[1]</sup> However, Kunc et al. highlighted that there is no true contact of the occipital bone with the vermis of the cerebellum and therefore suggested that the term "Vermian Fossa" should be replaced with "triangular eminence" (eminentia triangularis) and defined as flat triangular prominence at the inferior end of the internal occipital crest, formed by the attached margins of the falx cerebelli.<sup>[13]</sup> The descriptions of the VF in anatomical textbooks are either brief or absent altogether. There are several studies in the literature which describe the VF; however, these studies represent data largely from Asian populations. Murlimanju et al. suggested that VF morphology may be influenced by racial differences; it is therefore of value to analyze this structure within different population groups.<sup>[5]</sup> The results of this study represent data

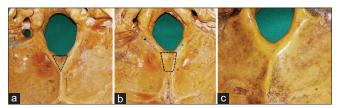


Figure 1: Morphological presentation of the Vermian fossa. (a) Type 1 - triangular; (b) Type 2 - quadrangular; (c) Type 3 – atypical

Author (year)	Sample	Population	Incidence (%)	of the Vermian fossa Morphology (%)			
Author (year)	size (n)	Topulation	Incluence (70)	Triangular	Ouadrangular	Atypical	
Cireli et al. (1990)	210	-	11.4%	-	-	-	
Berge and Bergman (2001) <sup>[4]</sup>	100	USA	4	-	-	-	
Kale and Öztürk (2008) <sup>[7]</sup>	158	Turkey	8.2	4.4	2.5	1.3	
Murlimanju <i>et al.</i> (2013) <sup>[5]</sup>	35	India	71.4	76	8	16	
Yadav et al. (2014) <sup>[8]</sup>	55	India	72.7	72.5	10	17.5	
Ranjan et al. (2015) <sup>[9]</sup>	110	India	80	70.5	8	21.6	
Archana <i>et al</i> . (2017) <sup>[10]</sup>	50	India	72	80.6	11.1	8.3	
Singh and Gupta (2017) <sup>[11]</sup>	60	India	66.7	80	20	0	
Pandey et al. (2018) <sup>[12]</sup>	40	India	80	90.6	6.3	3.1	
Kunc <i>et al.</i> (2020) <sup>[13]</sup>	1042	Czech Republic	29.7	-	-	-	
Weighted mean		*	32.9	54.2	8.1	9.2	
Present study (2019)	100	South Africa	62	27	8	27	

Table 2: Morphometry of the Vermian fossa						
Author (year)	Sample size ( <i>n</i> )	Length (mm)	Width (mm)			
Kale and Öztürk (2008) <sup>[7]</sup>	158	27.8	18.4			
Murlimanju <i>et al</i> . (2013) <sup>[5]</sup>	35	13.6	11.9			
Yadav et al. (2014) <sup>[8]</sup>	55	14.2	12.1			
Ranjan et al. (2015) <sup>[9]</sup>	110	13.4	12.1			
Archana et al. (2017) <sup>[10]</sup>	50	14.6	12.6			
Singh and Gupta (2017) <sup>[11]</sup>	60	17	13.5			
Pandey et al. (2018) <sup>[12]</sup>	40	16.7	18			
Weighted mean		18.8	14.7			
Present study (2019)	100	13.8	11.6			

from a select South African population to add to the global body of knowledge.

The literature revealed a weighted mean incidence of the VF in 32.9% of cases; however, the present study recorded the VF in 62% of cases [Table 1]. This finding is lower than that of recent literature, which records the incidence of the VF ranging from 66.7% to 80% [Table 1]. However, earlier studies by Cireli et al., Berge and Berman and Kale and Öztürk recorded significantly lower incidences of 11.4%, 4%, and 8.2%, respectively [Table 1].<sup>[4,7,14]</sup> It is notable that the latter studies were done in the USA and Turkey, respectively, whereas the studies reflecting higher incidences were conducted in India. The significant difference in incidence between the studies as well as the consistency in results from the Indian studies, supports the hypothesis that racial and geographic distribution may contribute to the variation of the VF.[5,7,8,12] The incidence of the VF within a select South African population, as is recorded in this study, has not been previously reported and is of clinical relevance to surgeons during the preoperative planning of surgeries within the posterior cranial fossa.

The morphological characteristics of the VF are variable and have been described as Type 1–3, according to the classification system proposed by Kale and Öztürk.<sup>[7]</sup> Type 1 represents a triangular fossa, Type 2 represents a quadrangular fossa, and Type 3 represents an atypically shaped fossa [Figure 1].<sup>[7]</sup> Type 1 was found in 27% of cases in the present study. This is the most commonly found shape in the literature, and the weighted mean incidence of Type 1 in the literature was 54.2% (range: 4.4%–90.6%) [Table 1].

Type 2, quadrangular VF, was found in 8% of cases in this study. This correlates closely with the findings of Ranjan *et al.* and Pandey *et al.*, who recorded Type 2 VF in 8% and 6.3%, respectively [Table 1].<sup>[9,12]</sup> The weighted mean incidence of Type 2, as depicted in the literature, was 8.1% (range 2.5%–20%), which correlates closely with the findings of the present study. Kale and Öztürk stated that a few authors termed the quadrangular VF as fossa occipitalis mediana since it was deeper than other cases.<sup>[7]</sup> However, in developing the classification of VF morphology, Kale and Öztürk classified fossa occipitalis mediana as Type 2

VF since the locations of the two are the same, the only difference being the depth of the fossa.<sup>[7]</sup> Furthermore, Kale and Öztürk hypothesized that the distinction in the depth of the VF may be attributed to the size and shape of the inferior cerebellar vermis and therefore concluded that this classification may be indicative of the size and shape of the inferior cerebellar vermis.<sup>[7]</sup>

Type 3 VF described atypical cases which presented with a morphology which was neither triangular nor quadrangular but did display a fossa. In the present study, Type 3 was recorded in 27% of cases; this finding is higher than the weighted mean incidence of 9.2% (range: 1.3%–21.6%) in the literature [Table 1].

The average length of the VF in the present study was 13.8 mm. This finding was lower than the weighted mean length of the VF in the literature, which was 18.8 mm. The average width of the VF was 11.6 mm, in the present study. This finding is less than the weighted mean width recorded in the literature (14.7 mm) [Table 2].

Sanudo *et al.* stated that both diagnosis and therapeutic performance may be augmented by studies of morphological variations.<sup>[15]</sup> Despite its high incidence, the VF is currently considered an anatomical variation and is an overlooked entity in many standard anatomical texts. Further to highlighting the VF as a distinguished structure of skull anatomy, the present study reported morphological and morphometric parameters of the VF. These results may aid the surgeon operating in the posterior cranial fossa during procedures such as the transventricular and supracerebellar infratentorial removal of midline tumors or the transvermian approach to tumors within the fourth ventricle.<sup>[9]</sup>

The closest anatomical structures to the VF are the posterior cerebromedullary cistern from the superior aspect and the division of the occipital sinuses continuing into the marginal sinuses from the lateral aspect. Surgeons operating in this area may benefit from knowing the incidence of variations of the VF, which may influence surrounding structures such as a duplicated falx cerebelli.<sup>[13,16]</sup> Furthermore, the detailed knowledge of the anatomical parameters of the VF may aid in the study of diseases which cause alterations in the size and morphology of the cerebellar vermis as cerebellar cortical dysplasia are associated with VF variations.<sup>[5]</sup>

#### Conclusion

Considering the paucity of literature regarding the VF in anatomical texts as well as the clinical relevance of this structure, this study highlights the need for a reappraisal of the VF. It is recommended that future studies include larger sample sizes, with biographical data for more comprehensive statistical analysis. Accurate knowledge of the anatomy and variations of the VF is of importance to anatomists, radiologists, and surgeons and may aid in mitigating iatrogenic injuries.

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#### **Conflicts of interest**

There are no conflicts of interest.

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# Considering the Surface Area and Sagittal Angle in a Pair of Lumbosacral Facets: Determining the Structural Relevance of Asymmetric Facets at the Lumbosacral Junction

#### Abstract

Introduction: The mechanism of spine dysfunction that was linked to asymmetry in facet joint planes remains poorly understood. We determined the surface area and sagittal angle in a pair of L4, L5, and S1 vertebral facets. We aimed to explain the structural relevance of asymmetric facets at the lumbosacral junction. Material and Methods: Vertebral columns of 45 adult male human cadavers were cut at the L3-L4 intervertebral disc. Each section was macerated and tied together in a sequence to obtain the value of sagittal angle of the superior facets of L4, L5, and S1 vertebrae and area of the inferior facets of L4 and L5 vertebrae, using a modified protractor and graph paper method, respectively. Asymmetry was determined using the formula propound by Plochocki (2002). Results: The mean value of surface area of the left and right inferior facets of L4 and the left and right inferior facets of L5 was 161  $\pm$  24 and 168  $\pm$  23 mm<sup>2</sup> and 200  $\pm$  28 and 218  $\pm$  33 mm<sup>2</sup>, respectively. The mean value of sagittal angle of the left and right superior facets of L4, L5, and S1 was  $37.71^{\circ} \pm 4.38^{\circ}$ ,  $36.18^{\circ} \pm 4.8^{\circ}$ ,  $46.96^{\circ} \pm 6.49^{\circ}$ ,  $48.51^{\circ} \pm 6.25^{\circ}$ ,  $52.49^{\circ} \pm 5.1^{\circ}$ , and  $54.67^{\circ} \pm 5.25^{\circ}$ , respectively. The degree of asymmetry in the area of the inferior facets of L4 and L5 ranges from 0% to 30% and 0%-32.26%, respectively, and that for sagittal angle of the superior facets of L4, L5, and S1 was 0%-37.93%, 0%-30.95%, and 0%-26.32%, respectively. Discussion and Conclusion: This study would suggest that despite the statistically significant mean differences in the paired variables, the vertebrae were free of any pathological change but with consequent adaptive features. However, the stress effects would suggest that the left lumbosacral facet joints are predisposed to dysfunction of mechanical origin.

Keywords: Adaptation, back pain, biomechanics, bipedal posture, body weight

#### Introduction

The extent of asymmetry in a pair of vertebral facets that becomes clinically relevant remains challenging to orthopedic surgeons and chiropractors. We cannot overemphasize the significance of facet asymmetry in medical practice. It may precipitate disc degeneration and hernia,<sup>[1]</sup> contributes to the development of different types of idiopathic scoliosis,<sup>[2]</sup> and predicts the occurrence of facet cyst and low back pain.<sup>[3,4]</sup> Although the incidence of asymmetry in a pair of the superior facets at the thoracolumbar region of the spine was rare,<sup>[5]</sup> some authors<sup>[6]</sup> queried its relevance in facet joint disease at the lumbosacral junction.

Second, in anthropology, lumbosacral weakness<sup>[7]</sup> and certain spine derangements

around the lumbosacral junction of a modern man were considered to be consequences of skeletal modifications to enhance stability, yet the reason remains vague.<sup>[7,8]</sup> Although little research has focused on asymmetry in the lower body,<sup>[9]</sup> this study could suggest a clear reason for the above proposal and explain in part some factors in the puzzle of lumbosacral adaptation to bipedal posture.

Third, the lumbosacral junction transmits the weight of the upper body to the pelvis, and it is the most frequent site of back pain and facet cyst.<sup>[3,4]</sup> Mechanical forces, static and dynamic stress,<sup>[10,11]</sup> higher body mass index,<sup>[12,13]</sup> and a smaller relative cross-sectional area of the paraspinal muscle<sup>[14]</sup> were linked to disc degeneration and back pain. Similarly, authors<sup>[8,15,16]</sup> explained the pattern of weight transmission at the lumbosacral

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junction and noted that lumbosacral facets were subjected to mechanical stress.

Given these findings, necessitated studies designed to determine the extent of asymmetry in surface area and sagittal angle in a pair of vertebral facets at the lumbosacral junction and explain how each facet responds to the stress of upper body weight. It would be of benefit in the management of spine derangement of mechanical origin.

#### **Material and Methods**

After institutional review board approval for this study in line with the conditions of the Medical Research Ethics Committee, a total of 45 adult male human cadavers (age range: 25–35 years, mean: 28 years) were involved since there were no female cadavers available during the study. The cadavers were being dissected by medical students during gross anatomy dissection curriculum.

The vertebral columns were cut at the L3–L4 intervertebral disc, using a hand saw [Figure 1]. Vertebrae that belong to each section were obtained through the process of maceration. All the vertebrae were reported by a pathologist to be fully ossified and free of any pathological change or inborn abnormality. The value of the left and right inferior facet areas of the fourth and fifth lumbar vertebrae was obtained in a sequence using graph paper method.<sup>[8,15]</sup> The number of squares covered on the graph paper was independently counted and determined by the three researchers to minimize interobserver error. The value of the sagittal angle of L4, L5, and S1 vertebrae was obtained in a sequence using a modified protractor.<sup>[5,17]</sup>

The extent of asymmetry in facet area and sagittal angle was calculated as follows: ([left side – right side]  $\div$  right side) × 100 as adopted by Plochocki.<sup>[9]</sup> The descriptive statistics of the data showed that the continuous random variables were normally distributed as shown in Figure 2a-c. Therefore, we adopted a paired sample *t*-test to compare the two means of paired inferior facet area and sagittal angle to establish the level of significance of the mean differences. Furthermore, regression analyses were conducted on paired variables.

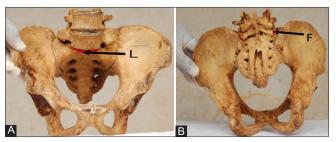


Figure 1: Anterior (A) and posterior (B) views of lower portion of the spine (L4 – coccyx) and pelvic girdle of a dissected cadaver, showing the Lumbosacral junction(L) and right L5/S1 facet joint(F)

#### **Results**

The statistics in Table 1 showed that the mean values of surface area of the left facet of the fourth lumbar vertebra (L4LF), the right facet of the fourth lumbar vertebra (L4RF), the left facet of the fifth lumbar vertebra (L5LF), and the right facet of the fifth lumbar vertebra (L5RF) were  $161 \pm 24$ ,  $168 \pm 23$ ,  $200 \pm 28$ , and  $218 \pm 33$  mm<sup>2</sup>, respectively. The mean values of the sagittal angle of the left (L4LSA) and right (L4RSA) superior facets of L4, the left (L5LSA) and right (L5RSA) superior facets of L5, and the left (S1LSA) and right (S1RSA) superior facets of S1 were  $37.71^{\circ} \pm 4.38^{\circ}$ ,  $36.18^{\circ} \pm 4.8^{\circ}$ ,  $46.96^{\circ} \pm 6.49^{\circ}$ ,  $48.51^{\circ} \pm 6.25^{\circ}$ ,  $52.49^{\circ} \pm 5.1^{\circ}$ , and  $54.67^{\circ} \pm 5.25^{\circ}$ , respectively. The result was characterized by increase in sagittal angle in a sequence from the fourth lumbar to the first sacral vertebra.

The range of asymmetry in the area of corresponding inferior facets of L4 and L5 was -23.16%-30.00% and -32.26%-20.99%, respectively, and that for sagittal angle of L4, L5, and S1 was -14.29%, 37.93%; -30.95%, 25.58% and -26.32%, 20.00%, respectively. [Figure 2a-c]. Having adopted the formula propound by Plochocki<sup>[9]</sup> to determine the proportion of asymmetry in a pair of corresponding structures, the negative value implied that the right parameter was greater than the left counterpart in some cases.

The correlation matrices and mean differences between the paired variables in Table 2 showed that the mean value of surface area of L4RF and L5RF was significantly (P < 0.05) higher than that of L4LF and L5LF, respectively. Likewise, the mean value of L4LSA, L5RSA, and S1RSA was significantly (P < 0.05) higher than that of L4RSA, L5LSA, and S1LSA, respectively.

The regression analyses in Table 3 showed that the regression coefficients of the predictors were highly significant (P < 0.01), indicating that predictions made on the resultant regression equations are reliable. Likewise, the multiple regression analyses revealed a linear relationship of the sagittal angle in a sequence from L4 to S1, which could be predicted with the following equations:

L5LSA (°) = -9.689 + 0.946 L4LSA + 0.399 S1LSA L5RSA (°) = 4.873 + 0.706 L4RSA + 0.331 S1RSA.

#### Discussion

The analysis of the work was a setback for lack of a statistical package determining asymmetry in a pair of structures among individuals in a given population. Although manual measurement of vertebral column angulations was valid in assessment of spine derangement,<sup>[18]</sup> a simple diagnostic tool and methodology determining the sequential relationship of the vertebral facets of an individual is essential. The regression equations in Table 3 could serve as functional matrices to adopt during identification of an individual sacrum and lower

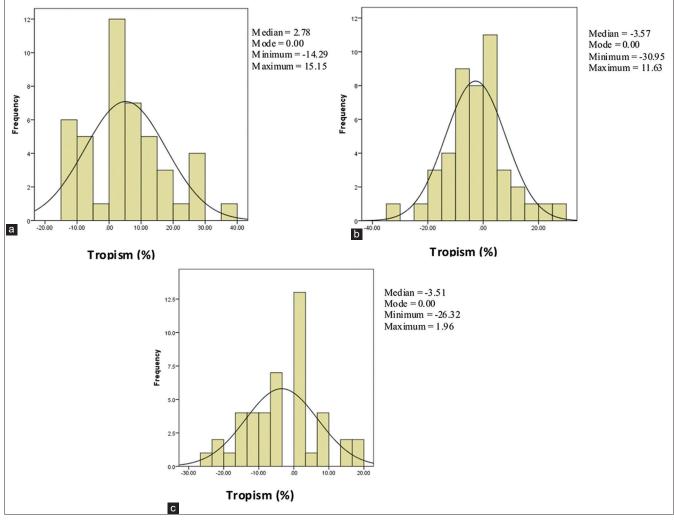


Figure 2: (a-c) Frequency distribution of degree of facet joint tropism of L4 (a), L5 (b), and S1 (c), showing a normal distribution curve

	Table 1: Descriptive statistics of L4, L5, and S1 variables									
	L4FL	L4FR	L4LSA	L4RSA	L5FL	L5FR	L5LSA	L5RSA	S1LSA	S1RSA
Mean	161.11	168.13	37.71	36.18	200.18	217.96	46.96	48.51	52.49	54.67
SEM	3.63	3.42	0.65	0.71	4.16	4.94	0.97	0.93	0.76	0.78
Median	156.00	172.00	38.00	36.00	202.00	218.00	48.00	48.00	53.00	56.00
Mode	156.00	172.00	40.00	37.00	208.00ª	216.00	50.00	43.00 <sup>a</sup>	54.00	57.00
SD	24.37	22.94	4.38	4.77	27.90	33.16	6.49	6.25	5.12	5.25
Range	120.00	92.00	17.00	21.00	116.00	124.00	32.00	26.00	21.00	23.00
Minimum	112.00	132.00	30.00	27.00	148.00	156.00	29.00	34.00	40.00	42.00
Maximum	232.00	224.00	47.00	48.00	264.00	280.00	61.00	60.00	61.00	65.00

a: Two modes exist and the least value was chosen. Number of valid cases=45. L4FL: Surface area of the left inferior facet of the fourth lumbar vertebra (mm<sup>2</sup>), L4FR: Surface area of the right inferior facet of the fourth lumbar vertebra (mm<sup>2</sup>), L4LSA: Sagittal angle of the left superior facet of the fourth lumbar vertebra (°), L4RSA: Sagittal angle of the right superior facet of the fourth lumbar vertebra (°), L5FL: Surface area of the left inferior facet of the fifth lumbar vertebra (mm<sup>2</sup>), L5FR: Surface area of the right superior facet of the fifth lumbar vertebra (mm<sup>2</sup>), L5FR: Surface area of the right inferior facet of the fifth lumbar vertebra (mm<sup>2</sup>), L5FR: Surface area of the right inferior facet of the fifth lumbar vertebra (mm<sup>2</sup>), L5FR: Surface area of the right inferior facet of the fifth lumbar vertebra (mm<sup>2</sup>), L5FR: Surface area of the right superior facet of the fifth lumbar vertebra (mm<sup>2</sup>), L5FR: Surface area of the right inferior facet of the right superior facet of the fifth lumbar vertebra (mm<sup>2</sup>), L5FR: Surface area of the right angle of the right superior facet of the fifth lumbar vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacr

lumbar vertebrae in a forensic anthropology laboratory. Second, the equations might be useful to orthopedic surgeons during reconstructive spine intervention procedures that aim to retain the original alignment of the facets. Such techniques include vertebroplasty and kyphoplasty,<sup>[19]</sup> in conjunction with pedicle screw-based instrumentation used for treating certain spinal geometric distortion.<sup>[20-22]</sup>

Table 2: The test of mean differences between L4, L5, and S1 corresponding variables								
	Paired differences							
	Mean	SD	SD SEM 95% confidence interval of the differences				Significant	
	difference			Lower	Upper		(two-tailed)	
L4FL-L4FR	-7.022	21.052	3.138	-13.347	-0.697	-2.238	0.030	
L4LSA - L4RSA	1.533	4.288	0.639	0.245	2.822	2.399	0.021	
L5FL-L5FR	-17.778	26.178	3.903	-25.643	-9.913	-4.556	0.000	
L5LSA - L5RSA	-1.556	4.998	0.745	-3.057	-2.054	-2.088	0.043	
S1LSA - S1RSA	-2.178	5.614	0.837	-3.864	-0.491	-2.602	0.013	

Based on the *P* value column, all the tests are significant (*P*<0.05). L4FL: Surface area of the left inferior facet of the fourth lumbar vertebra ( $mm^2$ ), L4FR: Surface area of the right inferior facet of the fourth lumbar vertebra ( $mm^2$ ), L4LSA: Sagittal angle of the left superior facet of the fourth lumbar vertebra (°), L5FL: Surface area of the right inferior facet of the right superior facet of the fourth lumbar vertebra (°), L5FL: Surface area of the left inferior facet of the fifth lumbar vertebra ( $mm^2$ ), L5FR: Surface area of the right inferior facet of the fifth lumbar vertebra ( $mm^2$ ), L5FR: Surface area of the right inferior facet of the fifth lumbar vertebra ( $mm^2$ ), L5LSA: Sagittal angle of the left superior facet of the fifth lumbar vertebra (°), S1LSA: Sagittal angle of the left superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°). SD: Standard deviation, SEM: Standard error of mean

	Table 3: Regression analysis summary for L4, L5, and S1 corresponding variables							
Var	Variables			Coef	ficients			
			Constant		I	ndependent variable	<u>,</u>	
Dependent	Independent	В	t-statistics	Р	В	t-statistics	Р	
L4FR	L4FL	76.313	4.101	0.000	0.570	4.989	0.000	
L4LSA	L4RSA	18.972	4.498	0.000	0.518	4.480	0.000	
L5FR	L5FL	64.611	2.308	0.026	0.766	5.529	0.000	
L5LSA	L5RSA	12.092	2.168	0.036	0.719	6.301	0.000	
S1LSA	S1RSA	30.364	4.085	0.000	0.405	2.990	0.005	

L4FL: Surface area of the left inferior facet of the fourth lumbar vertebra (mm<sup>2</sup>), L4FR: Surface area of the right inferior facet of the fourth lumbar vertebra (mm<sup>2</sup>), L4LSA: Sagittal angle of the left superior facet of the fourth lumbar vertebra (°), L4RSA: Sagittal angle of the right superior facet of the fourth lumbar vertebra (°), L5FL: Surface area of the left inferior facet of the fifth lumbar vertebra (mm<sup>2</sup>), L5FR: Surface area of the right inferior facet of the fifth lumbar vertebra (mm<sup>2</sup>), L5LSA: Sagittal angle of the left superior facet of the fifth lumbar vertebra (°), Sagittal angle of the right superior facet of the fifth lumbar vertebra (°), S1LSA: Sagittal angle of the left superior facet of the fifth lumbar vertebra (°), S1LSA: Sagittal angle of the left superior facet of the fifth lumbar vertebra (°), S1LSA: Sagittal angle of the left superior facet of the fifth lumbar vertebra (°), S1LSA: Sagittal angle of the left superior facet of the fifth lumbar vertebra (°), S1LSA: Sagittal angle of the left superior facet of the fifth lumbar vertebra (°), S1LSA: Sagittal angle of the left superior facet of the fifth lumbar vertebra (°), S1LSA: Sagittal angle of the left superior facet of the fifth lumbar vertebra (°), S1LSA: Sagittal angle of the left superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1RSA: Sagittal angle of the right superior facet of the first sacral vertebra (°), S1LSA=30.364 + 0.405 S1RSA

In support of the results in Table 1, some authors<sup>[15]</sup> observed that average inferior facet area of L4 and L5 was  $1.49 \pm 0.25$  and  $1.69 \pm 0.31$  cm<sup>2</sup>, respectively. Likewise, the mean value of the left sagittal angle of the superior facets of L4, L5, and S1 was  $35^{\circ} \pm 11^{\circ}$ ,  $44^{\circ} \pm 13^{\circ}$ , and  $55^{\circ} \pm 15^{\circ}$ , respectively, and that of the right counterpart was  $40^{\circ} \pm 14^{\circ}$ ,  $41^{\circ} \pm 13^{\circ}$ , and  $36^{\circ} \pm 17^{\circ}$ , respectively.<sup>[17]</sup> The values from this study were within the range of mean value of the figures above except for the value of the right sagittal angle of S1 vertebra. The difference was attributed to race<sup>[17]</sup> or biomechanical environment.<sup>[23,24]</sup> Moreover, inherent error due to approximation and difficulty in locating anatomic landmarks might have contributed to the difference. However, independent assessments by the three researchers were adopted in this study to minimize these sources of error.

Although some authors<sup>[7,25]</sup> observed tropism on the lumbosacral joint planes, others<sup>[2,26]</sup> had no evidence of asymmetry in the superior or inferior facets of the lumbar vertebrae in children. Most likely, asymmetry in the facet area or facet joint planes at the lumbosacral junction in adults could be an outcome of uneven distribution of upper body weight and stress on the pair of facets during

vertebral bone growth and remodel. Similarly, Rubbery<sup>[27]</sup> reported that the increase in sagittal angle of the lumbar vertebral facets in a sequence inclines the facet joint at the lumbosacral junction toward the coronal plane. Thus, the superior facet of S1 acts as an anterior wedge to the matching inferior facet of L5. The alignment was likely to enable a load on the body of vertebrae to gradually shift to their facets and also resist shear of the spine due to vertically directed axial load on the lumbosacral angle [Figure 1].

Notably, the vertebral column works as a driving shaft, and facet joints guide the extent and direction of its movements. If an axial torque rotation is applied at one end of it, every segment should be subjected to a twisting moment, in a direction perpendicular to the radius of the segment. Therefore, facet asymmetry presents an absence of common center of curvature for the left and right facets during hyperextension, flexion, and rotation of the spine. Precisely, it creates a rotational stress to the facet joint structures, leading to a plastic deformation,<sup>[28]</sup> especially on the counterpart with smaller radius and surface area. Stress is a measure of the force, an object experience per unit cross-sectional area, indicating an inverse relationship with surface area.

Therefore, mechanical strength of a vertebral facet, subject to the pressure from the pull of gravity on body weight and load, depends on its cross-sectional area. This would suggest that the left facet joint structures of the lower lumbar vertebrae were predisposed to stress effects and facet dysfunction of mechanical origin because of their smaller surface area when compared with the right corresponding facets.

However, the fifth lumbar vertebra gives origin to iliolumbar ligaments and relatively thickest mammillary processes and muscles that reinforce its superior facets.<sup>[10]</sup> The ligament and muscles attach to the pelvis. Ardently, we observed that the mammillary tubercle of the superior facet of S1 that aligned with the relatively larger inferior facet of L5 was likely to protrude more than its counterpart. The above-observed adaptive features might have reinforced the facets enabling them to accommodate the asymmetrical stress and also transmit it to the pelvis through the ligaments and muscles.

This study would suggest that despite the statistically significant mean differences in the paired variables in Table 2, the vertebrae were free of any pathological change. It could be an indication that stresses encountered by the pair of facets were within the range of their allowable and working stresses. However, it might have explained in part why earlier researchers in anthropology attributed weakness and low back pain to the lumbosacral junction. Moreover, it revealed a structural variation in the skeletal framework of a modern man, and how it could affect his biomechanical environment, as well as offered insight to this paleontological puzzle.

#### Conclusion

The increase in sagittal angle of the lumbar vertebral facets in a sequence led to a wedge alignment of the inferior facets of L5 and superior facets of S1. This arrangement could resist shear due to vertically directed axial load on the lumbosacral angle. Second, the mammillary tubercle of the superior facet of S1 that aligns with the relatively larger inferior facet of L5 was likely to protrude more than that of its corresponding facet. Furthermore, the mean value of the right facet area and sagittal angle greater than the left counterpart predisposed the left facets to stress effects and facet dysfunction of mechanical origin.

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Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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# *In vivo* Cross-Sectional Topographic Anatomy at Sternal Angle on Magnetic Resonance Imaging

#### Abstract

**Introduction:** The manubriosternal angle, first described by Louis in 1825, is an important landmark in the anatomy of the thorax and has been conventionally described as corresponding to the T4–5 IV disc level based on cadaveric dissections. The objective of this study was to document the level of the angle of Louis and various anatomic structures that also correspond to the same level in living individuals based on multiplanar magnetic resonance (MR) images. **Material and Methods:** We reviewed MR scans of the cervicodorsal spine of 262 individuals comprising 174 males and 88 females in the age range 14–76 years. For each individual, the vertebral level of the following structures was noted on T1-weighted (T1W)/T2-weighted (T2W) turbo spin echo (TSE) coronal and sagittal images, namely tracheal bifurcation (TB), aortic arch (AA), and sternal angle (SA). **Results:** The SA was most commonly seen corresponding to the T3 vertebral body level (45.20%) and at T4–5 IV disc level in only 20.45% of the individuals. The convexity of the arch of the aorta was seen in the majority of the individuals corresponding to the T3 vertebral body level (47.96%). TB was seen at T4 level in 34.35% and only in 22.69% at the T4–5 IV disc level. **Discussion and Conclusion:** The anatomical level of the SA, AA, and TB in living individuals as assessed on MR images is significantly different from the traditionally held belief based on cadaveric dissections.

Keywords: Aortic arch, magnetic resonance imaging, sternal angle, tracheal bifurcation

#### Introduction

Sternal angle (SA), the forward prominence formed by the manubriosternal joint, is an important landmark in the anatomy of the thorax. Based on cadaveric studies. conventional anatomical textbooks mention the SA plane and surface marking of SA to correspond to many anatomical structures including tracheal bifurcation (TB) and aortic arch (AA).<sup>[1-3]</sup> However, this belief has been challenged by many authors using computed tomography (CT)-based in vivo studies. All these studies conducted on different ethnic populations have shown results contrary to this traditional belief, and there has been a very wide variation in the vertebral levels of these structures in living human beings. Furthermore, there is a variable correlation among AA, TB, and SA plane. There is also variable evidence regarding age and gender differences in the levels of these structures.<sup>[4-8]</sup> Surface anatomy and markings are essential components of curriculum for medical

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students, and it is prudent to update this knowledge with availability of modern cross-sectional imaging modalities. This information is vital for planning and execution of many lifesaving interventions. There are few in vivo studies in medical literature that have assessed the anatomy of SA in human beings using magnetic resonance imaging (MRI) as an anatomical tool. MRI by virtue of its direct multiplanar imaging capability and superior contrast resolution is an excellent modality for depicting anatomy. The in vivo anatomical details of various anatomical landmarks that are traditionally believed to lie at the SA plane can be accurately depicted using MRI. In addition, results obtained from free-breathing MRI scans are likely to be closest to the normal physiology in human subjects. This study is an attempt to review the traditional anatomical descriptions of the SA plane and other thoracic structures (AA and TB) with respect to their corresponding vertebral levels and also in relation to each other using free-breathing MRI scans of the upper thorax in living individuals.

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#### **Material and Methods**

We reviewed 262 MRI scans of the cervicodorsal spine of patients done between September 2018 and December 2019 at our institution using a Philips Achieva 1.5 Tesla scanner (Philips Medical Systems, Veenpluis 4-6, The Netherlands). Institutional ethical committee clearance was obtained. The following categories of patients were excluded from the study:

- Patients with scoliosis of the cervical and/or dorsal spine
- Patients with basilar invagination
- Patients with collapse vertebrae
- · Patients with any congenital vertebral anomaly
- Patients with evidence of sternotomy
- Suboptimal visualization of structure under study.

All the MRI scans were performed in supine position with both arms lying parallel to and by the side of the torso. The routine protocol for MRI of the cervicodorsal spine at our institution consisted of T1-weighted (T1W) and T2-weighted (T2W) TSE sagittal and coronal and T2W gradient recalled echo (GRE) axial images, respectively, of which only the T1W and T2W coronal and sagittal images were reviewed for the purpose of this study.

The imaging parameters for the MR scan were as follows:

- T1W TSE sagittal: Repetition time (TR) 407 ms and time to echo (TE) – 14 ms
- T2W TSE sagittal: TR -4000 ms and TE -115 ms
- T1W TSE coronal: TR 400 ms and TE 15 ms.

All the scans were done at a field of view of 280 mm, slice thickness 3 mm, and with a matrix size  $512 \times 512$ .

For each individual, the vertebral levels corresponding to each of the following structures were noted: TB, AA, and SA. The vertebral levels were counted from above downward with the C2 vertebra as the reference due to its unique appearance on sagittal and coronal images. The sagittal images were used to evaluate the vertebral levels corresponding to the SA and AA, respectively, whereas the coronal images were used to evaluate the vertebral levels of the TB. A reference line was generated across the entire set of images in a series using an inbuilt software tool, which ensured that the line corresponded to the same coordinates on all the images of a series. A horizontal line drawn from the anterior aspect of the manubriosternal joint and another horizontal line drawn along the inferior surface (summit of concavity) of the AA were extended posteriorly across the vertebral column on sagittal images to assess the level of the SA [Figure 1] and the AA [Figure 2], respectively. The carinal angle was identified on coronal images, and a horizontal line corresponding to the inferior surface of the carina was generated across all the coronal images in the series to identify the corresponding vertebral level [Figure 3]. The vertebral body level or the IV disc level at which these lines passed through the vertebral column was noted. For

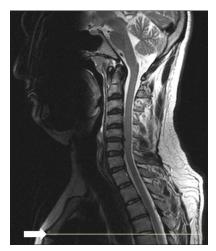


Figure 1: T2-weighted mid-sagittal image of the cervicodorsal spine with horizontal reference line to evaluate the vertebral level of sternal angle (white arrow)



Figure 2: T2-weighted sagittal images. (a) Reference line across the summit of inferior concavity of aortic arch (star). (b) Reference line extending up to the fourth dorsal vertebra

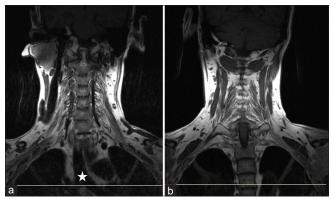


Figure 3: T1-weighted coronal images. (a) Reference line across carina (star). (b) Reference line extending up to the dorsal vertebra

ease of statistical analysis, these vertebral levels were further grouped into three broad groups [Figure 4]:

- Level 1: Superior to T4 vertebral body level
- Level 2: At the T4 vertebral body and T4–5 IV disc level
- Level 3: Inferior to T4–5 IV disc level.

Age groups were classified into three subgroups as <30 years, 30-50 years, and >50 years.

Chi-square test and mean test were used to assess the statistical significance in various data subsets. The relationship between the superior surface of AA and the manubrium sterni was also evaluated by drawing an additional horizontal line posteriorly from the sternal notch toward the vertebral column.

#### Results

We analyzed the MRI scans of 262 individuals comprising 174 males and 88 females of the age range 14–76 years. Out of the total study group of 262 individuals, only the number of scans, as shown in Table 1, was included in the data subset for studying the respective structures.

The SA corresponded to Level 3 in the majority of the individuals (58.91%) [Table 2]. A statistically significant difference was noted in the levels of the SA between males and females. Among females, the SA corresponded to Level 3 in 74%, whereas in males, it corresponded to Level 2 (46.71%) and 3 (49.63%), respectively (P < 0.001) [Figure 5]. The age distribution was as per Figure 6.

The undersurface of the AA corresponded to Level 2 in the majority of the individuals (72.73%) [Table 3]. In the majority of both males (76.11%) and females (67.44%), the concavity of the AA corresponded to Level 2 [Figure 5]. In the majority of the individuals of all the age groups, the undersurface of the AA corresponded to Level 2 [Figure 6].

The bifurcation of the trachea corresponded to Level 2 in the majority of the individuals (54.07%) [Table 4]. In the majority of both females (55.35%) and males (57.90%), the TB corresponded to Level 2 [Figure 5]. A statistically significant difference was noted in the level of the TB in individuals below 50 years (<30 years – 71.40% and 30–50 years – 57.73% at Level 2) and those above 50 years (57.14% at Level 3) (P < 0.05) [Figure 6].

No significant difference was noted in levels of TB and AA in males and females. Furthermore, no significant

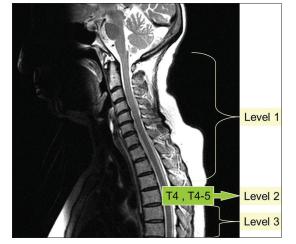


Figure 4: T2-weighted sagittal image showing grouping of vertebral levels

difference was noted in levels of SA and AA in different age groups (P > 0.05). On comparing levels of various structures in the same individual, AA and SA corresponded to the same level in 36.87% of the individuals (Level 2 in 29.03% and Level 3 in 7.83%). The AA was higher to the SA in 56.68% of the individuals (n = 217).

The TB and SA were at the same level in 41.13% of the individuals (Level 2 in 24.05% and Level 3 in 17.08%). In 42.4% of the individuals, the TB corresponded to a level higher to the SA (n = 158). The AA and TB were at the same level in 57.24% of the individuals (Level 1 in 46.20% and Level 3 in 11.03%). In 35.17%, the TB was below the SA (n = 145). Out of 133 individuals in whom we could evaluate the vertebral level of all the three structures, it was noted that they were at the same level 1 only 20.30% of the individuals (15.78% at Level 2 and 4.51% at Level

Table 1: Number of cases included in the data subset						
Parameter	Excluded	Studied				
Sternal angle	43	219				
Arch of aorta	42	220				
Tracheal bifurcation	90	172				

Table 2: Vertebral levels of the sternal angle							
Level	Number of individuals	Level-wise distribution (%)					
Т3	3	Level 1: 2.28					
T3-4	2						
T4	40	Level 2: 38.81					
T4-5	45						
Т5	99	Level 3: 58.91					
T5-6	16						
T6	12						
T6-7	2						

Table 3: Vertebral levels of the arch of the aorta			
Level	Number of individuals	Level-wise distribution (%)	
T3-4	9	Level 1: 4.09	
T4	94	Level 2: 72.73	
T4-5	66		
Т5	48	Level 3: 23.18	
T5-6	3		

Table 4: Vertebral levels of tracheal bifurcation			
Level	Number of individuals	Level-wise distribution (%)	
T2	1	Level 1: 2.90	
T2-3	1		
Т3	2		
T3-4	1		
T4	56	Level 2: 54.07	
T4-5	37		
Т5	53	Level 3: 43.03	
T5-6	19		
Т6	2		

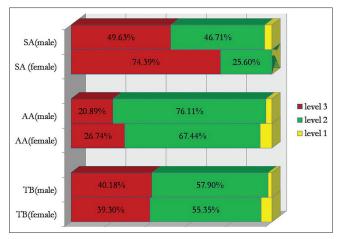


Figure 5: Clustered stacked chart depicting sex distribution of sternal angle, aortic arch, and tracheal bifurcation

3). In these individuals, the median level of the SA was at Level 3, whereas the median level of the AA and TB, respectively, corresponded to Level 1. This difference was statistically significant using median test (P < 0.001).

The relationship of the upper surface of the AA to the manubrium sterni was separately evaluated in 90 individuals as part of the study. It was noted that in all these individuals, the upper surface of the AA was consistently above the level of the SA. In 61.11% of the individuals, it corresponded to the superior half of the manubrium sterni, whereas in 31.11%, it corresponded to the level of the sternal notch or higher.

#### Discussion

Pierre Charles Alexander Louis (1787-1872) was an expert French morbid anatomist who has to his credit the description of the manubriosternal joint which is eponymously called the angle of Louis. It is a subject of debate among medical historians whether he actually ever described the angle at the junction of the manubrium and the body of the sternum (SA) due to insufficient medical literature on the same. Essom-Sherrier and Neelon<sup>[9]</sup> state that, in the year 1910, Edward Goodman reviewed Louis's work and found a comprehensive description of the SA. Louis had described the SA as a bulging in the chest of patients with emphysema in the Journal of the Society of Medical Observations in 1837. Morton and Norman state that Louis described the SA in "Recherches antomico pathologique sur la phthisie" in the year 1825,<sup>[10]</sup> but in an English translation of the same by Walshe<sup>[11]</sup> and in another article by Chukwuemeka et al.,[4] no specific description of the SA was found.

The mediastinum is strictly the partition between the lungs and includes the mediastinal pleura, although it is commonly applied to the region between the two pleural sacs. It is bounded anteriorly by the sternum and posteriorly by the thoracic vertebral column extending vertically from

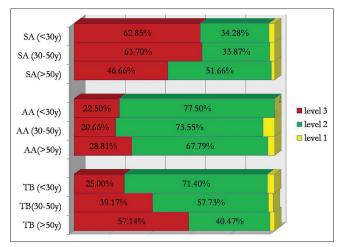


Figure 6: Clustered stacked chart depicting age distribution among various age groups of sternal angle, aortic arch, and tracheal bifurcation

the thoracic inlet to the diaphragm. Conventionally, most of the anatomical texts divide the mediastinum into the superior and inferior mediastinum by a plane which passes from the manubriosternal joint to the vertebral column. The vertebral level corresponding to this plane is another debatable issue. Grant et al. mention that "at this level the trachea bifurcates, the arch of the aorta traverses the thorax from right to left in a posterolateral direction."<sup>[1]</sup> Last RJ states that this is a horizontal plane passing horizontally through the SA backward to the lower border of the fourth thoracic vertebra.<sup>[2]</sup> The same author also mentions about the various other structures corresponding to this plane, stating that "the plane passes through the bifurcation of the trachea, the concavity of the arch of the aorta and just above the bifurcation of the pulmonary trunk. On the plane, the azygos vein enters the superior vena cava and the thoracic duct reaches the left side of the esophagus in its passage upward from the abdomen. Also lying in the plane are the ligamentum arteriosum and the superficial and deep parts of the cardiac plexus." Williams et al. state that "the plane of division into upper and lower mediastinum traverses the manubriosternal joint and the junction of fourth and fifth thoracic vertebra."[3] In a subsequent edition, Williams et al. state that "the plane of division into upper and lower mediastinum traverses the manubriosternal joint and the lower surface of the fourth thoracic vertebra."<sup>[12]</sup>

Various imaging modalities have been used to study the level of SA in relation to the other mediastinal structures and vertebral levels. Arora and Singh in an editorial have comprehensively reviewed the role of imaging in assessing the SA. They suggested that CT and MR are preferred modalities for accurate depiction of mediastinal anatomy. They also noted that there is significant individual variation in the level of mediastinal structures in various studies.<sup>[13]</sup> Various authors have studied the cross-sectional anatomy of various mediastinal structures on CT.<sup>[4-8]</sup> Similarly, Shabshin *et al.*<sup>[14]</sup> studied sagittal MRI sections of thorax

and localizer images of the cervicothoracic region to assess the reliability of various anatomical landmarks for accurate identification of dorsal vertebrae. In this study, the vertebral levels of the sternal apex, aortic bifurcation, and pulmonary bifurcation were assessed in 67 patients. In another study, Sharan et al.[15] studied the vertebral level of the sternal notch on T1 scout mid-sagittal MRI images in 106 consecutive patients to determine the appropriate surgical approach for thoracic spinal reconstruction without using thoracotomy or sternotomy. Connor et al.[16] have studied the utility of various anatomical landmarks in accurate identification of thoracic vertebrae on thoracic MRI. In this study, a total number of ten bony and soft-tissue anatomical landmarks such as sternal notch, inferior angle of the carina, and superior surface of AA were retrospectively studied in 100 thoracic MRI scans, and the authors found poor interobserver agreement, suggesting that the only reliable way of identifying the thoracic vertebrae is by identifying the C2 vertebra and counting down from thereon.

In the literature review, no other study could be found that has assessed vertebral levels of SA on MRI. However, many authors have assessed the same using CT scan.[4-8] Chukwuemeka et al. have shown that in the majority of the individuals, SA passed through the upper half of the T5 vertebral body (52.9%) with the level ranging from T4 to T6.<sup>[4]</sup> Similarly, Garg *et al.* have reported that the vertebral level of SA varied widely from T3/4 to T6/7, with the most common level being at T4/5 (35%).<sup>[5]</sup> Badshah et al. report the most common level to be at T5.<sup>[6]</sup> Uzun et al.<sup>[7]</sup> and Shen et al.<sup>[8]</sup> have reported the most common level of SA to lie at or above T4-5 IV disc. In our study, the SA passed through Level 3 in maximum cases (58.9%), followed by Level 2 (38.8%) with a range of T3 to T6/7 [Table 2]. Thus, the findings in our study broadly match the findings of other CT-based studies with respect to wide variation in level. Our findings are also similar to Chukwuemeka et al., Garg et al., and Badshah et al. with respect to the level of SA.[4-6] However, these findings are in variance with description of the SA in standard anatomy textbooks.<sup>[2,3,12]</sup> One of the possible explanations could be the movement of the thorax during inspiration, while CT images are acquired as opposed to previously described studies in cadavers. We found a statistically significant difference in vertebral levels of SA between males and females, wherein the SA was at a lower level in females. These findings are in variance with the findings of other authors where they found no statistical difference in the level of SA in relation to gender.<sup>[4,5,7,8]</sup> No plausible explanation could be found or suggested by authors for this difference. With respect to age distribution, our study also does not show any significant difference similar to other studies.[4-8]

Garg *et al.*<sup>[5]</sup> have reported the undersurface of AA to be at or higher than T3–4 IV disc level in 75% of the cases, whereas Uzun *et al.*<sup>[7]</sup> reported the same in 62%. Our study revealed that in a majority of individuals (72%), the undersurface of AA corresponded to Level 2 which correlates with the traditionally held belief described in most textbooks of anatomy. Similar results have also been shown by Badshah et al.<sup>[6]</sup> and Shen et al.<sup>[8]</sup> The concavity of the arch of the aorta has been variably reported to be corresponding to plane of the SA in 7%-59%.[4,5,7,8] In our study, however, the concavity of the arch of the aorta was at the level of SA in 36.8% and at a higher level in 56.68% of the cases. Interestingly, the level of the arch of the aorta did not show any such sex- or age-related variability in any of the studies. Although wide variation in the vertebral levels of TB has been reported with 63%-91% at or below T5.<sup>[5-8]</sup> Our findings are at variance to this as only in 43.03% of the cases TB were at or below T5. With respect to correlation with SA plane, TB and SA are at the same plane in 4%-31% of the individuals and lower in up to 91% of the individuals.<sup>[4,5,7,8]</sup> Our findings are at variance with these studies as we found TB and SA to be at the same plane in 41.13% of the cases. Furthermore, in another 42.4% of the cases in our study, TB was at a higher level than SA. The TB is seen to lie at a lower level with progression of age in our study. A significant difference was seen in the levels of TB in individuals below 50 years and above 50 years of age. The possible explanation for this difference could be age-related loss of elasticity of tracheobronchial cartilage, however, this needs to be studied further. Uzun et al. have reported a significant difference in levels of TB with respect to gender; however, no such difference was seen in our study.<sup>[7]</sup>

Shabshin *et al.*<sup>[14]</sup> correlated the superior margin of the aorta with the vertebral level and found it to vary from T2–T4 level, with only 8% of the cases above the level of T3 vertebra. Similarly, Connor *et al.*<sup>[16]</sup> have found that the mean vertebral level of the superior surface of AA is T3 with a range of T2–T5. In our study, the superior margin of AA was correlated with sternal notch instead of vertebral levels, and we found that in 31% of the cases, the superior margin of AA was above the level of sternal notch. AA was seen to correspond to the upper half of the manubrium or higher in more than 92% of the individuals. From an imaging perspective, the suprasternal approach for ultrasonography of the AA should be effective in the majority of the individuals due to this reason.

#### Conclusion

The conventional teaching in anatomy textbooks has been that SA, TB, and AA lie at the same cross-sectional plane which corresponds to T4–5 vertebral level. This description is based on historical cadaveric dissections and is probably governed by the dictates of convenience rather than on statistical data in living individuals in contrary to this traditionally held belief. Our study involving a sizeable number of living individuals using MRI as an anatomical tool has shown that these three anatomical landmarks lie at the same plane in only a minority of individuals. Even among these individuals, a further smaller number show that the corresponding cross-sectional plane is at T4-5 level.

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#### **Conflicts of interest**

There are no conflicts of interest.

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# Ectopic Pelvic Kidney and its Renal Artery from the Common Iliac Artery

#### Abstract

The pelvic kidney is the most common type of renal ectopia. It is clinically asymptomatic in general but are more prone to the urinary infections. It has also been associated with an increased risk of nephrolithiasis. The 37-year-old man was admitted to the emergency room with complaints of left flank pain, nausea and vomiting. In addition to this flank pain, the patient had an intermittent pain in the lower abdomen and pelvic region for 6 years with no history of fever. The renal ultrasound demonstrating several shadowing echogenic areas consistent with nephrolithiasis in both kidneys. In addition, while the right kidney was in the normal size and location, the left kidney was located on the ectopic site, just above the bladder. Doppler examination revealed double renal arteries originating from the aorta on the right side. Our case indicates the importance of the ectopic pelvic kidney location when planning surgical procedures in patients with renal and pelvic pathology, kidney transplantation and nephrectomy.

Keywords: Ectopic kidney, double renal artery, renal anatomy

#### Introduction

During the normal fetal developmental stages, the kidney does not yet reach its adult anatomical location.<sup>[1]</sup> If it continues to remain in the pelvic region after the fetal stages, it will be called pelvic kidney.<sup>[2]</sup> The pelvic kidneys which reside in the pelvis minor are clinically asymptomatic in general but are more prone to urinary infections and stone formation.<sup>[2,3]</sup>

The congenital anomalies of the kidney and the urinary system are common congenital organ malformations, with an incidence of 0.03%–0.16%.<sup>[3]</sup> The pelvic kidney is the most common type of renal ectopia. The ectopic kidney can rarely be found at the thorax.<sup>[2,3]</sup> The pelvic kidney can cause deep vein thrombosis due to its pressure on the iliac vein.<sup>[4]</sup> Ectopic kidney has also been associated with an increased risk of nephrolithiasis.<sup>[5]</sup> Furthermore, transitional cell carcinoma of a pelvic kidney is also observed, but it has a more rare occurrence.<sup>[6-8]</sup>

#### Embryology

Initially, the kidneys are located near the tail of the embryo in the early stages of embryogenesis.<sup>[1]</sup> The metanephric diverticulum and metanephric blastema, which are the main parts of the permanent kidney, develop from methanephrosis in the pelvis. The kidneys migrate to the lumbar region with the effect of the abdominal wall growth during the intra-uterine period. The kidneys also rotate medially during migration to renal fossa.<sup>[2,4]</sup> The vascular buds from the kidneys grow toward and invade the common iliac arteries. The genetic and teratogenic risk factors may affect the physiological development of the kidneys and lead to congenital disabilities. The congenital abnormalities of the kidney and urinary system are one of the common congenital organ malformations. It has two subgroups, which are the congenital malformations of the kidney and the anomalies of the lower urinary system.<sup>[2,3,9]</sup> The most common type of renal ectopia is a pelvic kidney. During the fetal period in the normal developmental stages, the kidney does not reach the anatomic location, and if it remains in the pelvic region is called the pelvic kidney.<sup>[5]</sup>

#### **Case Report**

In September 2018, the 37-year-old male was admitted to the emergency room with complaints of left flank pain, nausea, and vomiting. In addition to this flank pain, the patient had intermittent pain in the lower abdomen and pelvic region for 6 years with

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no history of fever. On careful investigation of the patient's medical history, it was discovered that he was admitted to the emergency department with the same flank pain complaint after seeing several physicians in different hospitals over many years. The patient was thin and tall. A kidney stone was suspected, and the renal ultrasound demonstrated several echogenic areas consistent with nephrolithiasis in both kidneys. In addition, while the right kidney was in the normal size and location, the left kidney was located on the ectopic site, just above the bladder. The left ectopic kidney was slightly malrotated with its pelvis oriented posteriorly. The size of the left kidney was 94 mm × 61 mm × 52 mm, and the renal pelvis was in the right and posterior position [Figure 1]. The size of the right kidney was 129 mm  $\times$  51 mm  $\times$  45 mm [Figure 2]. The thickness of the parenchyma of the right and the left kidneys were 11-12 mm and 12-13 mm, respectively. The cortical and medullary echogenicity of both kidneys were normal, and a parenchymal band was present in the middle, but there was no double collecting system. In addition, 8-10 stones were found in the calyces of both kidneys, ranging in size from 3 to 11 mm.

The computed tomography (CT) scan performed after ultrasound demonstrated multiple renal stones, but no double collecting systems in both kidneys [Figures 3 and 4].

Doppler examination revealed double renal arteries originating from the aorta on the right side. The diameter

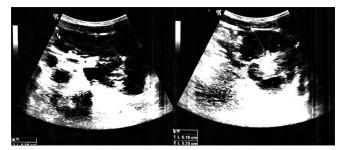


Figure 1: Renal ultrasonography demonstrating of the left kidney



Figure 3: Computed tomography urogram demonstrating the right kidney, the left kidney, and the pelvic part of the right ureter

of the right renal arteries was 4 mm and 3.5 mm. The diameter of the left renal artery was 5 mm, and it originated from the left common iliac artery immediately after the aortic bifurcation. The vascularization of both kidneys was symmetrical and normal. Segmental and interlobar arteries were normal, and resistivity indices up to arcuate arteries were normal. There was no renal artery stenosis in both kidneys.

#### Discussion

The kidneys that develop in the pelvis move to a more proximal site and merge with the adrenal gland. The growth of the lumbar and sacral regions of the embryo in length causes the kidneys to "ascend" to their final position in the upper lumbar region at the 9<sup>th</sup> week of gestation.<sup>[4]</sup> During this migration, as the kidneys rise, they continue to have new blood vessels at their new level and leave behind their old blood vessels to regress. As the kidneys migrate upward, the renal hilum turns 90° medially, and thereby, the renal hilum is finally located at the medial side of the kidneys.<sup>[1]</sup> Absence of the kidney in the renal

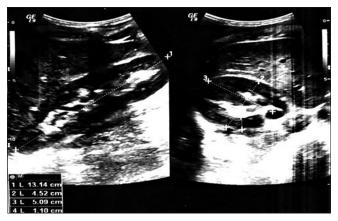


Figure 2: Renal ultrasonography demonstrating of the right kidney

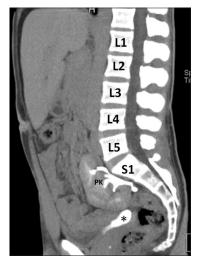


Figure 4: The sagittal section of the computed tomography urogram demonstrating the left kidney, which is located at the top of the bladder. \*: Urinary bladder

fossa (also called "Empty renal fossa") indicates renal agenesis; ectopic kidney is kidney located in an abnormal ectopic position which arises due to the failure of ascent or arrest of the ascent of the kidney.<sup>[3]</sup> The pelvic ectopia is a type of renal ectopia, which is caused by faulty migration from the pelvis during the embryological process. Although the ectopic kidney can be found in the lower lumbar, abdominal, and thoracic locations, the left pelvic region is the most common site for the ectopic kidney. In addition, contralateral and crossed ectopic kidneys can also occur.<sup>[5]</sup>

Urinary system anomalies can be detected in about 10% of the population. The presence of pelvic kidneys may not be noticed early in life unless it is detected by kidney ultrasound or abdominal tomography for different reasons. The pelvic kidney, which is the most common type of renal ectopia, can be seen in adults at the rate of 1/1000.<sup>[6]</sup> The incidence of a normal and a pelvic kidney ranges from 1:800 to 1:3000.<sup>[9]</sup> The pelvic kidneys are mostly asymptomatic. In the presence of an ectopic kidney, genital system abnormalities such as undescended testicles, urethral duplication, and hypospadias may also be defined. The malrotated ectopic kidney is one of the risks of stones formation, which may present as renal colic and hematuria, like in our case.

Since the renal vessels are shaped according to the normal anatomical position of the kidney, the position of the ectopic kidney also affects the origin of the kidney vessels. As we found in our patient, the artery of a distally located ectopic kidney may originate from a common iliac artery close to the aortic bifurcation, as well as from the inferior mesenteric artery, distal aorta, or external iliac artery.<sup>[9]</sup>

Although the ectopic kidney is asymptomatic, the patients should be monitored with ultrasound at regular intervals since these patients have a higher risk of developing nephrolithiasis. They should be alerted against complications such as secondary infection and hydronephrosis.<sup>[10-12]</sup> The treatment of hydronephrotic pelvic kidney involves various difficulties, as the kidney can be found in front of the sacrum, behind the lower gastrointestinal system and internal genital organs. Although the prevalence of renal cancer in the pelvic kidneys is exceedingly rare, it should also be kept in mind.<sup>[6-8,11]</sup>

Surgical removal of the pelvic kidney may be complicated because of its neighboring anatomical structures or the presence of a tortuous ureter in the presence of abnormal neurovascular structures.<sup>[10,12]</sup> For this reason, it is important to have information about the anatomical locations of the pelvic kidney. Our case indicates the importance of the ectopic kidney location when planning surgical procedures in patients with renal and pelvic pathology, kidney transplantation, and nephrectomy.

#### **Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

#### Financial support and sponsorship

Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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## **Turner Syndrome Associated with Cerebellar Abnormalities**

#### Abstract

We present an autopsied fetus with Turner syndrome (TS), obtained after a therapeutic abortion at the Clinic of Obstetrics and Gynecology at the University Hospital, Plovdiv, Bulgaria. It was a female fetus weighing 75 g. The chorionic villus sampling followed by karyotyping established monosomy XO. The fetal autopsy confirmed agenesis of the cerebellar vermis and cerebellar hypoplasia. The immunohistochemical study of the brain with S100 protein (S100), glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), and cluster of differentiation 68 was taken into consideration. The presence of extensive gliosis reaction is proved in the cerebellar structures of TS by the positivity of glial markers: (GFAP) and S100 protein. The quantity of neurons proved by NSE marker is probably associated with the deficiency of differentiation and cell migration caused by inhibition in the development of young cells. The presented case of TS found a new phenotype with cerebellar abnormalities described for the first time.

**Keywords:** Agenesis of the cerebellar vermis, brain, fetal autopsy, glial marker, immunohistochemical study, Turner syndrome

#### Introduction

The purpose of this study is to present a unique case of Turner syndrome (TS) associated with cerebellar abnormalities, utilizing modern investigation methods (ultrasound, amniocentesis, karyotype studies, and immunohistochemistry) in additionn to fetopathological autopsy as a final stage of diagnosis, as well as prognosis for future pregnancies

#### **Case Report**

The fetus was acquired from the therapeutic abortion of the first pregnancy of a 19-year-old female, at the Clinic of Obstetrics and Gynecology at the University Hospital "St. George," Plovdiv, Bulgaria. The control prenatal ultrasound of the fetus found a defect in the posterior cranial fossa Figure following 1], which a chorionic villus sampling and a karyotype examination were performed. The karyotype was found to be monosomy XO. Informed consent was obtained from both parents. A classical autopsy of the fetus immediately followed by the abortion and histological and immunohistochemical examinations of the brain structures were performed. All specimens were routinely

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fixed in 10% buffered formaldehyde and embedded in paraffin, and 3.5-µm-thick sections were stained with H and E [Figure 2]. After a longitudinal section of the cerebellum, the slides were selected for an immunohistochemical study by immunoperoxidase staining for S100 protein (S100), glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), and cluster of differentiation 68 (CD 68) markers using the DAKO "Link 48" immunostainer standard procedure [Table 1].

As a control specimen, a cerebellum was used without anomalies of a miscarried fetus at the 16<sup>th</sup> gestational week. Microphotographs were performed using the Nikon Microphoto-SA microscope (Japan), combined with Camedia-5050Z digital camera (Olympus, Japan). The measurements were performed with the help of the software"DP-Soft" 3.2 (Olympus, Japan). The microscopic field of study is divided by a rectangular grid with a X-horizontal and Y-vertical span. The parameters for this type of measurement and grid size of 20/20 pixels were set. Numerically, cellular densities in each square field (30 grids) of the same 300 mm<sup>2</sup> area were counted for each of the three markers for TS [Figure 3].

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The autopsy found biometric indicators confirming a fetal age of 16 gestational weeks and intrauterine growth retardation (fetal weight – 75 g). Facial dysmorphia (oblique eye slit, low-set and poorly formed auricles, long philtrum, and microretrognathia) and flat feet were present [Figure 4a and b]. Macrocephaly, pterygium, cystic hygroma of the neck , and horseshoe kidney malformations were also observed [Figure 4b and c]. The autopsy of the brain diagnosed

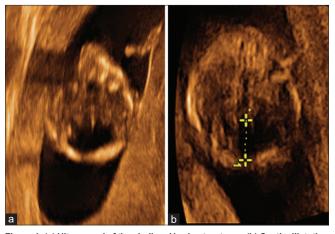


Figure 1: (a) Ultrasound of the skull and brain structures. (b) Cystic dilatation of posterior cranial fossa

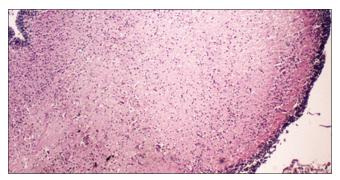


Figure 2: Cerebellum of Turner syndrome (H and E, 10 × 0, 25)

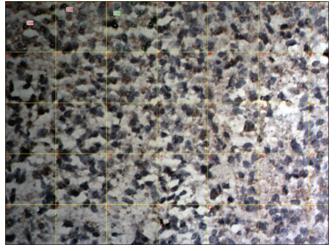


Figure 3: Method of morphometry

cerebellar hypoplasia (weight of cerebellum -1 g) and agenesis of the cerebellar vermis [Figure 4d].

#### Glial fibrillary acidic protein marker

The immunohistochemical study of the cerebellar structures with GFAP marker revealed increased gliosis in the case compared to the control [Figure 5a and b]. The morphometry established an increased cell expression in TS. Student's *t*-test revealed a statistically significant difference of expression of the GFAP marker in the case compared to the control [Figure 6 and Table 2].

#### S100 marker

The expression of the S100 marker in the cerebellum revealed an increased gliosis in the case with TS compared to the control [Figure 5c and d]. The morphometry established a weaker cell expression in the TS case compared to the control [Figure 6]. Student's *t*-test did not detect a statistically significant difference for the S100 marker in the case compared to the control [Table 2].

#### Neuron-specific enolase marker

NSE marker showed higher expression in the control specimen when compared to the case of TS [Figure 7a and b]. The morphometry established a weaker expression of NSE marker in TS [Figure 6 and Table 2].

#### **Cluster of differentiation 68 marker**

The lysosomal marker CD68 is negative in the control specimen, but in the case of TS, its expression is focal. Infiltration of mononuclear inflammatory cells in the fetal cerebellum with TS is increased [Figure 7c and d].

#### Discussion

Monosomy X is a chromosomal anomaly which represents about 65% of all aneuploidies.<sup>[1]</sup>

In the present study, the fetal autopsy confirmed agenesis of the cerebellar vermis and hypoplasia. The presence of extensive gliosis reaction is proved in the cerebellar structures of TS by the positivity of both glial markers GFAP and S100. Although only GFAF reaches statistical significance this is important, this is important because

Table 1: Distribution of the used products			
Used antibodies	Specific	Primary	
Anti-GFAP	Polyclonal rabbit anti-glial fibrillary acidic protein	Ready to use	
Anti-S100	Poly Rb a S100, RTU	Ready to use	
Anti-NSE	Mab a Hu NSE, cl BBS/ NC/VI-H14, RTU	Ready to use	
Anti-CD68	Monoclonal mouse anti-human, clone PG-M1	Ready to use	
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NSE: Neuron-specific enolase, GFAP: Glial fibrillary acidic protein, RTU: Ready to use

Table 2: t-test statistical significance for morphometry of glial fibrillary acidic protein, S100, and neuron-specific		
enolase markers		

Types	Variable	Control (n=14)	Turner syndrome ( <i>n</i> =13)	t	Р
GFAP	Mean (SD)	12.93 (12.07)	182.31 (49.54)	12.419	0.000
S100	Mean (SD)	102.4 (54.6)	103.8 (20.2)	0.093	0.927
NSE	Mean (SD)	91.6 (51.2)	86.0 (26.4)	0.376	0.710

SD: Standard deviation, NSE: Neuron-specific enolase, GFAP: Glial fibrillary acidic protein



Figure 4: (a) Fetus with Turner syndrome. (b) Facial dysmorphia – oblique eye slit, low-set and poorly formed auricle, and microretrognathia. Cystic hygroma of the neck. (c) Horseshoe kidney malformation. (d) Hypoplasia of the cerebellum, agenesis of the cerebellar vermis

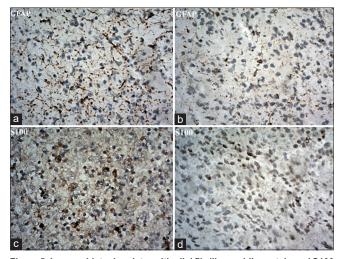


Figure 5: Immunohistochemistry with glial fibrillary acidic protein and S100 markers. (a) Glial fibrillary acidic protein: Turner syndrome. (b) Glial fibrillary acidic protein: control. (c) S100: Turner syndrome. (d) S100: control. The expression of both markers in the cerebellum reveals cellular architecture with increased gliosis in the case with Turner syndrome compared to the control ( $10 \times 40$ )

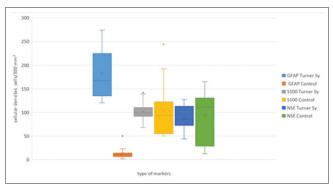


Figure 6: Graphic representation of the morphometric results with markers such as glial fibrillary acidic protein, S100, and neuron-specific enolase

GFAP is a predicative molecular signal for the degree of early developmental disturbances of fetal cerebellum.

The NSE marker does not show a statistically significant variation in the quantity of neurons, however, this does not deny the differences in cellular neuro-morphology. It is probably associated with deficiency of differentiation and cell migration, caused by inhibition in the development of young cells.

Cerebellar hypoplasia with glial proliferation suggests a disturbance in brain function, which does not correlate with the statement that there is no mental retardation in TS.<sup>[2]</sup>

Some authors explain the disturbance in the cerebellar development with intrauterine oxidative stress, while others with the absence of the X chromosome, which leads to a disturbance in the migration of neurons, but only once neuronal migration has been completed in the cortex of the cerebral hemispheres.<sup>[3]</sup> This explains the inefficiency of neuronal migration in the cerebellum and olive, which means that the process of neuronal migration is affected around the 4<sup>th</sup> month.<sup>[4]</sup> The morphogenesis of the cerebellum is impaired by the disturbances in neuronal migration and cell architectonics, long after the closure of the neural tube.<sup>[5]</sup>

According to the most recent studies, an increased immune reactivity of interleukin 1 and S100 markers is found in degenerative processes, such as Down syndrome and Alzheimer's disease.<sup>[6,7]</sup> Similar disturbances are found in Zellweger syndrome and trisomy 21. The association of TS with cerebellar hypoplasia is an additional evidence of a disturbance in the embryonic development of the brain. Although the prognosis for its clinical presentation in the postnatal period is difficult, the questions regarding the necessity of a prenatal genetic study and abortion due to medical reasons in cases of a positive result remain.

#### Conclusion

The presented case of TS found new phenotype with cerebellar abnormalities. This phenotype is a result of

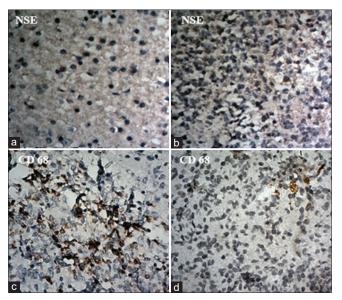


Figure 7: Immunohistochemistry with neuron-specific enolase and cluster of differentiation 68 markers. (a) Neuron-specific enolase: Turner syndrome. (b) Neuron-specific enolase: control. Neuron-specific enolase marker shows higher expression in the control specimen than in the case of Turner syndrome/CD68 Turner syndrome. (d) Cluster of differentiation 68: control. The lysosomal marker cluster of differentiation 68 is negative in the control specimen, but in the case of Turner syndrome, its expression is focal (10 × 40)

disturbances in the morphogenesis up to the 16<sup>th</sup> gestational week of embryonic development, due to impaired neuronal migration and cell architectonics. The most crucial aspect of the prenatal diagnosis remains the karyotype examination, even though chorionic biopsy is associated with an increased risk for the pregnancy.

#### **Declaration of patient consent**

The authors certify that they have obtained all appropriate

patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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Initiative	Type of Study	Source
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The manuscript could be of up to 1000 words (excluding references and abstract) and could be supported with up to 10 references. Case Reports could be authored by up to four authors.

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#### **Articles in Journals**

- Standard journal article (for up to six authors): Parija S C, Ravinder PT, Shariff M. Detection of hydatid antigen in the fluid samples from hydatid cysts by coagglutination. Trans. R.Soc. Trop. Med. Hyg.1996; 90:255–256.
- 2. Standard journal article (for more than six authors): List the first six contributors followed by *et al*.

Roddy P, Goiri J, Flevaud L, Palma PP, Morote S, Lima N. *et al.*, Field Evaluation of a Rapid Immunochromatographic Assay for Detection of Trypanosoma cruzi Infection by Use of Whole Blood. J. Clin. Microbiol. 2008; 46: 2022-2027.

3. Volume with supplement: Otranto D, Capelli G, Genchi C: Changing distribution patterns of canine vector

borne diseases in Italy: leishmaniosis vs. dirofilariosis. Parasites & Vectors 2009; Suppl 1:S2.

#### **Books and Other Monographs**

- 1. Personal author(s): Parija SC. Textbook of Medical Parasitology. 3rd ed. All India Publishers and Distributors. 2008.
- Editor(s), compiler(s) as author: Garcia LS, Filarial Nematodes In: Garcia LS (editor) Diagnostic Medical Parasitology ASM press Washington DC 2007: pp 319-356.
- Chapter in a book: Nesheim M C. Ascariasis and human nutrition. In Ascariasis and its prevention and control, D. W. T. Crompton, M. C. Nesbemi, and Z. S. Pawlowski (eds.). Taylor and Francis,London, U.K.1989, pp. 87–100.

#### **Electronic Sources as reference**

Journal article on the Internet: Parija SC, Khairnar K. Detection of excretory *Entamoeba histolytica* DNA in the urine, and detection of *E. histolytica* DNA and lectin antigen in the liver abscess pus for the diagnosis of amoebic liver abscess. *BMC Microbiology* 2007, 7:41. doi:10.1186/1471-2180-7-41. http://www.biomedcentral. com/1471-2180/7/41

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