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CONTENT

Instructions to Authors

Supplement to Volume 3; Issue II; 2019

12. HIPK2 may be a marker of neuronal stress in ALS/FTLD
    Mathangasinghe Y, Wright HB, Walker AK
13. High frequency of Klinefelter syndrome in a cohort of Sri Lankan males with azoospermia and oligozoospermia
    Samaranayake UMJE, Sirisena ND, Malalasekera AP, Dissanayake VHW
14. Effect of gestational age on the morphometric parameters of placenta
    Nazeefa HMFJ , Thirukumar M , Thayabaran M
15. The Morphology and Position of the Pterion in a sample of Sri Lankan Adult Population
    Nawarathna NGS, Dissanayake DMMLB, Sampath PH, Manawarathna MRK, Dissanayake JK, Peiris HRD, Nanayakkara CD
16. Cadaveric study of variations of origin of lateral circumflex and medial circumflex artery in a Sri Lankan Population
    Dassanayake DMTD , Chrishan AN, Dissanayake JK
17. Determination of sex using the humerus in a contemporary Sri Lankan population
    Dissanayake JK, Kodikara KAS, Peiris HRD, Ranasinghe DJ, Abeywardhana UNY, Chandrasiri DACT, Nawarathna LS, Nanayakkara CD
18. Vascular Anatomy of the Optic Disc: A Cross Sectional Study in a Sri Lankan Sample
    Ruwanpathirana PS, Sanjayan A, Dissanayake MM
19. Morphometric analysis of the foramen magnum in a sample of adult Sri Lankan skulls
    Fernando RCU, Ihalagedara DD, Vadysinghe A, Peiris HRD, Nanayakkara CD
20. Comparison of neck Range of Motion (ROM) and neck lordosis angle between spectacle users and non-spectacle users among first year undergraduates of the University of Peradeniya
    Kumarasiri KGSI, Jayasundara BP, Kalahagahawaththa KWDP, LisanaMMF, Wijesinghe NP, Karunathilaka KGLRAD, Senarath MKID, Sominanda A
21. Learning style preferences among second year medical students in Rajarata University of Sri Lanka
    Vijithananda HHTSM, Senarath DMKN, Perera HTC, Dharmarathne KND, Abeyrathne IANP, Adikari SB, Paththinige CS, Jayawardana SMA
22. Student perception of the educational environment in an evolving medical school in Sri Lanka
    Ranaweera SMKA, Nanayakkara NGGCS, Sanjeewa WA, Perera HTC, Warnasekara YPJN, Koralegedara KIS, Paththinige CS

23. A study on student perception on a formative 'mini-viva' examination in Anatomy
Ilangarathne IPSD, Dissanayake CS, Rodrigo PWDCH, De Silva CL, Koralegedara KIS, Jayawardana SMA, Paththinige CS

24. Knowledge, awareness and perception regarding physiotherapy services among clinical students in Rajarata University
Senarath DMKN, Sandamali AAK, Wijekoon WMMU, Ilangarathne IPSD, Ranaweera SMKA, Koralegedara KIS, Wickramage SP, Paththinige CS

25. Estimation of stature from hand and handprint measurements in a sample of Sri Lankan Sinhalese population
Ekanayake SD, Nandasena T, Nawarathna LS, Alahakoon S, Ariyasinghe S, Arambawatta AKS, Nanayakkara CD

26. Anatomical variations of the obturator artery in a group of Sri Lankan subjects: A cadaveric study
Samarawickrama MB, Rodrigo PM, Nanayakkara PGCL, Chamara AGD, Tharaka NGS, Karunadasa THAS

27. Anatomical variations of Peroneus tertius and Extensor digitorum longus muscles: Cadaveric Study
Kosgallana EW , Eshwara JM, Dissanayake JK

28. Anatomical variations of the sciatic nerve: A descriptive study
Chandrasiri DACT, Patabandige DJ, Ekanayaka EADA, Dissanayaka JK

29. Anatomical landmarks for the intercostal nerve blocks: A cross sectional cadaveric study
Mathangasinghe Y, Samaranayake UMJE, Wijayasinghe R, Manchanayake M, Seneviratne PD, Dassanayake VEG

30. Analysis of foot and footprint measurements to determine the sex of an individual
Jayaweera AS, Nandasena T, Nawarathna LS, Alahakoon S, Ariyasinghe S, Nanayakkara CD

31. Protocol for harvesting adult human hippocampus
Mathangasinghe Y, Handunnetti SM, Warawitage DH, Dissanayake DN

32. Location and Number of Nutrient Foramina in Human Long Bones of a Sri Lankan sample
Subasinghe SAJS, Weerasooriya WWRWMPK, Fernando RCU, Ekanayake EMSD, Jayaweera AS, Nawarathna NGS, Sampath PH, Manawarathne MRK, Banneheka BMHSK, Peiris HRD

33. Association of volume estimation accuracy obtained using USS, CT and volume calculation formulae for different shaped objects
Kodikara SKYI, Gamage DTK, Abeysekara I, Gamage LGM, Ilayperuma I

34. Psychological effects of Anatomy dissections among pre-clinical medical students in Rajarata University of Sri Lanka
Rodrigo PWDCH, Lasanthi SS, Vijithananda HHTSM, Dissanayake CS, Sanjeewa WA, Warnasekara YPJN, Koralegedara KIS, Paththinige CS

35. A survey on the attitudes of the Ayurveda medical students towards cadaver dissection
Abegunasekara NS, Chandimal KM

36. Optimizing a protocol for isolating fibroblasts from human oral cavity
Wickramasinghe WAJS, Dissanayaka VHW, Udagama PV, Goonasekera HWW

37. A case report of a high division of Extensor digitorum longus
Eshwara JM, Kosgallana EW, Dissanayake JK

EDITORIAL

Retention of Anatomy Knowledge

Anatomy teachers in medical faculties are often held responsible by clinicians especially surgeons for the inability of undergraduates to answer questions related to anatomy, once they start clinical training, having passed anatomy as a preclinical subject. This is a universal problem and anatomy teachers world over are faced with the challenge to overcome this issue. Having introduced more clinically oriented methods of teaching anatomy and including more clinically oriented questions at main examinations, the problem still remains.

Any subject, once learned, will not be retained indefinitely in one's mind without repeated re-exposure. Anatomy is no exception, and it would be interesting to see how knowledge in anatomy has been retained in those actively involved in the field of medicine for many years.

The Department of Anatomy at the Faculty of Medicine, Peradeniya embarked on a study few years back in collaboration with the Griffith School of Medicine, Australia to explore this issue. The preliminary results showed that the ability to recall basic anatomy is significantly affected by the length of time after passing anatomy as a subject. Medical students, few months after their main anatomy examination, as expected, retained better than final year students. Whereas consultants having completed their postgraduate examinations, in which basic anatomy is one important component, were much worse than the final year medical students and intern house officers who are getting trained under their own supervision. This finding could be an eye opener for those who plan to improve retention of anatomy knowledge in students where new strategies need to be introduced to overcome the issue.

A feasible solution that is being currently tested under the aforementioned research project is to introduce an online module that can be used by doctors and students as an in-service revision of their knowledge. The said module is being designed in such a way that based on the sub-field of medicine concerned, the user could revise the knowledge relevant to the field as the need arises. For instance, a medical officer transferred to a psychiatry unit after serving in the out-patient department for several years may revise the section on brain whereas a medical officer who is about to commence in the orthopedic unit will study the section on the skeleton and limbs. Once introduced, the medical students will be instructed to revisit the module from time to time based on their on-going field of training. In

parallel the extended clinical staff will be requested to reiterate the students whom they think have forgotten the basic facts to make use of the online module.

There is no question that it is our duty as anatomy teachers to be open-mined to criticism about our teaching and improve its quality and relevance, but at the same time it is not fair by the subject of Anatomy, which is one of the main pillars on which the field of medicine stands, to allow irrational and prejudiced attacks upon it.

Professor Sanjaya Adikari *(MBBS, PhD)*

President of the Anatomical Society of Sri Lanka
Professor of Anatomy

REVIEW ARTICLE

Hip Geometry on Femoral Neck Bone Strength; a Review Article

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Abstract

Introduction: Low bone strength of the hip, which depends on both bone mass and bone geometry, leads to risk for fragility hip fracture. Anatomical adaptations of the proximal femoral structure are important to maintain higher bone strength because they predict the fracture risk independent of bone mass. Therefore, it is crucial to evaluate the bone strength of proximal femur to predict the likelihood of fragility fracture risk based on both bone mass and geometric adaptations, as well as the biomechanical strength of femoral neck (FN).

Aim: To discuss the literature on geometric indices and biomechanical indices of the proximal femur to estimate FN strength

Material and methods: Dual energy x-ray absorptiometry (DXA) based hip structural analysis (HSA) technique derived indices of FN geometric adaptations and biomechanical strength at FN and their impact on FN bone strength were explored using the existing literature.

Bone mineral content (BMC, gram) and areal bone mineral density (aBMD, g/cm^2) can be measured using the HSA technique. The simple hip geometric indices such as hip axis length (HAL), femoral neck length (FNL), neck shaft angle (NSA), FN diameters, FN width and biomechanical indices such as cross-sectional moment of inertia (CSMI, cm^4), section modulus (Z, cm^3) and cross-sectional area (CSA, cm^2) can be estimated using the HSA technique to estimate FN bone strength.

Conclusion: Estimation of FN bone strength by HSA based geometric adaptations and biomechanical strength indices provides better understanding of bone strength at FN. HAL and FN bone size with BMD provide better hip fracture predictability.

Key words: femoral neck, bone geometry, bone strength, HSA

Introduction

Fragility hip fractures are associated with increased morbidity, mortality and impaired quality of life, leading to social and healthcare burdens. With the increasing elderly population, the incidence of hip fracture is estimated to be increased - especially in Asian countries (1). The strongest risk factor for sustaining a hip fracture is low bone mass, which can be assessed as, bone mineral content (BMC, g) and areal bone mineral density (BMD, g/cm2). However, unfavorable bone structure,

independent of bone mass, predicts FN fracture risk (2). Therefore, the ability of a bone to resist fracture, or whole bone strength, depends not only on the amount of bone mass but also bone size, bone shape or spatial distribution of bone mass, bone geometry, cortical and trabecular macro and microarchitecture, as well as the balance between bone formation and bone resorption (3) (Table 1). FN Bone diameter or bone size increases with advancing age. This increase in bone size by periosteal apposition is one important geometric adaptation that affects bone strength. FN diameter increases with aging, to compensate for the age related bone loss in order to maintain higher strength [9]. The fact that bone structure, independent of bone mass, predicts the fracture risk highlights the importance of considering hip geometry and biomechanical properties bone, when predicting bone strength and fragility fracture risk (2). Therefore, understanding the mechanism and anatomical factors associated with bone geometry and biomechanical indices of proximal femur at the hip is an important fracture preventive strategy. Thus, the purpose of this article is to review the literature on bone geometry and biomechanical properties of hip on predicting FN bone strength.

Table 1: Determinants of bone strength

Structural properties	Material properties
Geometry	Mineral
Size (bone mass)	Mineral-to-matrix ratio
Shape (distribution of bone mass)	Crystal size
Microarchitecture	Collagen
Trabecular architecture	Type
Cortical thickness/ porosity	Cross-links
Microdamage/ microfracture	

Determinants of bone strength:

Approximately 80% of the total skeletal mass is composed of cortical bone, mainly found in the outer surfaces of long bone shafts. The cortical bone provides the mechanical strength and stiffness of bone. The remaining 20% of the skeleton comprises trabecular bone, mainly found in bones of axial skeleton such as the ribs, the vertebrae and in the metaphysis, epiphysis and central part of the diaphysis of long bones (4). Trabecular regions, in the central part of the bone form a honeycomb-like network of thin rods and plates. These trabeculi contribute to form the trabecular network or trabecular microarchitecture, which provides architectural support to the bone. Compared to the cortical bone, trabecular bone comprises a larger surface area to volume ratio. Because

of it, the trabecular bone plays a more important role in the bone remodeling than the cortical bone. The process of bone remodeling is important to adjust bone strength in response to mechanical stress such as physical exercise, as well as to repair any micro-damages, consequently maintaining higher bone strength (5).

Anatomy of the hip:

The proximal femur is a complex structure. Femoral head and neck are predominantly composed of trabecular bone and the outer shell is formed by the cortical bone. The head of the femur articulates with acetabulum forming hip joint, a synovial ball and socket joint, allowing a wide range of movements in the hip. Such a range of mobility is pertained by having a neck that is much narrower than the diameter of the head. The FN, which connects the femoral head into the shaft of the proximal femur, is important to transmit body weight into lower limbs. Greater and lesser trochanters, which are the bony prominences closely related to FN, are important for attachment of muscles. In addition, the greater trochanter, as being the outermost projected bony part, is subjected to the first strikes on the ground when falling. In a cross-section, FN is roughly cylindrical in shape. It has a structure with varying cortical thicknesses (CT) and periosteal diameter (PD) due to the uneven spatial distribution of bone mass throughout its length (6). Because of that, FN shows varying diameters throughout its length. The narrowest neck represents the smallest PD and is identified as the weakest point. The narrowest neck, being the most vulnerable section for FN fracture, is used as the region of interest to determine the geometric indices and biomechanical measures of the FN, to calculate the FN bone strength (7, 8) (Fig 1)

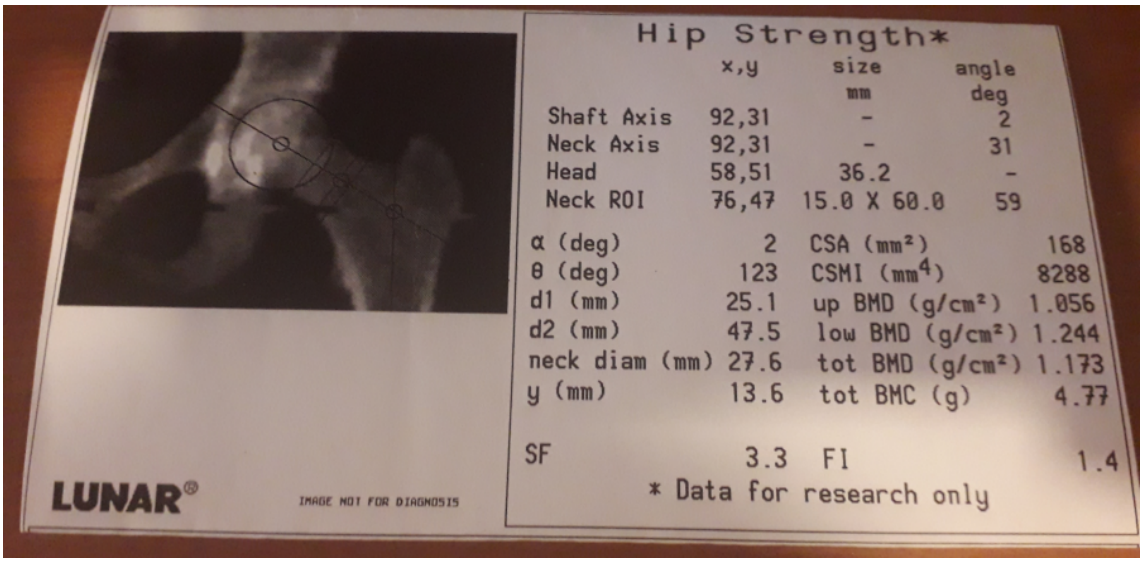

Figure 1: HSA output image showing the measurements of FN geometry and biomechanical properties

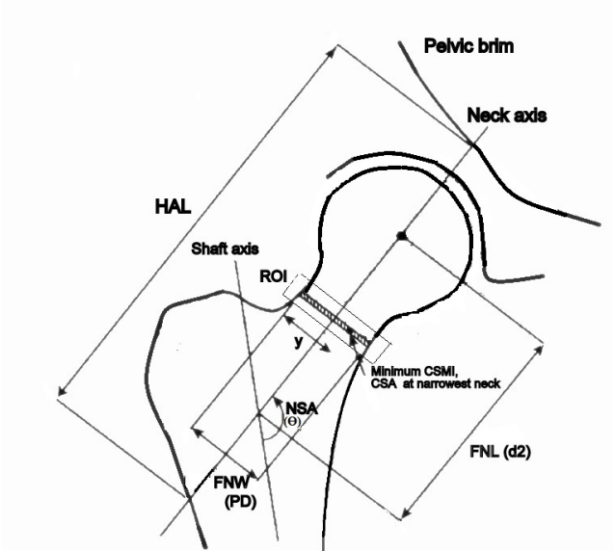

Figure 2: Measurements of Hip geometric indices; Hip axis length (HAL), femoral neck width (FNW), periosteal diameter (PD), femoral neck length (FNL), neck shaft angle (NSA), region of interest (ROI), cross-sectional moment of inertia (CSMI) and cross-sectional area (CSA).

Measurements of Hip geometry:

Hip axis length (HAL) is the distance from the base of the greater trochanter to the inner pelvic brim. Femoral neck axis length (FNAL) is the distance from the base of the greater trochanter to the apex of the femoral head. Femoral neck length (FNL/d2) is the distance from the user-defined center of femoral head to the intersection of neck and shaft axes. HAL increases with advancing age and longer HAL increases the risk of FN fracture (9, 10). Thus, HAL has been identified as an independent predictor of hip fracture (10, 11). Femoral neck shaft angle (NSA/θ) is the angle between the FN axis and the femoral shaft axis. The normal range of FNSA is approximately 120-135 degrees in adults. It is wider in young children and gradually becomes narrower with walking and mobility. Larger FNSA is associated with reduced bone strength and increased FN fracture risk in both men and women. An active lifestyle, by enhancing the level of PA is advisable to reduce NSA and therefore to increase bone strength at FN (12). Femoral neck width (FNW), which is the narrowest diameter across the FN, is also known as PD at the narrowest FN. Endosteal diameter (ED) at the narrowest FN can be estimated using the algorithm described by Thomas J. Beck (13). The mean CT is calculated as the difference between the periosteal and the ED, divided by two.

Biomechanical properties of bone:

Bone is structurally designed for resisting bending and torsional stresses. The resistance to bending and torsional loading is important to maintain bone strength against fracture. When a load is applied to a bone, it causes

deformation. The biomechanical properties of bone describe the impact of both bone mass and the distribution of bone mass based on the relationship between force/load applied to the bone and its deformation. Under normal conditions, long bones are predominantly loaded by bending and axial compressions (14).

The resistance to compressive loading depends on the cross sectional area (CSA, cm^2) of the bone. The resistance to bending strength of a bone is determined by cross-section moment of inertia (CSMI, cm^4) and section modulus (Z, cm^3), which depends on the spatial distribution of bone mass in relation to its neutral axis. The mechanisms of bending and axial strength have been explained using three circular bars, all composed of the same bone mass (Figure 3) (3, 15). For the same BMD, bone C has reported greater bending strength and axial strength than bone B and bone A as reported in the literature (3, 15). When the cortical shell moves further away from the bone's neutral axis, by periosteal apposition or endosteal resorption, resistance to bending can be improved (14). By using the HSA software the contribution of biomechanical parameters on hipbone strength independent of BMD can be estimated (Fig 1).

CSMI, the key biomechanical parameter, describes the ability of the bone to withstand bending forces or torsional forces independent of the material properties of the bone. The HSA software identifies the plane with the least CSMI across the narrowest FN, together with its center of mass. Z is another biomechanical index that also describes the resistance to bending forces of a bone. It can be estimated when CSMI is divided by "y"; the maximum distance from the neutral axis of the bone to perisosteam, or half of the PD (13) (Fig 2).

CSA is an index of the resistance of bone axial or compressive forces and as a result, the bone becomes shorter. CSA represents the minimum CSMI section of the narrowest FN and is equivalent to the BMC of that cross-section when all soft tissues are eliminated (13).

How to Measure hip geometry and biomechanical indices:

Dual energy x-ray absorptiometry (DXA) is used worldwide to measure BMC and BMD. DXA based advanced hip structural analysis (HSA) software is used to extract cross-sectional geometric indices and biomechanical parameters of the narrowest FN to calculate bone strength of the proximal femur (7). Bone geometric indices that determine the FN bone strength include the HAL, FNL, NSA, PD, ED and CT. These hip geometric parameters in combination with the biomechanical indices; i.e. CSMI, CSA and Z of the hip provide a better understanding of FN bone strength and fracture risk (2, 7) (Fig 1). Furthermore, these DXA based geometric indices at proximal femur have shown strong correlations with the biomechanical indices of hip strength of cadaveric proximal femur.

HSA is a cost effective approach, with minimum radiation exposure, to evaluate FN geometry and biomechanical behavior of hipbone strength. DXA technology is not designed to assess bone structure. Therefore, hip geometric indices obtained from two-dimensional HSA technology, have their inheriting deficiencies. The CT and the ED are estimated after making assumptions of a homogenous porosity in the cortical shell; a homogenous cross-sectional shape that assumes the FN to be cylindrical, not elliptical (16). However, DXA based HSA remains as a valuable tool to assess bone loss and fragility hip fracture risk. Inconsistent and inaccurate positioning of the femur, mainly due to the ante version of FN has been identified as the important limitation of the HSA technology. Therefore, careful attention should be paid, in repeated scans of the same person, to ensure the consistency among scans as much as possible, to minimize positional errors by the technician. Studies conducted using the peripheral quantitative computed tomography (pQCT) technique, provide novel insights into better anatomical bone structural changes when predicting fracture risk. However, because of high cost and risk of exposure to radiation, these more advanced technologies to assess FN bone geometry have not yet been well established. Further, in validations studies, HSA and QCT correlate favorably, which supports the validity of HSA derived geometrical properties of proximal hip (17).

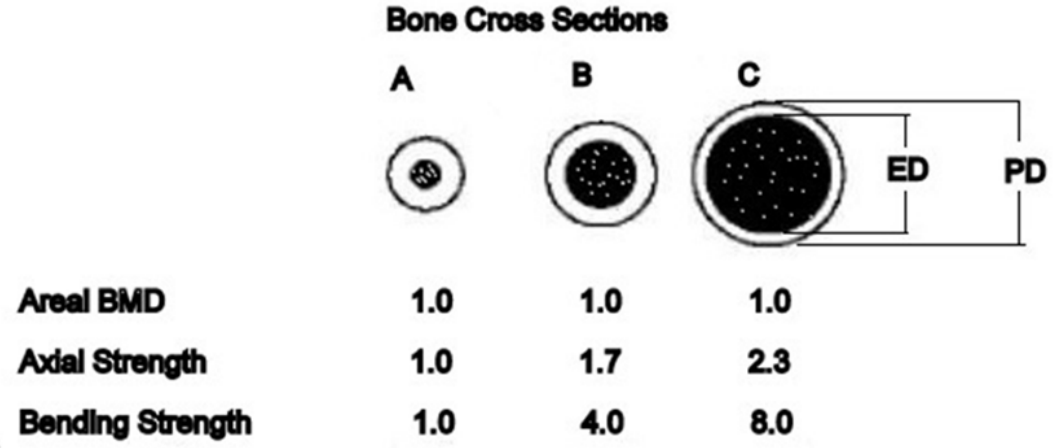

Figure 3: The effect of bone geometry on bone strength explained by changes of bone diameters with the spatial distribution of bone mass away from neutral axis. ED= endosteal diameter and PD= periosteal diameter (3).

Applications of hip geometry and biomechanical properties when predicting hipbone strength in adults:

Age related loss of bone mass is a common manifestation in the aging skeleton. In the bone mass life curve, even after reaching the plateau stage of bone mineral accrual where the bone formation equals the bone resorption in young adults, the bone size continuously increases into adulthood providing an important bone structural adaptation for fracture resistance (13,18). Larger bones are stronger than smaller bones, which support the importance of bone size when predicting fracture risk (Fig 2). Men and women reported different patterns of FN geometric adaptations and biomechanical strength at FN. This sexual dimorphism of bone structural changes through geometric adaptations in adult men and women has shown differences in hip fracture prevalence (13). Surface specific apposition of bone tissues on DP and ED is known as periosteal apposition and endosteal apposition respectively. Out of these two processes, increased PD due to periosteal apposition is more important to fracture resistance than the endosteal apposition. Periosteal apposition causes increased bone diameter or width, which is an advantage to have higher bone strength. This theory is implied when mechanical load such as physical exercise is applied on bone tissue to increase bone diameter by the process of periosteal apposition in boys and endosteal apposition in girls (12). In that, small increases in PD of a long bone, markedly improve its resistance to bending and torsional loading, by the fourth power of the radius (3, 15) and (Fig 3). In general, both BMD and CT decreased gradually but significantly with advancing ages whereas both PD and ED increased with advancing ages (13, 18). When the increase of ED is more than increase of PD, bone mass distributes further away from its neutral axis, which is an advantage because it positively influences bone resistance against bending and torsional loads (19). FN bone strength is more obvious in men than in women as periosteal expansion is more marked in men than in women. Furthermore, the age and sex discrepancies of bone size and spatial distribution of bone mass on bone strength between men and women highlight the importance of bone structural adaptations from childhood into older adults in order to predict FN bone strength and likelihood of fracture risk in elderly men and women (13, 18). In addition, the effects of changing skeletal load on hip BMD and geometry based on body weight have been studied in elderly women. It showed that the hip appears to adapt the body weight between weight gainers and losers by adjusting the Z, an index of bending strength, to new loading conditions (12). Recent studies have further shown that decreased CT, CSMI, and Z were predictive of hip fracture by changing FN diameters. It further showed that the significance was disappeared when the findings were adjusted with BMD. The study further suggests that both geometric properties and BMD are important to predict hip fracture risk (13).

Conclusion:

In conclusion, estimation of FN bone strength by HSA based geometric adaptations and biomechanical strength indices, provides better understanding of bone strength at FN. Simple geometric properties of the hip such as HAL and FN diameters may play a significant role in prediction of hipbone strength and FN fracture prediction ability. However, HAL and FN bone size in combination with BMD provide better hip fracture predictability. These observations have been shown using retrospective and cross-sectional studies. Nevertheless, future prospective and controlled studies are needed to confirm the potential of fracture predictive ability of both HAL and FN diameters. In addition, their applications in primary health care settings to evaluate FN bone strength and fracture risk need to be evaluated in future intervention studies.

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RESEARCH PAPERS

Optimization of DNA extraction protocol using skeletal remains found in Sri Lanka

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Abstract

Introduction

The preservation of DNA in old skeletal remains is reported to be very low in a tropical country like Sri Lanka due to prevailing climatic and environmental conditions such as high temperature, high rainfall and high humidity, etc. In this study, extraction of DNA from old skeletal remains dated back to 15 – 40 years was attempted by using previously published extraction protocols.

Materials and Methods

A 15-years-old humerus (15Y) excavated from Kuliyapitiya area in Kurunegala district and the 40-years-old tibia (40Y) received from Department of Anatomy, Faculty of Medical Sciences, University of Sri Jayewardenepura were used to extract old DNA. Human mitochondrial HVS I region of extracted DNA was amplified in PCR using four overlapping first round primers and second round nested primers respectively. A second-round nested PCR was performed. PCR amplification success was verified upon electrophoresis in 2% agarose gels.

Results and Analysis

DNA bands were obtained with correct size ranges for all systems in both first and second round PCR products of amplified DNA extract of old bones 15Y and 40Y from modified phenol-chloroform method. DNA bands were obtained from all four systems for 40Y bone DNA extract from DNA investigation Kit; QIAGEN, Germany.

Conclusion

In the present study, we have successfully extracted and amplified DNA from old skeletal remains by using modified phenol chloroform method and DNA investigation Kit – QIAGEN, Germany, nevertheless the preservation of DNA in skeletal remains in Sri Lanka is very low.

Keywords - Old DNA, Bone DNA extraction protocols, Forensic medicine,

Introduction

Nucleic acids in biological material are subjected to degradation and modification due to exposure to water, oxygen and, microbes and thus the survival of DNA in forensic remains/archaeological remains depends on geographical, climatic, environments and soil conditions.[1,2,3] The low preservation of DNA in forensic remains of old bones/teeth and their authenticity are the two main inherent problems recognized in extraction and analysis of old DNA.[4]

Nucleic acids slowly degrade over time mainly through processes of hydrolysis and oxidation.[2,5] Hydrolysis breakdown the N-glycosyl bond between the sugar and the base of DNA strand in the presence of water. Oxidation modify bases or distort the helix due to hydroxyl or superoxide radicals. As oxygen metabolism in the cell is taken place within the mitochondria, oxidation mainly affects the mitochondrial DNA compared to the nuclear DNA.[6]

Hydantoins (oxidized pyrimidines) are mostly done the destruction to DNA.[6] Hydantoins decreases the success in extraction and amplification of DNA in old and ancient skeletal remains. They particularly inhibit chain extension in PCR[7]. Therefore, recovery and, amplification of DNA in old bones, when possible, is usually limited to fragments of having sizes range from 300 to 500 bp in length[6].

The presence of a mineral matrix of hydroxyapatite which surrounds the osteon, preserves its DNA, therefore bone is generally considered an optimal DNA source for DNA studies in old samples[2,8,9]. Literature further supports that bones give better DNA yield than that of other soft tissues of the human body[10,11].

Globally, several DNA extraction protocols have been developed for old and ancient samples [4,12,13,14,15,16,17,18,19]. The Chelex based DNA extraction protocol has been attempted to extract highly fragmented aDNA from human and animal skeletal remains dated back to 5,400 YBP at temperate countries like Canada by Newman et al., (2002) [13]. The method of ethanol precipitation enhanced by Dextran blue has yielded aDNA of skeletal remains found in Iran in the Middle East [14]. Phenol-chloroform method has been used to successfully extract aDNA not only from Neanderthal infant (29,000 YBP) excavated from Russia but also from human bone samples (600-year-old) found in Malaysia situated near to equator [19,14].

The preservation of DNA in old or even archaeological remains in tropical countries like Sri Lanka is very low and the amount of recoverable DNA is scanty[20]. There are no documented studied done on successful extractions of DNA from old skeletal remains found in Sri Lanka. Therefore, the development of an efficient method to recover DNA from old skeletal remains found in a tropical county like Sri Lanka is timely needed.

Materials and Methods

Precautions to avoid contamination during DNA extractions

All extractions and PCR activities both pre and post were performed in three separate rooms with dedicated equipment according to the guidelines given for analysis of old and ancient DNA[4]. Physically isolated clean room was dedicated to DNA experiments for all manipulations prior to PCR. Access to DNA extraction room was strictly controlled according to the guidelines given by Paabo *et al*., 2004; Amory et al., 2012; and Zgonjanin *et al*., 2017[4,21,22].

Preparations reagents, bone grinding, DNA extractions and PCR were performed in laminar floor hood equipped with UV bulbs. The laminar floor hood was cleaned with 10% bleach, 96 % ethanol and UV irradiated before each use.

Barrier tips/aerosol-resistant tips were used in reagent preparation, DNA extraction and PCR and other all steps. Solutions used for DNA extraction, PCR reagents and post PCR reagent were stored in isolated rooms. Bone processing and DNA extraction was carried out in separate room. Polymerase Chain Reaction (PCR) was set up in a room dedicated for this sole purpose and post PCR gel electrophoresis was carried out in a third room.

The use of masks, coats, gloves and the use of disposable sterile materials for all manipulation were done to minimize potential contaminations. A negative control containing blank DNA extraction was performed along with each batch of extractions to eliminate contaminations during DNA extractions. A second negative control was used in PCR to verify the extraction and PCR authenticity[21,23].

Bone samples analysed

A 15-years-old humerus (15Y) excavated from Kuliyapitiya area in Kurunegala district and the 40-years-old tibia (40Y) received from Department of Anatomy, Faculty of Medical Sciences, University of Sri Jayewardenepura were used to extract DNA.

15 Y
15 Y (15-years-old humerus)

40 Y
40 Y (40-years-old tibia)

Bone samples used in extractions

Preparation of bones for DNA extraction

The preparation of bone was done according to the methods described by the Amory *et al*., (2012) [21]. The work station and all bone scraping tools were thoroughly cleaned with a freshly prepared 10 % bleach solution followed by washing with 96 % ethanol.

The selected bones were washed thoroughly with household bleach and distilled water respectively at least for two times and followed by air dried. The area of sampling was cleaned by using a sterile and UV irradiated sand-paper which has been stored in -20oC to remove the outer most bone surface to remove contaminants and other impurities. Then the purified bone surface was exposed to UV for 15 minutes. The UV irradiated surface was used to make fine powdered bone sample by using UV irradiated sand-paper.

DNA extraction protocols

Extraction of DNA from skeletal remains was attempted by using previously published extraction protocols such as Chelex[13], ethanol precipitation by Dextran blue[14], modified ethanol precipitation by Dextran blue[14], modified phenol-chloroform method [15] and one commercially available DNA extraction kit (DNA Investigation Kit; QIAGEN, Germany). Five extraction protocols were tried out to extract DNA of 15Y and 40Y bone samples.

PCR amplification of the human mtDNA HVS - I

The hypervariable segment - I in the human mitochondrial D - loop between the positions L15,978 and H16,355 of extracted bone DNA was amplified using first (1st) round oligonucleotide PCR primers Hum1.1, Hum1.2 and Hum1.3 each of which generating fragment sizes of 378 bp, 247 bp and 233 bp respectively[24]. PCRs were performed in 50 µl reaction volumes using 5 µl of extracted mitochondrial DNA per reaction in GeneAmp 9,600 thermal cycler (Applied Biosystems) using PCR amplification protocol as reported in Lertrit *et al*., 2008 [24].

The second (2^{nd}) round PCR was performed with 50 times diluted primary products by using Hum2.1, Hum2.2, Hum2.3 and Hum2.4. Each reaction generated fragment sizes of 247 bp, 135 bp, 161 bp, 228 bp respectively [24]. The second round PCR was performed in 50 µl reaction volumes using PCR amplification protocol as reported in Lertrit et al., 2008 [24]. Blank reactions were concurrently done for every PCR reaction in order to verify the reliability of the PCR process.

Verification of the success of 2^{nd} round PCR products was performed by running the PCR products along with a DNA size maker (50bp size marker) in a 2% agarose gel in 0.5 TBE, pH- 8 at 100v for 45 minutes after PCR amplification.

Results and Analysis

DNA was extracted from the old human bones of 15 years (15Y) and 40 years (40Y) by adopting the extraction methods of Chelex[13], ethanol precipitation with Dextran blue[14], modified ethanol precipitation with Dextran blue, modified phenol-chloroform method[15] and with a commercially available DNA extraction kit (DNA Investigation Kit – QIAGEN, Germany). The DNA was subjected to PCR amplifications. The success of each PCR amplification reaction was verified by 2% agarose gel electrophoresis.

Chelex based method

DNA was extracted from old bones of 15Y and 40Y using the method described by Newman *et al*., (2002). Seven point five microlitres of first and second round of PCR products were subjected to electrophoresis in a 2% agarose gel. First round products generated fragment sizes of 378 bp, 247 bp and 233 bp for (1st) round oligonucleotide PCR primers Hum1.1, Hum1.2 and Hum1.3 respectively. Second round products generated fragment sizes of 247 bp, 135 bp, 161 bp, 228 bp for (2nd) round oligonucleotide PCR primers Hum2.1, Hum2.2, Hum2.3 and Hum2.4 respectively.

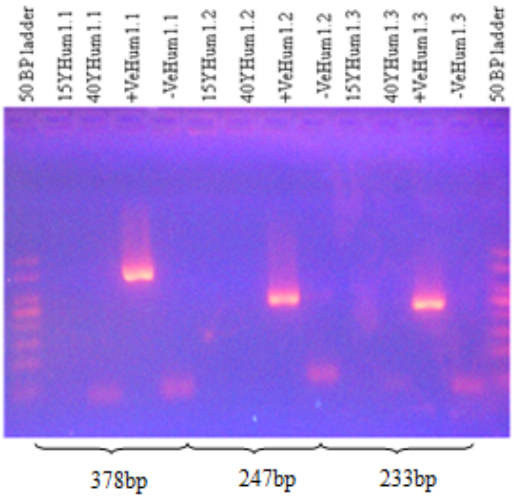

Fig 1.1a

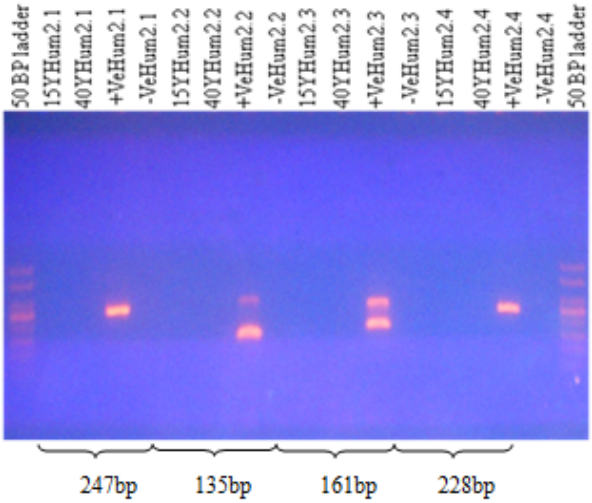

Fig 1.1b

Fig 1.1a Agarose gel containing 7.5 µl of each first round PCR product and Fig 1.1b Agarose gel containing 7.5 µl of each second round PCR product amplified from the extraction method described by Newman et al., (2002) [13] of 15Y and 40Y bones

PCR amplifications were not observed in the first round and second round amplifications of the DNA extracts obtained from the method described by Newman et al., (2002)[13]. This methodology is not success in DNA extraction from old skeletal remains.

Ethanol precipitation with Dextran blue based method

DNA was extracted from modern bones 15Y and 40Y using the method described by Mohendasan et al., (2004) [14]. Seven point five microlitres of first and second round of PCR products were subjected to electrophoresis in a 2% agarose gel. First round products generated fragment sizes of 378 bp, 247 bp and 233 bp for (1st) round oligonucleotide PCR primers Hum1.1, Hum1.2 and Hum1.3 respectively. Second round products generated fragment sizes of 247 bp, 135 bp, 161 bp, 228 bp for (2nd) round oligonucleotide PCR primers Hum2.1, Hum2.2, Hum2.3 and Hum2.4 respectively.

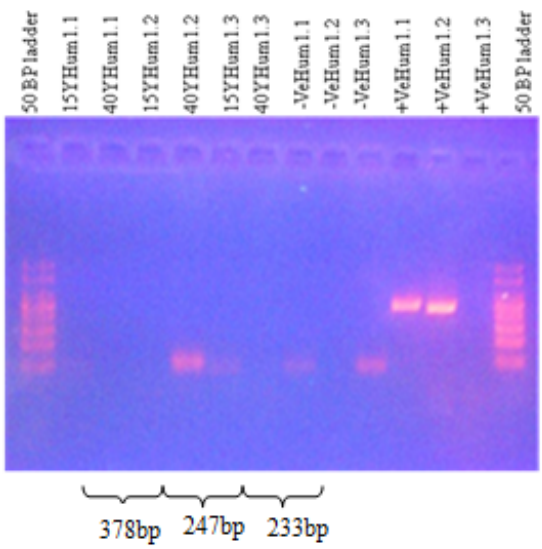

Fig 1.2a

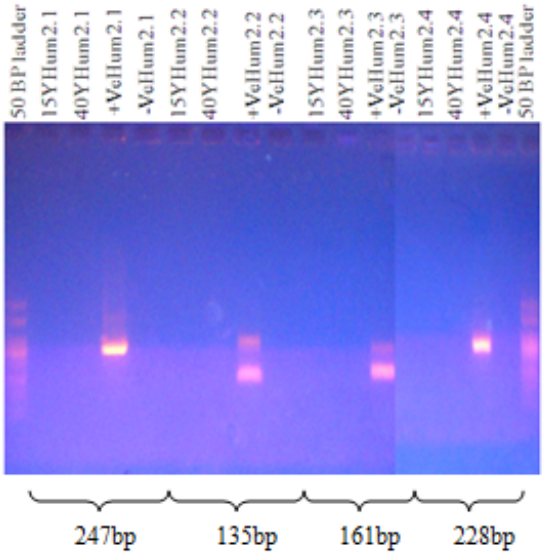

Fig 1.2b

Fig 1.2a Agarose gel containing 7.5 µl of each first-round PCR product and Fig 1.2b. Agarose gel containing 7.5 µl of each second round PCR product amplified from the extraction method described by Mohendasan *et al*., (2004) [14] of 15Y and 40Y bones

PCR amplifications were not observed in the first round and second round amplifications of the DNA extracts obtained from the method described by Mohendasan *et al*., (2004) [14]. This methodology fails to extract DNA from old skeletal remains.

Modified ethanol precipitation with Dextran blue based method

DNA was extracted from old bones of 15Y and 40Y using the modified ethanol precipitation with dextran blue method described by Mohendasan et al., (2004) [14]. Seven point five microlitres of each first and second round PCR products were subjected to electrophoresis in a 2% agarose gel. First round products generated fragment sizes of 378 bp, 247 bp and 233 bp for (1st) round oligonucleotide PCR primers Hum1.1, Hum1.2 and

Hum1.3 respectively. Second round products generated fragment sizes of 247 bp, 135 bp, 161 bp, 228 bp for (2^{nd}) round oligonucleotide PCR primers Hum2.1, Hum2.2, Hum2.3 and Hum2.4 respectively.

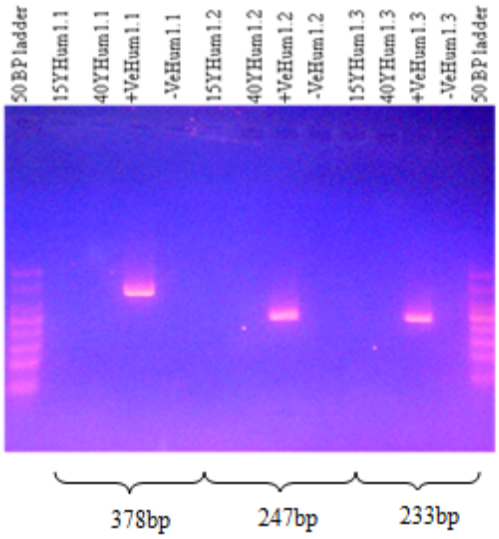

Fig 1.3a

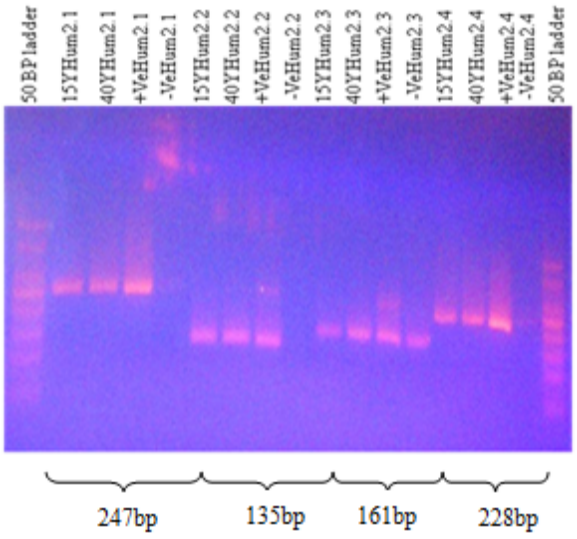

Fig 1.3b

Fig 1.3a. Agarose gel containing 7.5 µl of each first round PCR product and Fig 1.3b. Agarose gel containing 7.5 µl of each second round PCR product amplified from the modified extraction method described by Mohendasan *et al*., (2004) [14] of 15Y and 40Y bones

PCR amplifications were not observed in the first round PCR amplification of DNA extracts from the modified method described by Mohendasan et al., (2004) [14]. DNA bands were obtained with correct size ranges for all systems of second round PCR products.

DNA investigation Kit (QIAGEN, Germany)

DNA was extracted from old bones of 15Y and 40Y using commercially available DNA extraction kit; DNA Investigating Kit (QIAGEN, Germany). Seven point five microlitres of each first and second round PCR products were subjected to electrophoresis in a 2% agarose gel. First round products generated fragment sizes of 378 bp, 247 bp and 233 bp for (1^{st}) round oligonucleotide PCR primers Hum1.1, Hum1.2 and Hum1.3 respectively. Second round products generated fragment sizes of 247 bp, 135 bp, 161 bp, 228 bp for (2nd) round oligonucleotide PCR primers Hum2.1, Hum2.2, Hum2.3 and Hum2.4 respectively.

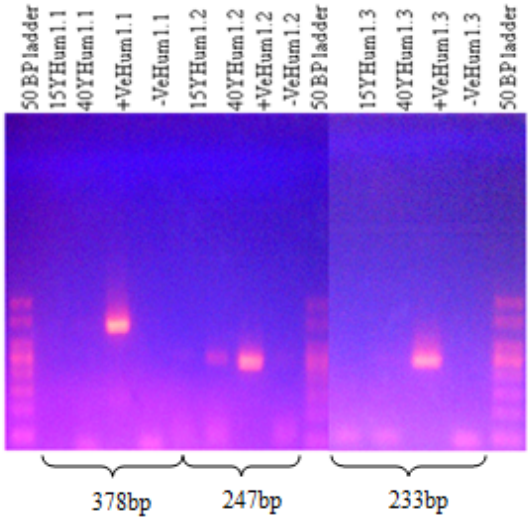

Fig 1.4a

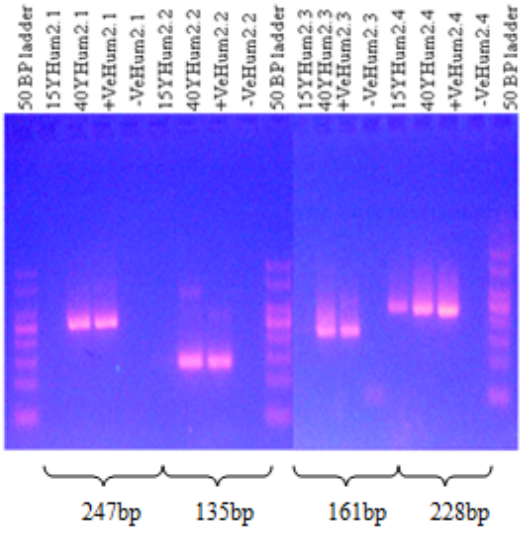

Fig 1.4b

Fig 1.4a. Agarose gel containing 7.5 µl of each first-round PCR product and Fig 1.4b Agarose gel containing 7.5 µl of each second round PCR product amplified from the extraction done by DNA Investigating Kit (QIAGEN, Germany) of 15Y and 40Y bones

DNA bands were obtained with correct size ranges for 15Y and 40Y bone amplified by PCR reactions containing ADHum1.2 primer of first-round PCR products.

DNA bands were obtained from all four systems for 40Y bone. However, there was no PCR amplification in 15Y bone except PCR system containing ADHum2.4 primer.

Modified phenol chloroform method

DNA was extracted from old bones of 15Y and 40Y using the modified method of phenol chloroform described by Ariffin *et al*., (2007) [14]. Seven point five microlitres of first round and second round PCR products were subjected to electrophoresis in a 2% agarose gel. First round products generated fragment sizes of 378 bp, 247 bp and 233 bp for (1st) round oligonucleotide PCR primers Hum1.1, Hum1.2 and Hum1.3 respectively. Second round products generated fragment sizes of 247 bp, 135 bp, 161 bp, 228 bp for (2^{nd}) round oligonucleotide PCR primers Hum2.1, Hum2.2, Hum2.3 and Hum2.4 respectively.

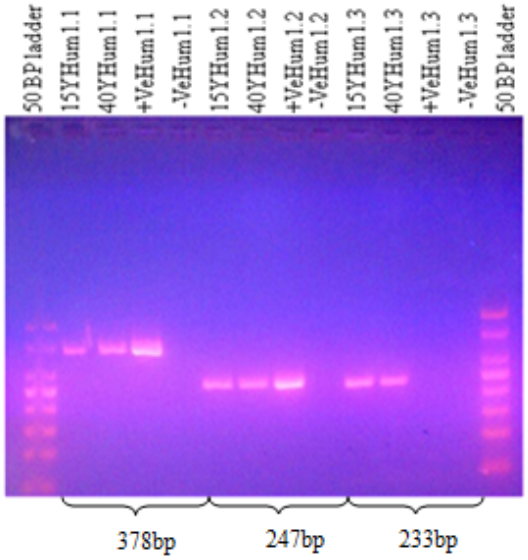

Fig 1.5a

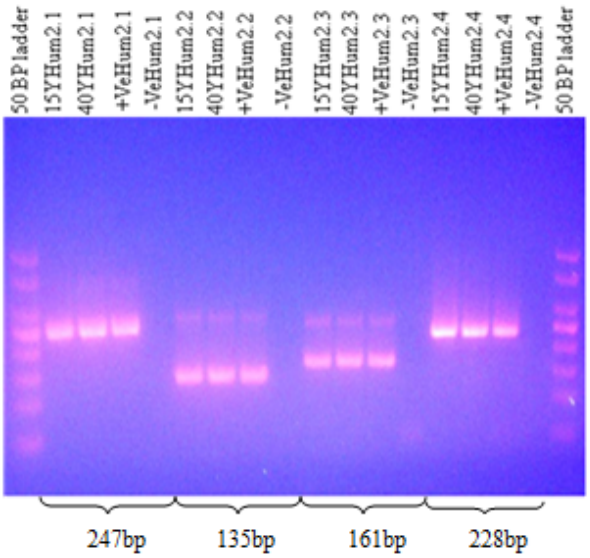

Fig 1.5b

Fig 1.5a Agarose gel containing 7.5 µl of each first-round PCR product and Fig 1.5b. Agarose gel containing 7.5 µl of each second round PCR product amplified from the modified extraction method of phenol-chloroform described by Ariffin *et al*., (2007) [14] of 15Y and 40Y bones

DNA bands were obtained with correct size ranges for all systems in both first and second round PCR products.

Discussion

Five DNA extracting methods were tested in order to check the feasibility of isolating DNA from bone samples of the present study.

Although, the Chelex based DNA extraction protocol has been successfully extracted highly fragmented DNA from human and animal skeletal remains dated back to 5,400 YBP at temperate countries like Canada by Newman *et al*., (2002) [13], this protocol did not yield any DNA from old bone samples used in this study (Fig 1.1a, and 1.1b).

The method of ethanol precipitation enhanced by Dextran blue has yielded DNA of skeletal remains found in Iran in the Middle East (Mohendasan *et al*., 2004) [14]. In this study, DNA extraction protocol - ethanol precipitation enhanced by Dextran blue was not able to yield DNA from both 40Y/ 15Y samples (Fig 1.2a, and 1.2b). The DNA extraction protocol - ethanol precipitation enhanced by Dextran blue was modified by changing the consistency of extraction buffer and this modified version of same protocol gave better results for second round PCR amplification (Fig 1.3a, and 1.3b).

The modified version of the protocol published by Ariffin *et al*., (2007) [15] reporting successful DNA extraction from a 400-year-old bone recovered from Wanli's shipwreck at Dungun sea

coast was found to be successful for the present study (Fig 1.4a, and 1.4b). A commercial DNA extraction kit; DNA Investigating Kit (QIAGEN, Germany) also yielded DNA from old bones in this study (Fig 1.5a, and 1.5b). Contamination with other DNA sources is of the utmost concern when working with old DNA [25,23]. Contaminant human DNA can be introduced at any point during the processes of sampling, extraction and finally PCR setup [25,23,28,29]. Therefore, strict laboratory procedures have been adhered to minimize such contaminations and this would affect the authenticity of the result of the DNA extract. Negative controls in extraction and amplification procedures were used to detect possible contaminations. PCR control amplifications were performed with multiple non – template PCRs containing aliquots of the reagent with distilled water. These controls were done together with actual template samples to detect sporadic or low copy number contamination as recommended by Eshleman and Smith, (2001) [26]. The absence of contaminants of human DNA was confirmed by the absence of amplification (DNA bands) in the negative controls (Fig 1.4a, 1.4b, 1.5a and 1.5b).

It is always recommended to have a dedicated laboratory to handle specimens for DNA studies of old or ancient samples. However, there is a possibility that chemicals, reagents and disposable items such as columns, tubes, pipette tips etc could be contaminated by modern DNA during manufacturing processes [26]. Therefore, contamination of DNA extracts with modern DNA can be possible even in a laboratory dedicated for forensic DNA analysis. In the present study, it was demonstrated that strict decontamination procedures were done that has not been used previously for modern DNA testing and decontamination process such as exposure to UV (Ultra Violet radiation) of reagents and equipment during the preparation of old DNA samples was utilized during analysing. Furthermore, 1.0% of chlorox was used to clean all bench surfaces to eliminate any surface contamination.

Detection of any PCR amplification even after 50 cycles of initial PCR amplification failed for both samples (Fig 1.1a). This observation was in contrast with that of the fresh biological sample (+ve control) (Fig 1.1a). This may be due to a result of very low amount of initial DNA template in the reaction. In order to compensate for the very low amounts of retrieved DNA, a second round (nested) PCR was optimized to increase the specificity and sensitivity of the technique as described in Lertrit *et al*., (2008) [24].

In this study, we successfully optimized DNA extraction protocol phenol-chloroform method which gave better DNA yield from old bone samples. DNA investigation kit gave better DNA yield from the old skeletal remains too. This optimized protocol could be used in forensic DNA analysis, archaeological DNA studies and even ancient DNA studies.

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Morphometric Evaluation of the Foramen Magnum in a Sri Lankan Population

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Abstract

Objective: The foramen magnum (FM) that transmits a large number of vital structures is an important anatomical landmark in neurosurgical procedures and forensic anthropology. The aim of the present study was to determine the dimensions and shape of the FM in a Sri Lankan population. Material and method: Forty-four adult skulls (32 males and 12 females) were selected for the study. Sagittal (FMSD) and transverse (FMTD) diameters of the FM were taken using a sliding digital vernier caliper to the nearest 0.01mm by one investigator. The area of the FM was calculated using Radinsky's formula; A=1/4 X 3.14 X FMTD X FMSD and FM index was calculated using the formula: FMTD x 100 / FMSD. The skulls were visually assessed to determine the shape of the FM. The shape of the FM was recorded as round, oval, egg-shaped, tetragonal, pentagonal, hexagonal or irregular.

Results: The mean sagittal and transverse diameter, area and index of the foramen magnum in males were 34.62 ±2.17 mm, 29.52±2.13 mm, 695.77±90.64 mm2 and 85.41±5.66, respectively, whereas they were 33.87±2.31 mm, 28.28±1.99 mm, 662.56±98.67 mm^2 and 83.69±6.13, respectively, in females. The shape of the foramen was found to be egg-shaped in 34.1%, and round in 27.2% of the cases.

Conclusions: The size and shape of the foramen magnum are highly variable among different world populations. Sri Lankan skulls showed smaller foramen magnum in compare with those of African, Mediterranean and European populations. Males displayed larger mean values than females for all measured variables.

Key Words: Foramen Magnum, Morphometry, Population difference, Sexual dimorphism

Introduction

The basicranium forms in a zone of interaction between structures with neural, skeletomotor, respiratory, auditory, masticatory, digestive, and visual functions. Because of the complex structural-functional relationships in the region, the morphology of the basicranium shows compromises resulting from the

competing demands of multiple soft tissue units. For example, during basicranium development, the structures traversing the endocranial-ectocranial boundary become encircled by cartilage and eventually by bone. The resulting bony foramina of the basicranium respond in an idiosyncratic but bilaterally symmetrical fashion to changes in the size and positioning of enclosed soft tissues [1].

The basicranium is complex, and the Foramen Magnum (FM), is the large opening in the posterior part of the cranial base, through which the cranial cavity communicates with the vertebral canal. It is situated at the craniovertebral junction and is oval, and wider behind with its greatest diameter being the anteroposterior [2]. It transmits many vital structures like the lower end of the medulla oblongata, meninges, vertebral arteries and spinal accessory nerve [2] and therefore, considered as an important landmark. Anteriorly, the margin of the FM is slightly overlapped by the occipital condyles which project down to articulate with the superior articular facets on the lateral masses of the atlas [2].

An awareness of the normal dimensions of the FM is clinically important as certain developmental and acquired craniovertebral junction disorders such as achondroplasia [3,4], craniometaphyseal dysplasia [5] assimilation of axis [6], and Jeune's asphyxiating thoracic dystrophy [7,8] associated with the FM resulting in variations in its morphometry. Besides its clinical importance, FM dimensions have also been used as significant criteria in sex determination in forensic investigations [9]. Recent studies report that morphometry is a fast and efficient method for the evaluation of demographic characteristics, such as ethnicity, gender, age, etc. Evidence suggests that the shape and dimensions of the FM exhibit sex dimorphism and population based variations [9-19]. Therefore, the shape and dimensions of the FM and their variations have been reported to be of diagnostic, anthropological, forensic and clinical importance [12]. Furthermore, FM dimensions are commonly used in anthropology and forensic investigations because FM with its surrounding cranial base can withstand physical damage than any other areas of the cranium as it is situated in relatively protected anatomical location with large masses of soft tissue over it [20].

Although the morphometry and shape of the FM have been studied with reference to various human population groups [9-19,21], to the best of our knowledge, none has been documented in the literature for Sri Lankans, despite its anatomical, anthropological and clinical relevance. Hence, the current study was conducted to determine the dimensions and shape of the FM in a Sri Lankan population, make comparisons with other population groups and investigate the sexual dimorphism of FM morphology.

Materials and Methods

Fifty-seven dry human skulls from the Division of Anatomy, Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka were assembled for this study. The sex and age of all skulls were retrieved from the personal records of body donors. The skulls with no apparent gross pathology, deformity, or traumatic lesions were included in the study. Criteria of exclusion were those in which the margins of the foramen magnum could not be clearly identified owing to breakage and those with deformities and trauma affecting the landmarks for measurement. Forty-four adult skulls 32 males and 12 females were selected for the study. Approval for this study has been granted by the Faculty Research Committee of the Faculty of Dental Sciences, University of Peradeniya (FDS-FRC/2014/06) and therefore, the study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and all subsequent revisions.

The measurements were taken using a sliding digital vernier caliper to the nearest 0.01mm. The following measurements of the FM were recorded adopting the landmark definitions described in previous studies [12,19];

1. Sagittal diameter (FMSD) - the distance between the basion and opisthion (Figure 1). Basion and opisthion are the points where the midsagittal plane intersects the anterior margin and the posterior margin of the FM, respectively
2. Transverse diameter (FMTD) - the distance between the lateral margins of FM at the point of maximum lateral curvature (Figure 1)

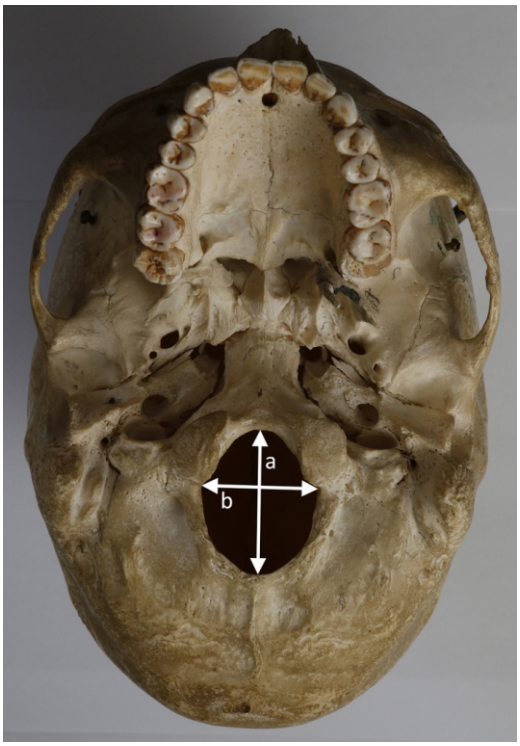

Figure 1. Measurements of the foramen magnum: (a) Foramen Magnum sagittal diameter (FMSD), (b) Foramen Magnum transverse diameter (FMTD)

The area of the foramen magnum (FMA) was calculated using Radinsky's formula; A=1/4 X 3.14 X FMTD X FMSD [22]. Additionally, the index of FM (FMI) was calculated by using the formula: FMTD x 100 / FMSD.

The skulls were visually assessed to determine the shape of the FM. The shape of the FM was recorded as round, oval, egg-shaped, tetragonal, pentagonal, hexagonal or irregular as described by Murshed *et al*. [12] (Figure 2).

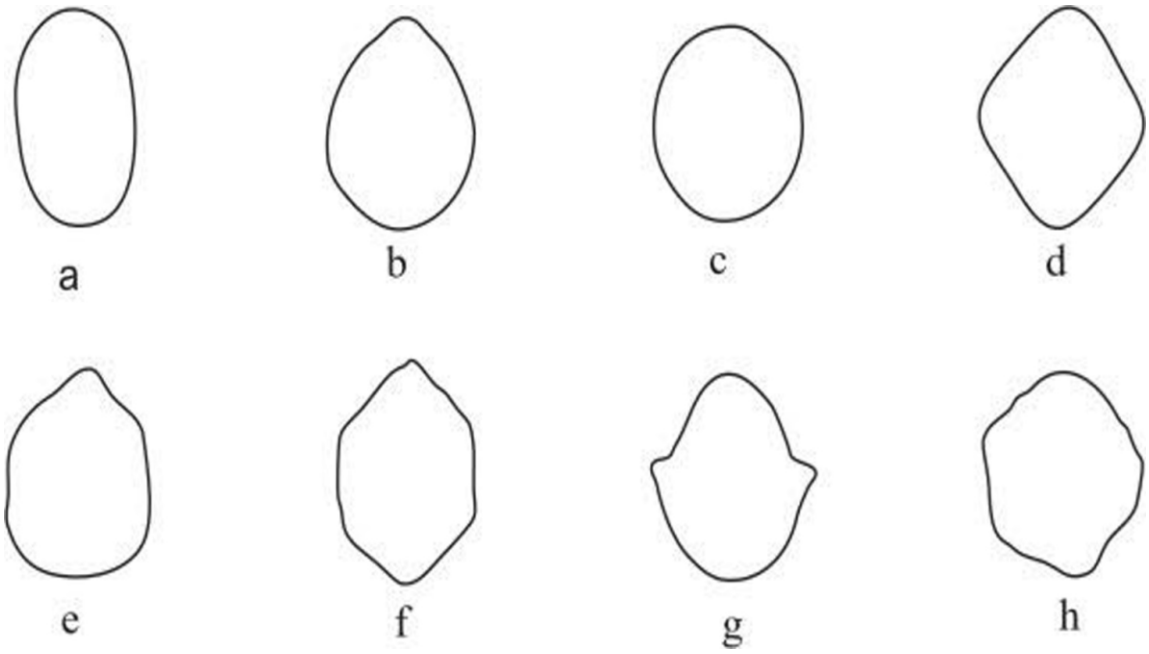

Figure 2. Foramen magnum shape variations

(A) Oval type; (b) Egg type; (c) Round type; (d) Tetragonal type; (e) Pentagonal type; (f) Hexagonal type; (g) Irregular type A; (h) irregular type B

All measurements and observations on the shape were recorded by one investigator. In order to minimize the intra observer error three repeated measurements were made for each parameter at different sittings with 1-week interval between sittings, and the average of the three measurements was taken for further analysis.

Results were expressed as means and SDs and the differences in the measurements between male and female were analyzed using the Statistical Package for Social Sciences (SPSS), 19th version. Students' t-test was used for the analysis and $p<0.05$ was considered as statistically significant.

Results

Descriptive statistics with values of the minimum, maximum, means and standard deviations of all measurements of the FM of males and females are presented in Table 1. Average age of the skulls in the present study was 65.25 years. Therefore, FM size and shape are considered to be fully established and age was not considered for further analysis. It is evident that males display larger mean values than females for all measured variables of the FM. Especially, the FMTD in male skulls varied between 24.24-33.48mm with a mean of 29.74mm, while in females the FMTD varied from 25.73-31.76mm with a mean of 28.28mm. The difference was statistically significant ($p<0.05$).

Although FMA was larger in males than in females the differences were not statistically significant. In addition, the mean FMI was higher in males (85.41±5.66) compared to females (83.69±6.13).

Table 1. Basic statistics of the FM dimensions and index in males and females

Measurement	Males (n=32)		Females (n=12)		Difference
	Range (mm)	Mean ±SD (mm)	Range (mm)	Mean ±SD (mm)	
FMSD	30.18 - 39.86	34.62±2.17	30.88 - 37.47	33.87±2.31	NS
FMTD	25.62 - 33.48	29.52±2.13	25.73 - 31.76	28.28±1.99	*
FMA	608.67 - 901.44	695.77±90.64	519.84 - 867.56	662.56±98.67	NS
FMI (FMTD/FMSDx100)	68.80 - 94.62	85.41±5.66	69.59 - 95.56	83.69±6.13	NS

*P<0.05; NS, Not Significant

FMSD; Foramen Magnum Sagittal diameter, FMTD; Foramen Magnum Transverse diameter, FMA; Foramen Magnum Area, FMI; Foramen Magnum Index

We combined the male and female data to analyze the shape of FM because the shape difference between the two was not statistically significant. The various shapes of the FM observed in the present study are shown in Table 2.

Table 2. Frequency of different shapes of FM

Shape of foramen magnum	Frequency (%)
Egg	34.1
Round	27.2
Oval	13.6
Tetragonal	13.6
Irregular	9.1
Hexagonal	2.3

Discussion

In any attempt to figure out the population differences of FM morphology it is important to consider about the development pattern of the basicranium because it makes us understand the balance between genetic and environmental contribution on its development in different world populations. It is evident that in the early foetal stages the size of the brain stem increases relative to the cerebrum and cerebellum. Therefore, the youngest foetuses have a voluminous brain stem relative to the size of the cerebellum. This growth pattern results in a larger FM being associated with a small cranial vault in early foetal stages [23]. However, Richards and Jabbour [24] showed that this relationship is restricted only to early foetal stages because growth increases are minor in late foetal stages relative to the increases observed in the cranial vault. It has also been confirmed that the brain stem completes the majority of its growth at the termination of the first year of life and brain size doubles during this time period [25]. In addition, the rates of growth in breadth of both the FM and the cranial vault which dominated early in the development stabilize while lengths continue to increase slowly during 1-3 years of age. After 3 years of age FM has completed most of its growth while the cranial vault continues to increase [24]. This confirms that pattern of increase in FM length and breadth vary during foetal life and even during the first year after birth. However, FM sizes and shapes tend to vary throughout the ontogenetic sequence and these variations are correlated with the changes in the brain size and cranial capacity during the growth period and also linked to specific cranial morphologies [24].

In the meantime, Smith and Beals [26] explained the association between cranial capacity and latitude showing larger cranial capacity with more rounded head in people habitat in cold climates. They further emphasised the association between the cranial capacity and body size with large cranial capacity with increasing body size. This is further supported by a study done by Gonda et al. [27] on association of brain size and environmental factors in nine-spined sticklebacks. They reported that variation among tested populations of wild nine-spined sticklebacks is large, both in terms of brain size and in the size of separate brain parts. Furthermore, the incongruence between the wild and common garden patterns suggested that much of the population variation found in the wild may be attributable to environmentally induced phenotypic plasticity.

These evidence suggest that FM size and shape may vary among different world human populations and both genetic and environmental factors contribute for these differences. In addition, it is affirmed that FM shape and size are correlated with the brain size, cranial capacity, and specific hemodynamic, hydrodynamic and locomotor functions in the region and the structural-functional relationships

between some of these are modified during ontogeny. This is further emphasized by the present study which shows clear population variation in both FM size and shape (Table 3).

Table 3. Comparison of FM diameters of the present sample and with those of other

Population	FMSD (mm)		FMTD (mm)	
	Male	Female	Male	Female
Turkey [12]	37.2±3.43	34.6±3.16	31.6±2.99	29.3±2.19
Britain [19]	35.91±2.41	34.71±1.91	30.51±1.77	29.36±1.96
Spain [13]	36.2±0.3	34.6 ±0.4	31.1±0.3	29.6 ±0.3
Brazil [14]	36.5±2.6	35.6 ±2.5	30.6±2.5	29.5±1.9
Iran [15]	37.72±1.01	34.37±1.47	31.67±1.27	28.34±1.4
Poland [29]	36.89 ± 3.17	34.76 ± 2.96	32.47 ± 3.08	30.96 ± 3.01
India [30]	35.5±2.8	29.6±1.9	32.0±2.8	27.1±1.6
Nigeria [16]	36.36±2.3	34.39±3.88	30.9±2.5	28.16±1.9
Kenya [28]	40.0	34.0	38.0	28.0
Sri Lanka (Present study)	34.50±2.16	33.87±2.31	29.74±1.98	28.28±1.99

FMSD; Foramen Magnum Sagittal diameter, FMTD; Foramen Magnum Transverse diameter

Interestingly, the mean values of FM dimensions of Sri Lankans are lower than those reported for Brazilian, Turkish, Spanish, Iranian, Nigerian, Kenyan and British populations (Table 3). Loyal et al. [28] showed that Kenyans demonstrated a large FM having FMSD, 40mm and FMTD, 28mm in males. This is followed by Mediterranean, Middle east and European populations who showed moderately larger FM. Murshed et al. [12] reported that FMSD, FMTD and FMA as 37.2mm, 31.6mm and 931mm^2 in males and 34.6mm, 29.3mm and 795mm^2 in females, respectively in a Turkey population. Meanwhile, for an Iranian population Kazemzadeh et al. [15] showed that FMSD as 37.72mm and 34.37mm and FMTD as 31.67mm and 28.34mm for males and females, respectively. In a similar study Burdan *et al*. [29] showed that FMSD and FMTD as 36.89mm and 32.47mm in males and 34.76mm and 30.96mm in females, respectively with FMA having 881mm^2 and 765mm^2 in males and females, respectively in a Poland population. In the meantime, FM dimensions of the present study in a Sri Lankan population were less than 35mm in males and 30mm in females in all

parameters with FMA having less than 700mm^2 in both the sexes. However, FM dimensions of Indian populations showed close affinities with those of Sri Lankans [30]. These differences may be attributed to genetic, environmental or socio-economic differences among these population groups. The broad range of variation demonstrated in relation to FM sizes emphasize the importance of establishing populations specific values as those established for one population group may not be applicable to another population group.

Previous studies of FM shape have shown it to be oval in infant and oval to round in adults. Rates and patterns of fetal growth are in accord with FM elongation and its oval shape [31]. Postnatal growth rates and patterns were found to result in increased breadths, reduced growth in length, and a general tendency for a broadening of the FM. Growth increases in the FM mostly occur at the interocciptal synchondroses anterior and posterior and neither the positioning nor the growth patterns of these synchondroses are such as to promote evenly distributed breadth increases [32]. Therefore, in general, the tendency during postnatal growth is for foramina to become ventrally wide, not circular [24].

Standard texts of anatomy describe the foramen magnum to be usually oval in shape [2]. Although this is in agreement with the majority of skulls, it ignores a large body of evidence regarding the other shapes of the FM present among different populations. In the present study, majority of the FM were either egg shaped (34.1%) or round (27.2%). In a Turkish population, Murshed et al. [12] observed oval shaped FM less frequently, 8.1% and more occurrence of round (21.8%), irregular (20%), and hexagonal (17.2%) shapes. In the meantime, Zaidi and Dayal [33] reported that 64% of the FM was oval in shape in an Indian population. Interestingly, Loyal et al. [28] analyzing 202 skulls of Kenyans reported the shape of the FM to be polygonal in 63% of the cases. It is evident from these studies that there is great variation regarding the predominant morphological type of FM among different world population groups and these differences are related to brain size, cranial capacity, specific hemodynamic, hydrodynamic and locomotor functions in the region and even genetic differences of these populations.

The FMI derived from the sagittal and transverse diameters is a measure of the foramen shape [24]. Moreover, the index is also helpful in distinguishing oval-like or round-like types of foramina [29]. The round-like form is characterized by the index 0.9-1.1 (90-110%), while foramina with a value below or above it is called as longitudinal and horizontal oval like ones, respectively [29]. The FMI of the present study was 85.41±5.66 in males and 83.69±6.13 in females which is indicative of the longer FMSD in the skulls of the present study.

As the fusion of the bony elements forming the FM completes early in life

it is unlikely to respond to any significant secondary sexual changes [19,34]. Moreover, as the nervous system attains maturity at a very young age there is no requirement for an increase in size. Sex dimorphic characteristics in the FM region must therefore occur during an early stage of development at a time when other skeletal elements have not attained sex-specific traits [19,24]. This feature of the FM is significant in gender determination in young individuals using FM parameters.

All measured variables of the FM have been reported to be greater in males than in females in almost all previous studies [9-19,21]. However, the level of significance observed in the sex differences varies in different studies. These differences observed in various studies have been attributed to the diverse ethnic groups involved and to the variations in methodology and statistical analyses adopted [11,17]. In the present study, males displayed larger FM dimensions than the females. However, statistically significant difference between the sexes was seen only for the measurement of FMTD ($p<0.05$). Although this finding is consistent with that of Macaluso Jr. [11] in a French sample and Vedanayagam and Sathyamurthy [18] in an Indian population, it contrasts with the results presented in similar investigations on diverse populations, including the Nigerian [16], Spanish [13], Indian [17], Turkish [12], Brazilian [21] and the historic British sample from St. Bride's Church [19], where males exhibited significantly larger dimensions than females, not only for the FMTD, but also for the FMSD and area of the FM. Contrary to these views, in studies of Gruber et al. [10], Tubbs et al. [6] and Loyal et al. [28], the diameters of the FM did not display any significant sexual dimorphism. Gruber et al. [10] attributed this difference to the smaller sample size and greater variability in dimensions in their study on Central European dry specimens dating from Pleistocene to modern times. Furthermore, in the present analysis, the mean FMA was higher in males is than in females. This is in accordance with the findings reported in preceding studies [9,11,12,17,28] (Table 4). However, in contrast to their findings, the difference between the means of males and females was not statistically significant in our study. Uthman et al. [9] reported that the FMA could be used in sex determination with an overall accuracy of 69.3%. Discriminant function analyses carried out in previous studies have demonstrated varying degrees of sex predictability by these dimensions of the FM. Hence, it is the view of many authors that the measurements of the foramen should be used in conjunction with other sexing evidence available to ascertain the sex of unidentified individuals [17,19,21]. In the meantime, it should be noted that smaller female sample size is one of the limitations of the present study and future studies with larger sample is recommended to make concrete conclusions.

Table 4. Comparison of FMA of the present sample and with those of other populations as reported in previous studies

Population	FMA (mm^2)	
	Male	Female
Turkey [12]	931	795
Iraq [9]	765	670
South India [17]	705	622
France [11]	854	807
Poland [29]	881	765
South India [35]	748	711
Sri Lanka (Present study)	695	662

FMA; Foramen Magnum Area

Conclusion

This study presents valuable reference data regarding the shape and morphometry of the FM of an adult Sri Lankan population which indeed will be of great anthropological, forensic and clinical interest. The size and shape of the foramen magnum are highly variable among different world populations. Sri Lankan skulls showed smaller foramen magnum in comparison with those of African, Mediterranean and European populations. The sex dimorphic measurements of the foramen may be used in conjunction with other sexing evidence available to ascertain the sex of bony fragments of unidentified individuals.

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Evidence of Restricted Maternal Gene Flow of Purana (Old) Population in the Suburbs of Sigiriya, Sri Lanka

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Abstract

Introduction

Based on historical records suggesting that the ancestry of the *Purana* (old) population living in the suburbs of Sigiriya in Sri Lanka can be traced back to the times of the Sinhalese Kings of the 5th century A.D. (1,450 YBP) and studies reporting that the *Purana* are biometrically different from the rest of present Sri Lankans and other populations in the world, we investigate the maternally inherited Mitochondrial DNA (mtDNA) of *Purana* population in the suburbs of Sigiriya to better understand genetic affinities of them to present day Sri Lankans.

Materials and Methods

Forty six *Purana* inhabitants belonging to *Purana* pedigrees were recruited in the study. Mitochondrial DNA was extracted, amplified and sequenced. Polymorphisms of mtDNA hypervariable segment I (HVS –I) between nucleotide positions 16,004 – 16,411 were identified using CLUSTALX option of MEGAVA 4.0 sequence alignment software. In order to investigate genetic affinity of *Purana* population, Arlequin software version 3.11 and analysis of molecular variance (AMOVA) were applied using reported similar genetic data of present Sri Lankans such as Sinhalese, Sri Lankan Tamils, Indian Tamils, Sri Lankan Moors and *Vedda*. Genetic relationships of *Purana* population with other Sri Lankans were further explored by phylogenetic analysis.

Results and Analysis

Genetic dissimilarity among groups was higher (2.81%) when populations were grouped into two as modern and *Purana* than grouping them according to their ethnic basis (0.00%). This indicates a restricted mtDNA flow between the two groups *(Purana* and rest of the Sri Lankans) that made *Purana* population was maternally isolated from the rest of Sri Lankans.

Conclusion

Detailed phylogenetic analysis of the study revealed that they are maternally more related to Sri Lankan Tamil than to any other present Sri Lankans.

Keywords: Mitochondrial DNA, Maternal Inheritance, *Purana* population, Sigiriya Sri Lanka

Introduction

Mitochondrial DNA (mtDNA) provides a potent tool for studying human evolution, in view of characteristics such as a maternal mode of inheritance, a mutation rate ~10x higher than nuclear DNA, presence in high copy number, and lack of recombination[1,2,3,4]. As mtDNA is strictly maternally inherited, sequence variation evolves through the sequential accumulation of base substitutions along radiating maternal lineages[1,3,4,5]. Polymorphisms which occurred thousands of years ago results in the geographic structuring of mtDNA haplogroup distributions therefore it is possible to investigate an individual's matrilineal ancestry [6,5].

Mitochondrial DNA is composed of two regions: the D-loop control region (the non-coding segment of mtDNA), and the coding region, and that while the coding region is much larger, we more often study the polymorphisms in the D-loop region[7]. The D-loop region is 1124 bp in length and is made up of hypervariable segments I and II (HVS - I and HVS - II)[8]. In human mitochondrial genome, HVS - I is found between the nucleotide positions 16, 024 to 16,365 and HVS II from positions 73 to 340[7].

The HVSs reveal about 3 % variability between individuals. Within the HVSs, the polymorphic sites are not distributed uniformly. Because of high number of polymorphic sites, the analysis of HVS - I and II enable to study matrilineal ancestry and explore patterns of female movement and interactions in the past[9]. The HVSs are highly valuable markers in terms of population genetics or phylogenetic studies[10,11].

The first mtDNA study on *Vedda* in Sri Lanka a minority indigenous group of people in Sri Lanka was done by Harihara[12]. Since then several national level genomic studies have been conducted representing major ethnic groups and *Vedda* in Sri Lanka[13]. The Genome Variation Database containing information on Single Nucleotide Polymorphisms (SNPs) found in major ethnic groups in Sri Lanka (i.e. Sinhalese, Tamils and Moors) has been established[14]. The genetic database of Sri Lankan population, containing information including genotype frequencies of 34 genomic variations encompassing 14 medically important genes, has been reported[15].

“The ancestry of the present-day *Purana* inhabitants of villages *Talkote, Pidurangala, Diyakepilla, Nagalaweva, Alakolaweva, Ilukweva, Kosgahaela* in Sigiriya area has been a subject of debate. There are two potential hypotheses regarding their ancestry can be tested. They are descendants of the prehistoric hunter-gatherer populations from the region or descendants of contemporary *Vedda* community in Sigiriya region.

The Purana populations in Sigiriya represented by having *Purana* surnames such as *Aluthgedara, Gamagedara, Undiyagedara, Beddegedara, Millagahagedara, Kongahagedara* etc, maintain their caste system by strictly practicing their marriages among themselves. They are

considered as isolated breeding units with extended family endogamy. They trace their ancestry to the times of the Sinhalese Kings of the 5th century A.D. (1,450 YBP)[16]. Therefore, one hypothesis is that the *Purana* population have been occupying this locality for quite a long time and might have preserved the genetic signatures from ancient pre-Sinhalese settlers due to their practice of endogamy. Exploring the genetics of the *Purana* population of Sigiriya is important it's that their unique genetic signature (if there is one) will become admixed with other Sri Lankans and the current population structure (if there is any) will change. Studying mtDNA of the HVS - I from the *Purana* population in the suburbs of Sigiriya is important to explore the genetic structure and to construct the phylogenetic tree of the *Purana* population.

Materials and Methods

The present study analyses the mtDNA HVS-I control region of the *Purana* population in Sigiriya suburbs. The ethical clearance to study certain anthropological measures and collection and analysis of blood samples of the *Purana* population in Sigiriya has been granted by the Ethics Review Committee of Faculty of Medical Sciences, University Sri Jayewardenepura, Sri Lanka (Ref No: 491 / 10).

Study population and selection criteria

Forty-six individuals from *Purana* populations living in *Nagalaweva, Diyakepilla, Pidurangala* and *Talkote* at Sigiriya suburbs, who gave their informed consent, were included in DNA study. The *Purana* pedigrees such as *Gamagedara, Undiyagedara, Liyanagedara, Beddedara, Millagahagedara, Kongahagedara* and *Aluthgedara* of the subjects of the present study identified as *Purana* pedigrees and confirmed according to *Purana* pedigree recorded in *Bandaranayake et al*., (1994) [16]. Each pedigree was traced back to at least three generations prior to the recruitment to the study.

Blood sample collection and DNA extraction

Capillary blood was collected from 46 adult individuals from *Purana* villages in Sigiriya suburbs. Samples were taken from the fingertips of each individual onto labelled filter paper from each selected individual under strict sterile conditions. A diameter of four millimetres (4 mm) of blood stain from filter paper was used to extract DNA from the leukocyte fraction of the blood according to Walsh *et al*., (1991)[17].

PCR amplification of the human mtDNA HVS - I

The hypervariable segment - I (HVS - I) of the D-loop control region between the positions L16,000 and H16,450 of the human mtDNA was amplified by PCR using the following primer pair targeting a 446 bp DNA fragment[18].

PCRs were performed in 50 µl reaction volumes using 5 µl of extracted

mitochondrial DNA for each reaction in GeneAmp 9,600 thermal cycler (Applied Biosystems) using stranded PCR amplification protocol.

Automated DNA sequencing of mtDNA HVS - I

Each PCR product was purified by following the methods described in the MinElute PCR product purification kit (QIAGEN, Germany). Automated cycle sequencing was performed in an ABI 310 genetic Analyzer (Applied Bio systems, USA). Each automated DNA cycle sequencing mixture contained 1µl of 10 µM forward primer/ reverse primer 4 µl of cleaned PCR product, 2.5 µl nuclease free water, 1.5 µl sequencing buffer and 1µl Big Dye terminator solution (http://www.appliedbiosystem.com).

Determination of nucleotide sequence variation

Sequence data retrieved from the data collection software of ABI 310 instrument were aligned with the human mitochondrial DNA revised Cambridge Reference Sequence (GenBank accession number – NC 012920 Andrews et al., 1999)[19] using CLUSTALX option of MEGAVA 4.0 sequence alignment software[20]. Mitochondrial DNA HVS -I sequences were aligned using CLUSTALX option of MEGAVA 4.0 sequence alignment software [20]. Additional sequences from modern Sri Lankan reported by Illeperuma, (2009) [13] were also included in the study for genetic comparison. Arlequin software version 3.11 was used for the calculation of haplotype frequencies, AMOVA, pair wise Fst values and associated probability values were estimated from 10,000 mutations calculated using the software ARLEQUIN Version 3.11 [21].

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Construction of phylogenetic tree of the Purana population using mtDNA markers

The genetic relationship among Purana population and major ethnic groups in Sri Lanka was explored by drawing Neighbour-joining tree based on DA distances by using the reported data of

Illeperuma (2009) [13]. The distance matrices namely Nei's DA [22] were calculated using GENDIST option in PHYLIP v3.5c software package [23] using mtDNA HVS -I sequences. Neighbour – joining tree was constructed using DA distances and its robustness was established by bootstrap re-sampling procedure. The option NEIGHBOUR in PHYLIP v3.5c was used to draw the phylogenetic trees from the distance matrices created by GENDIST.

In this method, statistical bootstrap involving 1000 replicates was carried out using SEQBOOT option PHILIP v3.5c. Finally, a consensus of 1000 Neighbour-joining trees was drawn using CONSENSE option of PHILIP v3.5 [23] and viewed using the program TREEVIEW.

Results and Analysis

Mitochondrial HVS - I sequence polymorphisms of the Purana population at Sigiriya suburbs

Each sequence comprised of 407 nucleotides spanning nucleotide positions (bp) 16,004 – 16,411 of the human mtDNA. The variable positions recorded upon compared with rCRS were presented in Table 1.

Table 1. Mitochondrial HVS - I sequences of 46 samples of the Purana population aligned with rCRS. Only variable positions are given. Positions are numbered accorded to rCRS.

	1111111111111111111111111111111111111111111111111111111111111111
	6666666666666666666666666666666666666666666666666666666666666666
	0000000000000111111111222222222222222223333333333333334444
	0345678888899223458888012333345667777899011122245555668900 11
	4721950134503696843479943014925060248945418905742368121989 01
rCRS	CAGACTAACGCTTTGTCTACCTTCCATCCCGCCCAGCACCTTAGCTCCTCTCGTTACTCC
SITH_143	.....T..C.T.....C.....G
SITH_145	.....C.....T.C.....C.....G
SITH_155	.....T.....C....C..TCG.
SITH_184	.....TT.....T..C.T.....C.....G
SITH_180	.....T..C.T.....C.....G
SITH_181	G.....C.....T.....G
SITH_195	G.....T.....CC.....C.....G
SITH_179	.....C.....T.....
SITH_166	.....T.....CC.....C.....G
SITH_157	.....T.....CC.....C.....G
SITH_159	.....T.....CC.....C.....G
SITH_201	.....T...A.....T.....G
SINA_214	.....T...T.....A.....AT.....C..T..G
SINA_216	.....CT...T.....TC.....G
SINA_219	.....T...T.....A.....AT.....C.....G
SINA_217	.....CT...T.....TC.....
SINA_221	.....CT...T.....TC.....G
SINA_218	.....T.....CT...T.....TC.....G
SINA_215	....T.....C.....A.....T.....G
SINA_223	.....T.....G
SINA_224	..A.T.....A.....T.....TC.G
SINA_220	....T.....A.....T.....TC.G
SINA_222	....T.....A.....T...T .....TC.G
SIPI_120	.....C.C.....C..T.....T.....T.....G
SIPI_125	.....T..C.T.....C.....G
SIPI_56	.....T.....T.....C.....G
SIPI_42	...G.....T.....C.....TG.....C.....
SIPI_39	.....C.....T.....T.A .....A....C.....
SIPI_61	.T.....T.....T.....C.....G
SIPI_36	.....T..T...T..G..G.....C.....G....
SIPI_109	.....T..C.T.....C.....
SIPI_137	.....T.....T.....C.....G
SIPI_140	.....A.....T.....G
SIPI_141	.....A.....T.....G
SIPI_138	.....C.....T.....T.A.....A....C.....G
SIPI_32	.....T.....T.....C.....G
SIPI_52	.....C..T....T.....C.....TC.C..CGG
SIDI_17	...G.....C.....T.A.....CT.....G
SIDI_20	...G.....T.....CG.T.....C.....
SIDI_12	...G.....C.....T.A.....CT.....G
SIDI_13	.....CC.....T.....T.....
SIDI_21	...G.....C.....T.A.....CT.....G
SIDI_4	.....CCTT.....C.....T.A.....CT.....
SIDI_8	.....CCTT.....T.....A.....G
SIDI_5	.....CCTT.....C..C..T.....T.....
SIDI_14	.....CCTT.....C.....T.A.....CT.....G

Nucleotide position of humanmtDNA

SITH - Sigiriya, *Talkote*
SINA - Sigiriya, *Nagalaweva*
SIPI - Sigiriya, *Pidurangala*
SIDI - Sigiriya, *Diyakepilla*

Comparison of mtDNA HVS - I sequence polymorphisms of the *Purana* population with modern populations in Sri Lanka

The sequences of the mtDNA HVS - I in 46 individuals belonging to the *Purana* population living at Sigiriya suburbs was compared with reported data available for major ethnic groups: Sinhalese (n = 55), Sri Lankan Tamils (SL Tamils) (n = 40), Indian Tamils (IN Tamils) (n = 34) and Sri Lankan Moors (SL Moors) (n = 55) [13]. Arlequin software version 3.11 was used for the calculation of haplotype frequencies, mean number of pair wise differences, nucleotide diversity and gene diversity among *Purana* and modern Sri Lankans [21] (Table 2).

Table 2. Genetic indices of mtDNA HVS - I of the Purana population and modern Sri Lankan populations

Genetic Indices	*Purana*	Sinhalese	SL Tamils	IN Tamils	SL Moor
Number of haplotypes	28	46	28	34	31
Mean number of pair wise difference	7.459903 ±3.549980	5.989226 ±2.899290	4.819231 ±2.402766	5.601329 ±2.742017	3.734007 ±1.913702
Nucleotide diversity	0.020780 ±0.010978	0.016683 ±0.008959	0.013424 ±0.007435	0.015603 ±0.008482	0.010401 ±0.005914
Gene diversity	0.9720 ±0.0103	0.9939 ±0.0046	0.9628 ±0.0193	0.9878 ±0.0080	0.9542 ±0.0170

Gene diversity of the *Purana* population was low (0.9720 ± 0.0103) with compared to Sinhalese (0.9939 ± 0.0046) and IN Tamils (0.9878 ± 0.0080). However, diversity was higher than that of SL Tamils (0.9628 ± 0.0193) and SL Moors (0.9542 ± 0.0170).

Haplotype frequencies of the *Purana* population and modern populations in Sri Lanka

The Purana population living at Sigiriya suburbs showed the highest percentage of population specific mtDNA haplotypes:- Single unique, Multiple unique and Non unique (24 haplotypes out of 28 – prevalence of 86%). Fourteen percent of haplotypes that were shared among individuals between populations (non unique haplotypes) of the Purana populations shared among Sinhalese (50%), SL Tamils (25%) and SL Moors (25%) (Table 3).

Table 3. Haplotype sharing statistics of the Purana population among modern Sri Lankan populations

Haplotype	Sinhalese	SL Tamil	IN Tamil	SL Moor	*Purana*
Single unique	27	15	20	15	17
Multiple unique	06	01	02	02	07
Non unique	13	12	12	14	04

Inter population and intergroup mtDNA genetic variation of the Puranapopulation with modern population

Intergroup mtDNA genetic variation was analyzed using Analysis of Molecular Variance (AMOVA) by grouping them on the basis of their ethnicity, *Purana* and modern status using the data of present study with the data published for the modern Sri Lankan population groups [13].

Table 4. Inter population mtDNA genetic variation of the *Purana* and modern Sri Lankan populations

Grouping	Source of variation	Percentage variation	F_{ST}
Purana & modern groups	Among groups	2.81	
Group 1 – *Purana*	Among populations in groups	0.74	0.03556
Group 2 – Sinhalese	Within population	96.44	
SL Tamil / IN Tamil			
SL Moor			
Ethnic groups	Among groups	0.00	
Group 1 – *Purana*	Among populations in groups	1.91	0.01869
Group 2 – Sinhalese	Within population	98.13	
Group 3 – SL Tamil / IN Tamil			
Group 4 – SL Moor			

The percentage of genetic variation was higher (2.81) when comparing *Purana* against the rest of the Sri Lankan population than grouping the four populations (0.00). This difference between *Purana* and the other populations was clearly affected by its elevated F_{ST} (0.03556) compared to 0.01869.

Table 5. Population pair wise F_{ST} values (below the diagonal) and their significance level (above the diagonal)

	Sinhalese	IN Tamils	SL Tamils	SL Moors	*Purana*
Sinhalese		0.71171±0.0516	0.01802±0.0121	0.38739±0.0490	0.00901±0.0091
IN Tamils	-0.00322		0.00901±0.0091	0.45946±0.0515	0.00000±0.0000
SL Tamils	0.01817	0.02115		0.00000±0.0000	0.01802±0.0121
SL Moors	0.00072	0.00021	**0.03504**		0.00000±0.0000
Purana	0.02447	**0.02899**	0.02476	**0.03909**	

Note – Highly significance P values (P< 0.00005) are in bold face

The lowest pair wise FST has been observed among IN Tamils and purana populations (0.02899, P<0.00000). The highest significant pair wise was among Moors and *purana* population. Other values are not significant.

Phylogenetic analysis of mtDNA for the Purana population

Genetic relationships of the Purana population with modern Sri Lankans and *Vedda* (Adivasis) groups reported by Illeperuma (2009) [13] were further explored by drawing neighbour joining trees based pair wise DA distances between populations[22]. An un –rooted neighbour joining (NJ) tree was constructed on the basis of DA matrices and visualized by TREEVIEW 1.6.1.

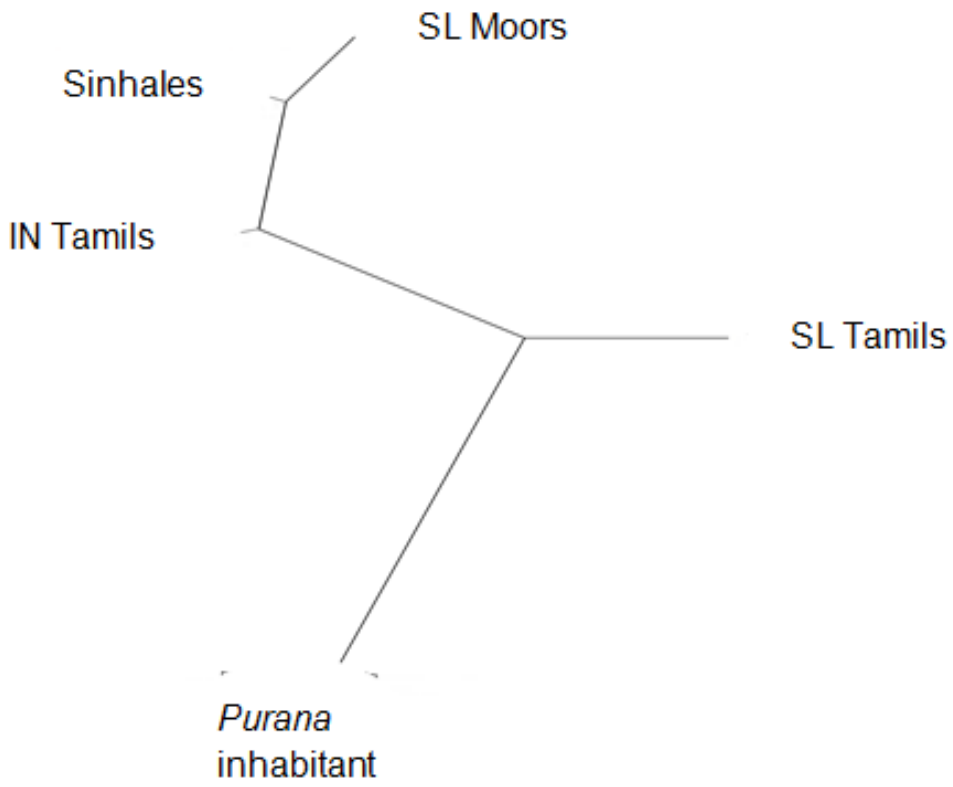

Fig 1. Neighbour – joining tree based on DA distance values of mtHVS - I sequence variation of the *Purana* population and four modern Sri Lankan populations

The significant observation of the DA based neighbour – joining tree was the clustering of Sinhalese, Indian Tamils and the Sri Lankan Moors together in a single clade on the tree compared to others. Although the *Purana* population was isolated in the tree, the genetic distance between the *Purana* population and SL Tamils was low compared with that of others.

Upon inclusion of the aboriginal *Vedda* population to the analysis, the genetic distance of *Purana* to Sri Lankans was high other than the SL Tamil and *Vedda.*

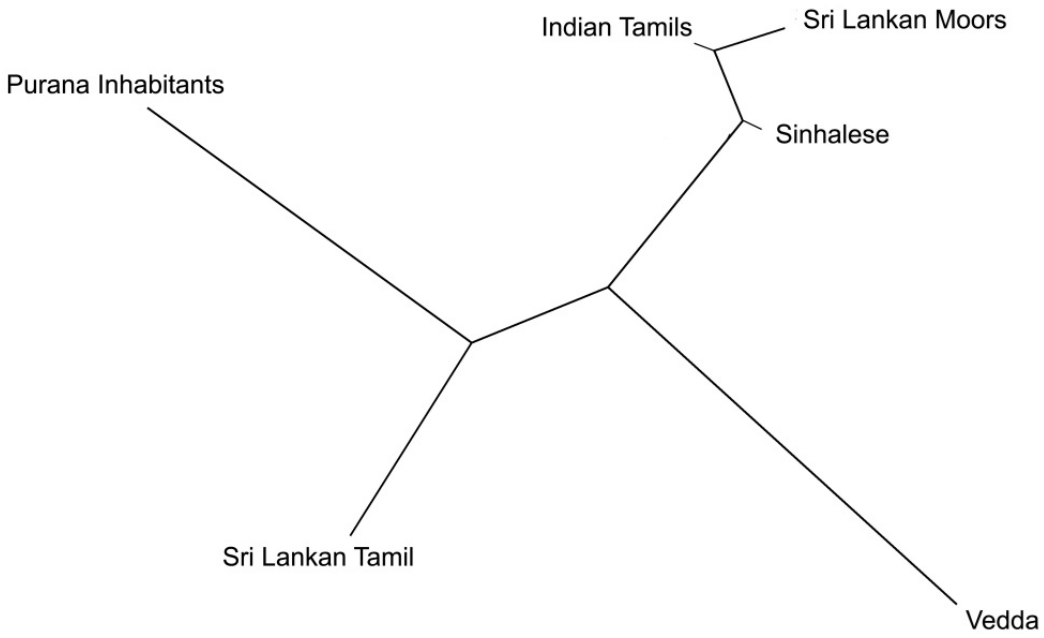

Fig 2. Neighbour – joining tree based on DA distance values of mtHVS - I sequence variation of the Purana populations, *Vedda* and four modern Sri Lankan populations

Note – Close affiliation of the *Purana* population with the SL Tamils was observed. *Purana* population was closer to SL Tamils than *Vedda* and to other Sri Lankans.

Each sequence comprised of 407 nucleotides spanning nucleotide positions (bp) 16,004 – 16,411 of the human mtDNA. The variable positions recorded upon compared with rCRS were presented in Table 6.3.

Discussion

A high percentage of population specific haplotypes observed in the *Purana* population in comparison to modern Sri Lankan populations reported by Illeperuma (2009) [13] suggested that the maternal gene flow of the *Purana* population was restricted among them (Table 3.). This may be a reflection of the endogamous caste system and intra-group marriages that are common among *Purana* populationPurana population at Sigiriya suburbs presently belonging to Govigama and Nekathi castes make marriages restricted to the same caste[24]. Therefore presently maternal gene flow has taken place among the *Purana* population in Sigiriya suburbs and gene flow among other Sri Lankans was limited. The isolation and endogamy observed with mtDNA is also consistent with the reported morphological and morphometrical variations such as dominant mesocephalic cephalic phenotype (35%), leptoprosopic facial phenotype (38%), mesorrhine nasal phenotype (56%), blood group O (46%) etc when

Purana population is compared with present Sri Lankans. The effects of isolation of the *Purana* population were further demonstrated by their other morphometrical and morphological findings [25,26].

Intergroup mtDNA genetic variation was analyzed using AMOVA by grouping them on the basis of their ethnicity, *Purana* and modern status using the data of present study with the data published for the modern Sri Lankan population groups [13]. When populations were grouped according to their ethnicity, AMOVA revealed a 98.13% variation within populations is higher and the variability among four ethnic populations was only 1.91 % (Table 4). This was in agreement with the AMOVA results of HVS - I polymorphisms of the four major ethnic groups in Sri Lanka (Sinhalese, Sri Lankan Tamils, Indian Tamils and Sri Lankan Moors) [13]. Among Sri Lankan ethnic populations, the percentage variation of AMOVA was high (98.97%) within population and the variability among four ethnic populations was 1.03 % [13]. The AMOVA analysis of HVS - I showed less differentiation (FST -0.01869) among the four ethnic populations in this study (Table 5.). This might be due to a higher amount of admixture among them in their maternal linage.

The *Purana* population was reported to be isolated from 5th Century A.D. from the rest of the Sri Lankan population [27]. Therefore, grouping of the population for AMOVA was done with reference to modern and *Purana* basis and increased genetic variation could be identified among groups (2.81%) (Table 4.). Differentiation among populations when grouped by the basis of the origin of the population was higher (F_{ST} - 0.03556) than among population grouped according to ethnicity (F_{ST} - 0.01869) (Table 4.). Bandaranayake *et al*., (1994) [27] recorded that the marriages of the *Purana* inhabitants have been continuously to occur only within each *Purana* community due to strict practicing caste system. Hence present findings demonstrate the genetic consequences of practiced endogamy of the *Purana* population at Sigiriya suburbs in population isolation.

Population pair wise F_{ST} values and their related statistical significances (Table 5.) confirm that the *Purana* population at Sigiriya suburbs was genetically differentiated from the rest of the various modern Sri Lankan tested. The DA distance based neighbour – joining tree (Fig 1. and Fig 2.) further justifies this isolation. High DA distance of the *Purana* population compared to the other Sri Lankans is an indicative of a recent possible occurrence of a population bottleneck. Genetic distance values are known to increase rapidly when bottleneck has occurred [28]. Reduced population size, cultural isolation due to strict practicing caste system are probably the other reasons for the occurrence of the *Purana* population in Sigiriya suburb as an outlier in mitochondrial neighbour – joining phylogeny.

D_A based neighbour joining tree showed that the *Purana* population

were genetically closer to Sri Lankan Tamils than to any other population probably because of an occurrence of increased maternal gene flow among them than other respective populations in Sri Lanka (Fig 2). The antiquity of the *Purana* population has been reported as old as 5th Century AD (1,450 YBP) [27]. The Purana population those who had lived long before, might have made a close association with the Tamils those who migrated from India at the historic time. In Mahavansa, Tamil adventurers such as Elara invaded the Anuradhapura area around 2,100 – 2,200 YBP. In the 9th and 10th centuries, *Pandya* and *Chola* incursions into Sri Lanka culminated in the *Chola* annexation of the island, which lasted until the latter half of the 11th century. Tamil soldiers from Southern parts of India were brought to Anuradhapura between the 7th and 11th centuries by the Tamil dynasty. Such a large influx of Tamils those who came from India in historic time tend to be intermixed with Sri Lankans living in dry zone (Anuradhapura and Polonnaruwa kingdoms).

The present study explores the genetic affinity of the *Purana* population at Sigiriya suburbs in respective to the genetic variation seen only in the HVS - I of the mitochondrial genome. As the mtDNA represents the maternal gene flow, the finding of the present study restricts to the maternal lineage of the studied subjects. Hence the findings of the present study should be further verified with genetic data derived from nuclear and male-specific Y chromosomal DNA markers which may furnish information regarding patrilineal relationships.

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Morphological variations of human adult spleens in a cohort of Sri Lankan population: A cadaveric study

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Abstract

Introduction

Spleen is an organ enriched in vascular and lymphoid tissue located in left hypochondrium. It has morphological variations which may be misinterpreted as a disease condition involving the spleen. Therefore, knowledge on such morphological differences in a population is vital to distinguish normal variations from the disease conditions. Objective of this study was to identify the morphological variations such as notches, fissures and lobulations in cadavers.

Methods

Ten-percent formalin fixed, self-donated cadaveric human spleens (n=13) in the Department of Anatomy, Faculty of Medicine, University of Colombo were included in the present study. In the study population, male to female ratio was 10: 3 with age ranging from 26 to 95 years. The morphological features such as shape, notching of borders, fissures and lobulations were photographed, dissected and analyzed according to Michels NA classification. The morphological types of spleens were categorized by measuring hilar lengths.

Results

Notches were present in superior (n=8), inferior (n= 1) or intermediate (n=2) borders in ten spleens, while notches were absent in three. Five spleens had supernumerary notches. Six spleens contained fissures in both visceral and diaphragmatic surfaces. Four spleens had more than three lobules, while three had more than one hilum. The splenic types included compact, intermediate and distributed, which accounted for four, three and six respectively.

Conclusion

Inconstant location of notches, presence of supernumerary notches, fissures and lobules were a common morphological variation observed.

Keywords: cadavers, morphology, spleen

Introduction

Spleen is an intraperitoneal, encapsulated organ enriched with vascular and lymphoid tissue. It usually found in the left hypochondrium under the left dome of the diaphragm. The extent of the spleen is from the ninth to eleventh rib.

The embryonic development of the human spleen is not fully understood. Around the 5th week of gestation, in the dorsal mesogastrium the mesenchymal cells proliferate to form a mesenchymal mass which gives rise to hematopoietic tissues. By the 8th week, the spleen has a segmented morphology of lobules, which gradually disappear around week 30 as the spleen develops its lymphoid structures. A few lobules eventually fuse to form the spleen proper.

During development, due to its relationship with the surrounding structures, the shape of spleen varies from crescentic to quadrangular [1]. It has a visceral and a diaphragmatic surface. Visceral surface is located at the undersurface of the stomach, with impressions for stomach, colon, left kidney and pancreas. Its diaphragmatic surface is completely covered by peritoneum and usually does not contain fissures [1]. The visceral surface is also covered by peritoneum, except at the hilum which allows blood vessels to enter and exit.

It has three margins; superior, inferior and intermediate. The superior margin of the spleen possesses characteristic notches. The notches located in the superior border of the spleen are representatives of growth that separated the lobules during fetal life [2]. Notches however, can be observed in inferior and intermediate margins as well [1]. The position and curvature of the hila are variable.

Michels NA classification categorizes the spleens according to its morphology as compacted, intermediate and distributed by measuring the length of the hilum in centimeters [3].

Spleen has morphological variations in different populations, which may be misinterpreted as various disease conditions involving the spleen. Therefore, knowledge on such morphological differences in a population is vital to distinguish normal variations from the disease conditions. Objective of this study was to identify the morphological variations such as notches, fissures and lobulations in cadavers.

Material and methods

This descriptive cross-sectional pilot study using human cadavers was conducted at the Department of Anatomy, Faculty of Medicine, University of Colombo, Sri Lanka from April 1, 2019 to May 1, 2019. This study included 13 cadavers that were 10% formalin fixed, self-donated, with a male: female ratio of 10: 3 of and with age ranging from 26-95 years. Human spleens that had destructed surfaces and margins by any mechanical, pathological or other conditions were excluded from the study.

The spleens were inspected macroscopically in detail for shapes, surfaces, borders, hilum and presence or absence of accessory spleen and lobulation. Each spleen sample was photographed perpendicular to the specimen using a 16.0 mega pixel

camera. (Pictures available in Figure 1) The presence of notches on the superior, inferior and intermediate borders and the presence of fissures on the diaphragmatic or visceral surface were noted. Spleens were analyzed according to Michels NA classification. The morphological types of spleens were categorized by measuring hilar lengths.

Standard descriptive statistics were used to analyze the data using SPSS software.

Results

Among the 13 spleens (Figure 1) 4 were oval, 3 triangular, 3 wedge, 1 tetrahedral, 1 elliptical and 1 crescent spleen were noted. Notches were present in superior (n=8), inferior (n= 1) or intermediate (n=2) borders in ten spleens, while notches were absent in three. Five spleens had supernumerary notches. Six spleens contained fissures in both visceral and diaphragmatic surfaces. Four spleens had more than three lobules, while three had more than one hilum. The splenic types included compact (n=4), intermediate (n=6) and distributed (n=3), which accounted for four, three and six respectively.

Discussion

Different populations are found to have numerous morphological variations of the spleen. Notches in the superior border was a common finding in many studies (Table 1) which was a similar finding in ours.

The average number of superior border notches to be reported is 2-4 [5]. In our study we observed two spleens, one with four and another with three notches in the superior border. However, in rare instances it is possible to have superior notches up to nine as reported by Thanya et al [4].

In a Malaysian study, deep fissures extending to the diaphragmatic surface were sparse and it occurred only in 1% of cases [5]. In an Ethiopian Study of 21 spleens, in two specimens (9.52%), the deep notches were observed on the superior and inferior borders extended towards the diaphragmatic surface in the form of two fissures [6]. In our study, six spleens had fissures extending to diaphragmatic surface. Out of them, three had two fissures. Nayak et al [8] reported a lobulated spleen with five fissures and this is the highest number of fissures reported in the literature.

Out of 32 spleens of a North Indian population, 3 spleens (9.3%) had multiple lobules [9]. In the present study, 3 spleens had five lobules, 1 spleen had four lobules, 3 had three lobules, 3 had two lobules and 2 had single lobule.

Ivan described, Lobulations of the spleen as a normal variation of spleen shape and it was not associated with any pathological conditions. However, these lobulations may be misinterpreted as splenic lacerations in patients with abdominal trauma [10]. Also, splenic

lobulations may be misdiagnosed as gastric or left renal tumors [11]. This is further proven by Smidt [12] with the presence of a congenital fissure mimicking splenic hematoma. Abnormal lobulations, therefore could be misinterpreted as a mass originating from the kidney by the radiologists [11]. Therefore, we believe that the findings of our study are of great importance with regards to treatment during trauma care. Table 2 compares on incidence morphologic types of spleen based on Michels NA classification [13] with other studies. In our study more than one hilum could be seen in three spleens. A study in Kerala, also mentions multiple hila. Similarly, there were other studies that reported multiple hila [13].

Presence of supernumery notches, fissures and lobules were a common anatomical variation in this cohort of cadavers of Sri Lankan origin. Studies done in future with a larger sample size will aid in the identification of common morphological variations in the Sri Lankan population.

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Anatomical Variations of the Terminal Branching of the Brachial Artery

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Abstract

Objective

Knowledge regarding arterial variations is important during surgeries, intra-arterial injections, venipuncture and imaging of the upper limb. Our objective was to document brachial artery variations.

Methods

We studied 54 upper limb specimens during routine dissection. The anterior compartment of the arm, cubital fossa and the forearm were dissected and the pathway and the distribution of the brachial artery and its branches were documented.

Results

Variations of the brachial artery anatomy were noted in 3 specimens (5%). Unilateral high bifurcation of brachial artery was noted in two specimens on the left side. In one of these, bifurcation was directly from the axillary artery at the axilla just proximal to the formation of the median nerve. Here the brachial artery divided into its terminal radial and ulnar branches. In the other specimen, the brachial artery bifurcated into radial and ulnar arteries at the level of the mid humerus. In the other specimen, the brachial artery was seen to trifurcate into radial, ulnar and common interosseus arteries at the level of the neck of the radius.

Conclusions

High brachial artery bifurcation and trifurcation of the brachial artery in the upper arm was noted in this study. Reporting of such variations in different populations is important for the practicing clinician during diagnostic and surgical procedures.

Introduction

Arterial distribution of the upper limb shows great anatomical variation [1]. Variations of the radial artery are the commonest followed by the ulnar artery and then the variations of the brachial artery [1]. Knowledge about the these anatomical variations is important during surgeries such as flap surgeries of forearm, amputations, fasciotomy for compartment syndromes and using radial artery for coronary artery bypass grafting as well as during intra-arterial injections and venipuncture [2]. Further a thorough knowledge of the arterial anatomy prevents diagnostic error in imaging of limb vasculature[1].

Brachial artery is the continuation of the axillary artery beyond the lower boarder of the teres major muscle. It divides into the radial and ulnar arteries its terminal branches, opposite the neck of the radius in the anterior cubital region [4].

High bifurcation of the brachial artery is not a rare occurrence and accounts for 15% of the brachial artery variations [4]. It may bifurcate as high as the axillary artery, but most commonly it divides at the proximal 1/3 of the upper arm [4]. Recent studies have revealed that the high bifurcation can cause several clinical implications including increased failure rate of AV fistulae created for hemodialysis [3]. High origin of ulnar artery is described as superficial ulnar artery where it arises as a branch of the axillary artery or from the brachial artery. Usually it lies superficial to the brachial artery and the median nerve. In the forearm it crosses the forearm flexors to the medial side of the arm [4]. Trifurcation of the brachial artery is relatively rare, where it trifurcates into the radial artery, ulnar artery, radial recurrent artery, common interosseous artery or into the median artery [5].

With this background, the study was designed to examine and document the variations of the terminal branching of the brachial artery.

Methods

The study was conducted on 54 upper limb specimens in 27 cadavers, 16 of which were male, during routine dissection at the Department of Anatomy, Faculty of Medicine, University of Peradeniya. The anterior compartment of the arm, cubital fossa and the forearm were dissected according to routine dissection procedures. The skin, superficial facia, deep facia and the muscles were dissected and separated. The brachial artery was carefully traced from its origin to it bifurcation. Observed anatomical variations of the terminal branching of the brachial artery were examined, documented and photographed.

Results

Variations of the branching of the brachial artery were noted in 4 specimens (5.5%). Unilateral high bifurcation of brachial artery was noted in 2 cadavers (3.7%). The bifurcation into radial and ulnar arteries was directly from the axillary artery in the axilla, just proximal to the formation of the median nerve in one cadaver (figure 1). Thereafter both the branches were seen to travel in the anterior compartment of the arm to the cubital fossa and followed their normal course for the rest of their lengths. In the other cadaver brachial artery was seen to bifurcate into radial and ulnar arteries at the level of the mid humerus (figure 2). After the bifurcation, the radial and ulnar arteries continued in their normal course. Ulnar artery was seen to disappear deep into the deep head of pronator teres and ran medially and distally on flexor digitorum profundus. The radial artery passed distally and medial to the biceps tendon.

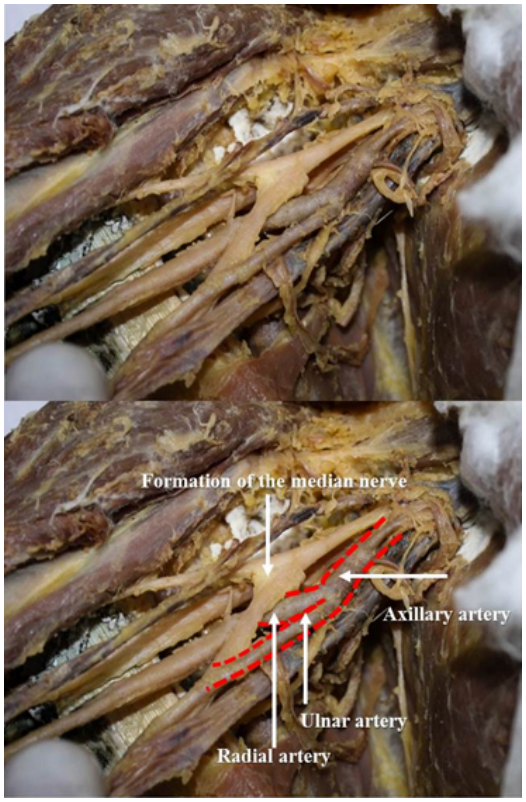

Figure 1: High bifurcation of the brachial artery

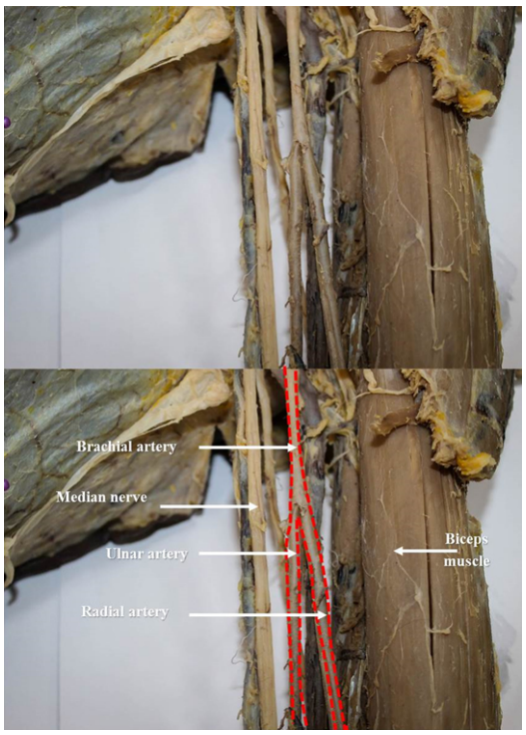

Figure 2: High bifurcation of the brachial artery at mid humerus level

Unilateral trifurcation of brachial artery was noted in one cadaver (1.85%). The brachial artery trifurcated into radial, ulnar and common interosseus artery at the level of the neck of the radius (figure 3).

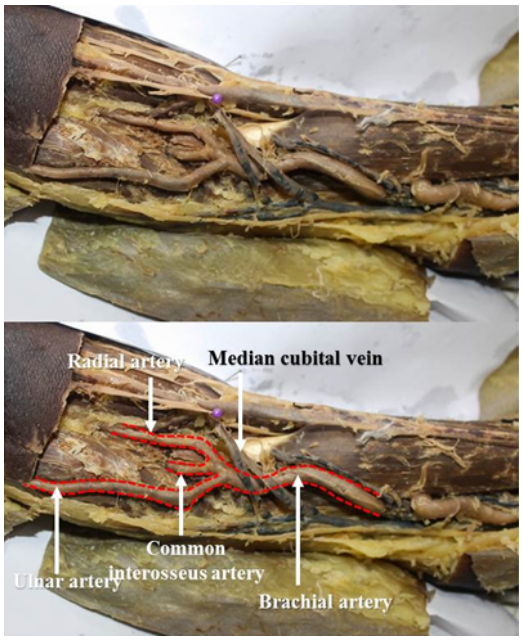

Figure 3: Trifurcation of the brachial artery in the cubital fossa

The radial artery was further traced and found to be of standard description while the ulnar artery continued superficial to the flexor digitorum superficialis and flexor carpi ulnaris up to mid forearm: 7cm from trifurcation and then ran deep into the plane between flexor carpi ulnaris and flexor digitorum profundus. The common interosseus artery too ran its usual course

Superficial ulnar artery was detected in one specimen (1.85%) (figure 4). The ulnar artery commenced at the level of the neck of the radius and passed

superficial to the pronator teres, fibrous arch of the flexor digitorum superficialis, flexor carpi ulnaris and continued lateral to flexor carpi ulnaris tendon at the distal forearm, just deep to the skin and the deep facia throughout its course and continued as the superficial palmar arch.

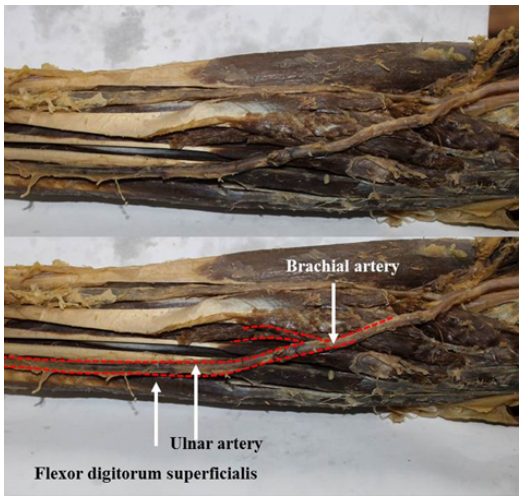

Figure 4: Superficial ulnar artery

Discussion

Local hemodynamic factors are said to influence the development of blood vessels during embryological period and alteration of these factors causes anatomical variations in the arterial system (6). Variations were observed in 5.5% of specimens in our study. Ciervo et al stated that the vascular variations of the upper limb are estimated to be between 9% to 18.5% which is consistent with our study (7).

High bifurcation of the brachial artery is estimated to be between 3% to 15% (7). The percentage of high bifurcation of the brachial artery in our study was 3.7% and is consistent with reported studies.

Williams et al had reported that the high bifurcation of the brachial artery is more frequent than trifurcations and if it is a trifurcation, the third artery is the common interosseous artery (8). Huber et al mentioned that the third branch could be median artery, common interosseous artery or the radial recurrent branch (9). However in our study, the percentage of the trifurcation of brachial artery was 1.85% and the third branch was the common interosseous.

Senanayake et al presented a rare case of ulnar artery arising from the medial side of the upper brachial artery and descending deep to the deep fascia and superficial to the bicipital aponeurosis (10). De Costa et al stated that the incidence of the superficial ulnar artery is 0.7%- 7% (11). It was 1.85% in our sample similar to these reports.

Clinicians need to be aware of these anatomical variations when diagnosing as well as during therapeutic procedures to avoid unnecessary complications and injuries and therefore timely reporting of such variations is of paramount importance.

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CASE REPORTS

Review of anatomy in managing complications of chronic pancreatitis-case report

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Abstract

The main problem of chronic pancreatitis is managing the chronic pain. When pharmacological means fail, ablative procedures like coeliac plexus ablation or bilateral splanchnicectomy becomes necessary. These procedures are designed based on anatomy. Pancreatic duct and common bile duct obstruction are known complications of chronic pancreatitis and methods of treatment are essentially based on interpretation of images. We present a case history of a patient with chronic pancreatitis who underwent bilateral thoracoscopic splanchnicectomy and later laparoscopic cholecysto-jejunostomy for common bile duct obstruction.

The importance of anatomy in diagnosis and treatment are highlighted.

Introduction

In chronic pancreatitis there is calcification and fibrosis of the gland (1). The main presentation is due to pain which is sensed by the splanchnic nerves. In addition there is loss of endocrine and exocrine function due to fibrosis and atrophy of gland. Fibrosis can cause obstruction of the pancreatic duct which aggravates pain and functional impairment. As distal common bile duct travels through the pancreas gland it may also get involved in fibrosis leading to obstruction (1).

Bile duct stricture (also called biliary stricture) is an uncommon but challenging clinical condition Most benign bile duct strictures are iatrogenic, resulting from operative trauma (2). Bile duct strictures may be asymptomatic but, if ignored, can cause life-threatening complications, such as ascending cholangitis, liver abscess, and secondary biliary cirrhosis (3,4). The common bile duct (CBD) and the ventral portion of the pancreas have a close relationship as both develop from the same anlage. In about 80 to 85 % of people, the lower CBD traverses the pancreatic head, and in 15 to 20 % cases, it is adjacent to the posterior surface of the pancreatic head (5). Thus, the lower bile duct may be involved in neoplastic as well as inflammatory processes of the pancreatic head. The stricture of the lower common bile duct in chronic pancreatitis is not rare. The reported incidence ranges from 3 to 46 % [6–13]. But symptomatic stricture develops in about 10 % of cases [14]. CBD stricture in chronic pancreatitis may be due to edema, pseudocyst

formation, and encasement of the bile duct by fibrous processes. Management of such stricture is a matter of controversy. Once the patient develops symptoms related to biliary obstruction, some form of biliary drainage is necessary (15-17). Options for biliary drainage include endoscopic stenting and operative bypass (18). Stricture resolution was observed in the long term in 12 to 38 % of patients (19-23). In the long run, two thirds of the patients require surgical intervention for persisting stricture after stent removal. Repeated stent changing or use of metallic stent for this benign disease is associated with numerous hospital admissions and substantial morbidity. Currently, stenting is at best a temporizing procedure particularly for cholangitis. On the other hand, surgery offers long-term relief of symptoms related to biliary obstruction. So, surgery is the best option in good-risk patients, keeping endoscopic stenting for patients with serious comorbid conditions or those unwilling to undergo surgery.

Management of pancreatic pain is challenging, many not responding to oral analgesics and may get dependent to narcotics. In such situations splanchnicectomy provides satisfactory and long standing pain relief (24).

We present a case report of patient who underwent thoracoscopic splanchnicectomy for relief of pain and subsequently laparoscopic cholecysto-jejunostomy for obstructive jaundice. In both interventions understanding anatomy is the key for successful performance of procedure.

Case Report

38 year old male presented with episodic severe epigastric pain during past six months. He also had dyspeptic symptoms during past ten years. Analyzing symptoms anatomically pain has to be from a foregut structure. As the pain was radiating to back with relief from bending forwards it was more suggestive of pancreatic origin. He had diabetes which too favored chronic pancreatitis. He was used to consume alcohol a known etiological factor. Examination was unremarkable except mild epigastric tenderness. Ultra-sound scan of abdomen revealed chronic pancreatitis. Contrast enhanced computerized tomogram(CECT) confirmed the diagnosis. His pain was treated with multiple analgesics.

As the response to analgesics was poor he was planned for bilateral thoracoscopic splanchnicectomy. At thoracoscopy anatomy of the splanchnic nerves were identified. Nerve trunks were divided with diathermy hook. This provided a good pain relief with reduction for need of analgesics.

About one year later he presented with obstructive jaundice. Most likely anatomical site of the extra-hepatic bile duct obstruction was the intra-pancreatic part due to fibrosis by chronic pancreatitis, which was confirmed by imaging. Placement of a stent via the ampulla of Vater was attempted by endoscopically which failed. Therefore a biliary by pass was planned. Looking at the anatomy of the

obstructed system on CECT it was decided to perform a cholecysto-jejunostomy, laparoscopically. At laparoscopy transverse colon was lifted up to identify the duodeno-jejunal flexure and jejunum traced down to identify a suitable loop. A loop cholecysto-jejunostomy was performed.

Discussion

Management of pain in chronic pancreatitis is challenging. In patients with a poor response to pharmacological treatment nerve ablation procedures need to be considered (24). The options according to nerve supply are either coeliac plexus ablation or bilateral splanchnicectomy(25). In the patient presented the latter was selected and produced a good pain relief. By performing by thoracosocpy other than a huge reduction of morbidity of bilateral thoracotomy, identification of the splanchnic nerves is easy due to the magnified clear image (26).

Biliary strictures as a consequence of CP have long been recognized. After pseudocyst formation, bile duct stricture is the second most common complication in CP. It is most commonly found in chronic pancreatitis with inflammatory head mass or when there is dense calcification in the pancreatic head (27). Noninvasive imaging of the hepatobiliary tree is important in evaluating a patient with presumed biliary tract obstruction. Ultrasonography will demonstrate bile duct obstruction in 80 % of cases (28).

The definitive evaluation of biliary obstruction can be done by cholangiography. ERCP or MRCP can outline both the pancreatic duct and bile duct. ERCP is more important when endoscopic stenting and brush cytology are required. Asymptomatic patients can be managed conservatively and monitored by 6-monthly liver function tests and biliary ultrasound. As there is no reliable means for predicting progression to secondary biliary cirrhosis (3), biliary drainage is indicated when the patients have symptomatic biliary stricture. Biliary drainage can be achieved by ERCP with stenting, percutaneous transhepatic biliary drainage, or surgery. Long-term success rate of surgery (failure rate <3 %) is much higher than that in endoscopic stenting (29). So, we think that surgery should be offered to all good-risk patients with symptomatic biliary obstruction. In the patient discussed ERCP and stent insertion failed and cholecysto-jejunostomy was done.

Conclusion

Anatomy plays a key role in making a clinical diagnosis, interpretation of anatomy and surgical treatment which is illustrated by the patient who had chronic pancreatitis and needed surgical treatment for relief of pain and obstructive jaundice

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Classical presentation of Renal cell carcinoma: A case report

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Abstract

Renal cell cancer (RCC) represents 2-3% of all cancers with male predominance affecting old age. Risk factors include smoking, obesity, hypertension and genetic factors. Presentation can vary from non-visible hematuria to symptoms from metastatic disease to bones, lung, brain and lymph nodes. Only 6-10% presents with classical presentation of loin pain, loin mass and hematuria (3). We presents a case of a patient who had non-visible hematuria later progressing to metastatic renal cell carcinoma.

Introduction

Renal cell cancer (RCC) represents 2-3% of all cancers with the highest incidence in Western countries and male: female ratio of 1.5:1 (1). Peak incidence age in Sri Lanka is 50-60 years. Etiological factors include smoking, obesity and hypertension. Having a first-degree relative with RCC will increases the risk of RCC. More than 50% of RCC are asymptomatic and found incidentally on imaging (1). The classic triad of flank pain, visible hematuria, and palpable abdominal mass is rare (6-10%) and compatible with aggressive histology and advanced disease (3). Another population may present with features of paraneoplastic syndrome and disseminated disease. Persistent non- visible hematuria may indicate underlying urological malignancy which needs further evaluation.

Case Report

Mrs. S 61 years old previously healthy female presented to the urology clinic with the complaint of left loin pain for 3 weeks duration. For the last 1 week she complains of gradually increasing left loin area swelling and hardness to touch. For the same duration she complained of a left side neck lump which was gradually enlarge in size and did not cause any symptoms. On further questioning she revealed a history of dysuria 6 months ago where she was managed as a urinary tract infection. Follow up urine investigations showed blood in the urine which was persistent for 2 months, but she defaulted until current presentation. No symptoms were to suggest distant spread of a malignancy apart from above.

On examination she was pale and having mobile left supraclavicular lymph node enlargement. Abdomen was asymmetrically distended with a well-defined firm intraabdominal mass measuring 10cm×8cm in the left hypochondrium extending into left flank. Other systems examination were normal.

Urine analysis showed microscopic hematuria and complete count remarkable for 7.7g/dl hemoglobin level. USS abdomen revealed a solid mass measuring 8×10×12 cm arising from left kidney. Fine needle aspiration of supraclavicular LN demonstrated malignant smear. CECT IVU and Chest was ordered subsequently. It confirmed the mass to be in consistent with left side renal cell carcinoma with evidence of loco regional LN metastasis. (T2b N1 M0).

After multi-disciplinary team discussion left side open radical nephrectomy was done. There was no ipsilateral adrenal gland involvement so it was preserved. There were extensive para-aortic LN enlargement which were not dissected. Renal vessels and IVC appeared clear of tumor. Patient recovered fully within 2 days and no complications were encountered. Histology revealed type 1 papillary carcinoma of Fuhrman grade 1. Tumor emboli were seen in perihilar vessels. Later patient was referred to oncology care and started on systemic therapy with tyrosine kinase inhibitors.

Discussion

Loin is the area of abdomen between the 12th rib and the iliac crest. Lump in the loin can be intraabdominal or extra abdominal in origin. Revising the organs in that region differential diagnosis includes lump arising from spleen, kidney, descending colon, small bowel or rarely retroperitoneum. Classical examination findings like moving with respiration and alternative bands of resonance over the lump may help to differentiate renal masses from others. This patient belongs to the rare group of classical presentation of RCC, hematuria, loin pain and loin mass. This patient has had microscopic hematuria preceding loin mass, but lost to the follow up. Palpable renal mass indicates advanced disease and poor prognosis (1). Curative surgery could be feasible in this patient if identified early. That indicates the importance of evaluating non-visible hematuria as it can harbors underlying malignancy. Staging imaging puts the patient to locally advanced (stage 3) disease category, where curative surgery is not possible. But cytoreductive nephrectomy with systemic therapy has better survival than systemic therapy alone (2). That is the reason for nephrectomy in this patient with the added benefit of symptoms alleviation. This patient had type 1 papillary cancer which has good prognosis compared to clear cell carcinoma but overall prognosis seems to be poor.

Conclusion

This case report emphasizes the importance of evaluation of non-visible hematuria which may identify early disease for curative intervention.

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Anatomical considerations during Laparoscopic excision of a splenic cyst: Case Report

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Introduction

Splenic cysts are an uncommon disease entity of the spleen. Most of the patients are asymptomatic and are diagnosed incidentally in abdominal ultra sound and CT scans that are performed for other reasons. Some present with vague abdominal pain with or without mass.

Performing excision by laparoscopy reduces morbidity. To perform laparoscopic excision anatomy of the spleen provides the key for positioning of patient, port placement and subsequent procedure.

We present a patient who underwent a laparoscopic excision of a splenic cyst.

Case Report

A 58 year old female presented with a vague aching type persistent epigastric and left hypochondrial pain for two months. There was no history of abdominal trauma. General and abdominal examinations were normal. Ultrasound scan revealed a unilocular thin walled encapsulated cyst in the lower pole of spleen measuring 7.9cm x 7.1cm x 5.9cm suggestive of a benign cyst. Contrast CT abdomen confirmed an encapsulated unilocular thin walled cyst at lower pole of the spleen.

Laparoscopic splenic cyst excision was planned after obtaining informed consent. As the spleen and cyst occupies the left hypochondrium patient was placed on right lateral decubitus position and head up tilt to let other organs fall away from field of dissection. With stomach's close relationship with the spleen decompression of stomach with a naso-gastric tube facilitates dissection. Surgery was performed using three ports. After inserting the camera port related anatomy was appreciated. Stomach, spleen, transeverse colon, splenic flexure, gastro-splenic ligament, gastro-colic ligament and spleno-colic ligament were identified. As the cyst was large it was initially decompressed by aspiration. Excision of the cyst with a thin rim of splenic tissue was done using ultra-sonic dissector and bipolar diathermy. Hilar area with splenic vasculature was preserved. The specimen was retrieved within a retrieval bag through enlarged 10mm port site. The surgery was completed in 45 minutes without any measurable blood loss.

Patient was commenced on oral feeding on same day and mobilized out of bed. Pain was managed with oral analgesics and Diclofenac sodium suppositories.

The patient was discharged on the following day. The histology revealed a pseudocyst with chronic granulomatous inflammation suggestive of Tuberculosis. She was referred to chest clinic where Mantoux test was strongly positive and was started on anti-TB therapy.

Discussion

According to Fowler's classification, the splenic cysts are primary or true and secondary or false cysts depending on the presence or absence of true epithelial lining. Primary cysts can be congenital, traumatic, inflammatory, neoplastic or parasitic and secondary cysts can be traumatic, inflammatory or degenerative[1].

Most of the splenic cysts are asymptomatic. Patients can present with vague left hypochondrial pain, left side shoulder tip pain and postprandial abdominal fullness. They also can present with complications of cysts which includes trauma leading to rupture and haemorrhage and infection.

Contrast enhanced CT is the investigation of choice for splenic cysts specially in differentiating nonparasitic cysts from parasitic cysts[2]. The key to interpret the CT is understanding cross-sectional anatomy of abdomen. Non parasitic cysts are unilocular densely cystic and thin walled whereas parasitic cysts are multilocular with daughter cysts within the main cysts[3].

Asymptomatic cysts less than 4 cm can be managed conservatively. Symptomatic cysts and asymptomatic cysts more than 4 cm warrant intervention[4].

Treatment options include percutaneous drainage, laparoscopic total cyst excision, laparoscopic partial splenectomy, laparoscopic partial cyst excision with marsupialization and hand assisted laparoscopic splenectomy. The laparoscopic approach is associated with lower morbidity when compared to open surgery[1]. Though laparoscopic total cyst excision or partial splenectomy removes the cyst completely and reduces the risk of recurrence, it is more time consuming and has higher bleeding risk compared to partial cyst excision with marsupialization. However with perception of anatomy of spleen and related organs the procedure was completed in forty five minutes without any measurable blood loss. The recurrence rates are 64% and 78% respectively [5]. Measures such as Argon laser coagulation of splenic surface and omental packing do not completely eliminate the risk of recurrence.

The patient discussed underwent a complete cyst excision with a thin rim of splenic tissue. The histopatholgy revealed a chronic granulomatous pathology suggestive of Tuberculosis, Melioidosis or parasitic infection. Patient was referred to a chest physician and was started on anti-TB therapy as mantoux test was strongly positive.

Conclusion

This case illustrates that appreciation of anatomy is the basis of making a surgical diagnosis including interpretation of CT images. Complete excision of a splenic cyst can be performed by laparoscopically and detailed appreciation of anatomy provides the key.

Key words: Laparoscopy, Splenic cyst

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LETTER TO EDITOR

A brief history of neuroscience

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The study of neuroanatomy dates back to the Egyptian era in early 3000 BC. Edwin Smith Papyrus is one of the oldest medical scripts written in this period and is often referred to as the first treatise on surgery. There are references to the "brain" throughout the papyrus, and it is evident that these surgeons were aware of connection between traumatic brain injuries and accompanying motor weaknesses. In 500 BC, Croton in Greece, a contemporary of Pythagoras studied brain development in chick embryos. Furthermore, he believed that sensory perception was linked to the brain. However, the art of dissection of animals was pioneered by Herophilus of Alexandria (325-255 BC), hence was named the father of Anatomy. He identified the nerves that emerged from the brain as motor and sensory cranial nerves and the fact that paralysis of muscles would follow if the former are damaged. His younger contemporary Erasistratus continued Herophilus' legacy and conducted the first ever comprehensive scientific dissection of the human body, including "vivisections" on criminals. He differentiated cerebrum and cerebellum based on external features. He attributed the increased number of gyri and sulci in human brain to the greater cognitive capacity in humans when compared with other non-human vertebrates.

Alexandria and Egypt were conquered by the Caesar of Imperial Rome and many Alexandrian anatomists were persecuted. Following the era of Herophilus and Erasistratus, anatomical dissections were abandoned for almost 350 years until the time of the celebrated physician Claudius Galen (131-201 AD). During his time, human dissections were banned in Rome. Therefore, he extensively dissected non-human primates and published two treatises which gained an enormous publicity among regional scholars for many years and even after his death. These treatises were considered the gold standard in anatomy until his writings were challenged by Vesalius in the 16th century. He identified all the cranial nerves except olfactory and trochlear nerves, nevertheless he erroneously described almost all of them to be sensory nerves in contrast to the spinal nerves, which he believed to be motor in origin. Furthermore, Vesalius extensively studied the anatomy of non-human primate brain and described the ventricular system, corpus callosum, septum pellucidum, pineal gland and fornix in great detail. Human dissections were recommenced only after the dawn of renaissance in

1530 AD. It is not clear whether Leonardo Da Vinci conducted human dissections himself, however his sketches reflect the detailed understanding of the complexity of human brain. Moreover, he conducted seminal experiments on the spinal reflex in frogs and demonstrated that the reflex could be eliminated by damaging the spinal cord. Subsequently, Thomas Willis (1625-1725) made a remarkable contribution to the field of neuroscience. He is famous for describing the arterial circle located at the base of the brain, which is known as the "Circle of Willis". Having completed dissections across numerous species of animals including both vertebrates and invertebrates, he pioneered the field of comparative neuroanatomy.

While these scholars were studying the gross anatomical features of the human nervous system, Robert Hooke described crude features of cells using a prototype microscope in 1665. Following invention of the modern microscope by Leeuwenhoek in 1670s, anatomists were interested in exploring the complexity of structure of the human nervous system. In early nineteenth century, the scientists recognized the cell as the structural unit of the human body. The Italian biologist Golgi initiated the concept of "reticular theory" in which he stated that nervous tissue was formed by a fundamental structural unit of "nerves" connected to each other by numerus processes forming a network. Spanish neuroscientist Santiago Raymon Y Cajal was the principal opponent of this reticular theory. Cajal often debated that there should be discrete nerve cells as opposed to a reticulum and was able to stain isolated neurons using silver staining technique, which was pioneered by Golgi himself. Nevertheless, for their enormous contribution to the field of neuroscience, both Golgi and Cajal were awarded with the Nobel Prize in Physiology and Medicine in 1906. With the discovery of neurone, Cajal paved the path for the discoveries made in modern neuroscience.

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INSTRUCTIONS TO AUTHORS

The Sri Lanka Journal Anatomy publishes the following categories of articles which have relevance to Anatomy and allied sciences.

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Original and review articles must contain an abstract of approximately 250 words with four specified subtitles:

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Chapter in book Boyde A. Amelogenesis and the structure of enamel. In: Cohen B. Kramer KH(eds). Scientific Foundations of Dentistry. William Heinemann Medical Books Ltd. London. 1976: 335-352.

No author given International statistical classification of diseases and related health problems, 10th revision, vol 1. Geneva: World Health Organization, 1992; 550-564.

Supplement to Volume 3; Issue II; 2019

Abstracts Presented at Annual Academic Sessions 2019

HIPK2 may be a marker of neuronal stress in ALS/FTLD

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Amyotrophic lateral sclerosis (ALS) and fronto-temporal lobar degeneration (FTLD) are neurodegenerative diseases often characterized by TAR DNA-binding protein-43 (TDP-43) pathology in neurons. Accumulation of misfolded proteins causes endoplasmic reticulum (ER) stress, which may lead to cell death in disease. Here, we aimed to optimise antibodies to enable their use to reliably detect ER stress proteins C/EBP homologous protein (CHOP), protein kinase R-like ER kinase (PERK) and homeodomain-interacting protein kinase-2 (HIPK2) in an ALS/FTLD transgenic mouse model at different stages of the disease course. Tissues from the cerebral cortex and lumbar spinal cord were harvested from two-week off doxycycline NEFH-tTA/tetO-hTDP-43ΔNLS B6C3H mice with neurological manifestations of ALS/FTLD. Paraformaldehyde fixed tissues were immune stained with commercially available CHOP, PERK and HIPK2 primary antibodies with appropriate negative controls and visualized under an epifluorescence microscope. Alpha-motor neurones in the spinal cord of bigenic mice showed nuclear staining for HIPK2, consistent with previous findings where expression of HIPK2 was altered in disease compared to healthy tissue. Conditions for detection of PERK and CHOP require further optimisation. Therefore, we conclude that HIPK2 is expressed in the central nervous system of the bigenic mouse model of ALS/FTLD at an early age of the disease. These findings suggest that activation of HIPK2 may contribute to the early stages of diseases associated with TDP-43 pathology.

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High frequency of Klinefelter syndrome in a cohort of Sri Lankan males with azoospermia and oligozoospermia

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Klinefelter syndrome (KS) is the most common sex chromosomal aneuploidy which affects 1:667 new born males. It is a major cause of male factor infertility due to the associated testicular atrophy. This study aims to describe the karyotypes identified in a cohort of Sri Lankan infertile males referred for cytogenetic testing prior to undergoing testicular biopsy. The karyotype reports of males with severe oligozoospermia (sperm cell count<5×106 cells/ml in seminal fluid analysis) or azoospermia, who were referred for karyotyping between January 2010 and February 2019 were maintained prospectively in an anonymized database and analysed retrospectively. All patients were referred from a single urological practice prior to undergoing testicular biopsy. Karyotyping was performed on routinely cultured lymphocytes after GTG-banding according to the guidelines of the International System for Human Cytogenetic Nomenclature (2016). A total of 69 infertile males underwent karyotyping. Among them, forty (58.0%) had azoospermia and 29 (42.0%) had severe oligozoospermia. Abnormal karyotypes were seen in 14 (20.3%) males, comprising of 11 (78.6%) with KS (47, XXY) and 3 (21.4%) with 46, XX karyotype. Polymerase chain reaction for SRY gene was positive in one male with 46, XX karyotype. Among the KS patients, 6(54.6%) had azoospermia and 5 (45.5%) had severe oligozoospermia. KS accounted for more than two-thirds of the chromosomal anomalies in this cohort of infertile males. Cytogenetic testing of severe oligozoospermic and azoospermic males may help avoid extensive and invasive investigations in both partners. Multicentre studies with a larger sample size would provide more generalizable data on the karyotype profile among Sri Lankan infertile males.

Effect of gestational age on the morphometric parameters of placenta

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Placenta plays a vital role in the foetal growth and development. Alteration in its morphological parameters is associated with adverse pregnancy outcome. Hence, in the present study the influence of gestational age on the weight, thickness and number of cotyledon formation of the placenta were assessed. The study was conducted on 289 singleton normal vaginal deliveries in Teaching Hospital, Batticaloa. The gestational age was determined from last menstrual period and confirmed by early ultrasonography. The placentae were washed to remove the blood clots and the weight and thickness were measured. The number of cotyledons was counted through careful examination. The collected data were analysed by Spearman's correlation test using SPSS software. The gestational age exhibited a positive relationship with the weight of the placenta ($p=0.007$). However, the correlation coefficient (0.176) was very weak according to Evans (1996) suggestion. The thickness and number of cotyledons showed significant relationship ($p=0.000$) with the weight of the placentae than gestational age. The weight of the foetus increases with the weight of the placenta. This indicates that placental growth restriction leads to restriction in the intrauterine growth of foetus. The study result shows that advance gestational age support the growth and development of the placenta. However, the maternal factors such as nutritional values of the diet, BMI, gestational weight gain and pregnancy induced conditions might influence the weight of the placenta. Therefore, the effects of maternal factors on the placental weight need to be studied.

The Morphology and Position of the Pterion in a sample of Sri Lankan Adult Population

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The pterion, defined as a H-shaped sutural confluence formed by the frontal, parietal, temporal and sphenoid bones on the lateral surface of the skull overlies both the anterior branch of the middle meningeal artery and the lateral cerebral fissure. The present study was conducted to determine the morphology of the pterion, and its precise position with reference to surrounding anatomical landmarks in an adult Sri Lankan population. Sixty pteria of thirty skulls of known sex in an adult Sri Lankan population were investigated. The sutural pattern of the pterion on both left and right sides of each skull were recorded based on Murphy's classification. Linear distances were measured using a digital vernier caliper to the nearest 0.01mm. The predominant type of pterion observed in male and female combined sample was sphenoparietal (82.4%), followed by stellate (9.8%), and frontotemporal (3.9%), and epipteric types (3.9%). The mean distances from the center of the pterion to the frontozygomatic suture were 31.11 ± 5.02mm and 26.98 ± 3.45mm; to the zygomatic arch, 38.92 ± 3.55mm and 36.16 ± 3.83mm; to the mastoid process, 81.54 ± 4.62 mm and 77.79 ± 3.88mm; and to the external acoustic meatus, 53.62 ± 2.58mm and 51.91 ± 2.98mm, in males and females, respectively. All the differences were statistically significant at the level of $p<0.05$. The pterion in the males was positioned at a higher point from the zygomatic arch than in females. The present study has shown that the sphenoparietal type of pterion predominates in this sample.

Cadaveric study of variations of origin of lateral circumflex and medial circumflex artery in a Sri Lankan Population

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Lateral circumflex artery (LCA) and medial circumflex artery (MCA) are two main branches of Profunda Femoris artery (PFA) given off on its lateral and medial aspect, respectively. Knowledge on its possible variations is important in preventing iatrogenic injury during surgeries in the hip and bypass surgeries. Twenty seven formalin fixed pre dissected cadavers from the Department of Anatomy, Faculty of Medicine, University of Peradeniya were used. Further dissection was done to expose the site of origin of LCA and MCA. The pattern of origin of the arteries was observed. In cadavers where the MCA and LCA were originating from the Femoral Artery (FA), the distance from the inguinal ligament to the origin of their respective branch was measured using a tape and a string. Eighty three per cent of LCA and 96.2% of MCA were originating from the PFA but 16.9% of LCA and 3.7% of MCA were originating directly from the FA, above the origin of PFA at distances from the inguinal ligament varying from 1.6cm-4.2cm. Two limbs showed a high origin of LCA from FA within 2cm from the inguinal ligament. LCA had a common origin with PFA in two cadavers, where the MCA had a normal origin. LCA also had a common origin with MCA from femoral artery in 1 cadaver. This study revealed considerable variations where 16.9% of LCA and 3.7% of MCA originated from the FA. This knowledge on variations is important in various surgical and radiological interventions in the femoral triangle.

Determination of sex using the humerus in a contemporary Sri Lankan population

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Identification of the biological profile includes determination of sex, age, ethnicity and stature estimation. It is important when unknown skeletal remains are recovered. Metric characteristics of humeri also have received attention in forensic studies for this purpose. The study was conducted in the Department of Anatomy and Department of Forensic Medicine, Faculty of Medicine, and Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya. A sample of 136 dry humeri was studied. Six measurements were taken including the weight, maximum length, circumference at the proximal 1/3, middle 1/3, distal 1/3 and the length of the shaft using standard techniques. Descriptive statistics were obtained separately for each sex. Two sample t-test was used to assess whether there is a significant difference between males and females. Univariate and stepwise discriminant function analyses were performed to assess the sex classification potential of humeral measurements. The data sets were normally distributed. All 6 dimensions were statistically greater in males ($p<0.05$). Weight measurement gives the highest accuracy of sex classification with an accuracy of 83.3%. Circumference at the middle 1/3 and distal 1/3 also gives more than 80% accuracy of sex determination. The best multivariate equation for the sample was the combination of weight and circumference at the proximal 1/3 with a cross validated accuracy of 82% and 84.7% original group accuracy. Sex determination is important to narrow down the possible identities of the unknown skeletal remains. The humerus has a good sex predicting potential for the Sri Lankan contemporary population.

Vascular Anatomy of the Optic Disc: A Cross Sectional Study in a Sri Lankan Sample

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The optic nerve head receives the blood supply from the central retinal vessels and posterior ciliary vessels. There is a watershed between the two predisposing to ischaemia. Angiogenesis on the optic disc (OD) is seen in proliferative diabetic retinopathy. Despite the clinical significance, the vascular patterns on the OD have not been established. Fundoscopic images (n=150) of normal individuals, after excluding images with pathologies by an ophthalmologist were evaluated for the vascular anatomy of the OD. Hundred and four images from 54 people were analysed of which 67.3 %(n=70) were of females. The majority (92.3%, n=96) received the main arterial supply through one artery while the rest had two arteries entering. The number of veins exiting were also either one (84.6%,n=88) or two. Majority of the eyes had the arteries (74.0%,n=77) and veins (90.4%,n=94) centrally in the optic cup while the rest had them at the cup, disc margin. Only three (2.9%) eyes had pre-papillary looping. The arteries arborized leading to 0–6 arteries (mode=4,31.7%,n=33) and 0-3(mode=0,51.9%,n=54) arterial junctions to cross the OD margin. Zero to six veins (mode=4,36.5%, n=38) crossed the margin while 0-3 had a junction (mode=0,54.8%, n=57). Majority had 4-7(83.7%, n=87) minor vessels at OD (range=3-10). The mode and the median were 5(26.9%, n=28) and 6(21.2%, n=22) respectively. Establishing normal vascular anatomy of the OD contributes to developing digital technology to identify retinal vascular pathologies early. The association between the vascular variations and pathologies should be sought.

Morphometric analysis of the foramen magnum in a sample of adult Sri Lankan skulls

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The foramen magnum (FM) that transmits a large number of vital structures is an important anatomical landmark in neurosurgical procedures and forensic anthropology. The objective of the present study was to determine the dimensions and shape of the FM in a sample of adult Sri Lankan skulls. Forty-four adult skulls (32male and 12female) were selected for the study. The sagittal (FMSD) and transverse (FMTD) diameters of the FM were measured using a sliding digital vernier caliper to the nearest 0.01mm by one investigator. The area of the FM was calculated using Radinsky's formula; A=1/4×3.14×FMTD×FMSD and the index was calculated using the formula: FMTD×100/FMSD. The skulls were visually assessed to determine the shape of the FM. The shape of the FM was recorded as round, oval, egg-shaped, pentagonal, hexagonal or irregular. The mean sagittal and transverse diameter, area and index of FM in males were 34.62±2.17mm, 29.52±2.13mm, 695.77±90.64mm2 and 85.41±5.66 respectively, whereas they were 33.87±2.31mm, 28.28±1,99mm, 662.56±98.67mm2 and 83.69±6.13 respectively, in females. The shape of the foramen was found to be egg-shaped in 34.1% and round in 27.2% of cases. The size and shape of the FM are highly variable among different world populations. Skulls in the studied sample showed smaller FM in comparison with those of African, Mediterranean and European populations. Males displayed larger mean values than females for all measured variables.

Comparison of neck Range of Motion (ROM) and neck lordosis angle between spectacle users and non-spectacle users among first year undergraduates of the University of Peradeniya

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Due to prism effect of glasses, objects are viewed in a different line of sight than the normal. Spectacle users might compensate this by lifting the chin or by leaning forward. Repetitive use of incorrect postures can become "daily habits" affecting the normal physiology and biomechanics of the neck. Present study was focused to determine the effect of spectacle wearing on neck ROM and lordosis angle and further to determine correlation between the cervical ROM and lordosis angle. A randomized sample of 100 first year undergraduates of University of Peradeniya (age=19-24) were divided into a study group who wore spectacles (n=50) and an age sex\ matched control group who did not wear spectacles (n=50). The male:female ratio was 1:1. Cervical ROMs were measured using universal goniometer. Flexible ruler was used to measure the cervical lordosis. Significant differences were not found in cervical ROM ($p>0.05$) and lordosis angle ($p>0.05$) between spectacle users and non-users. Comparison of data across the sub-groups; male wearers and non-wearers showed a significant difference ($p=0.012$) among flexion angles. Weak positive correlations between forward flexion ($p<0.001$), right lateral flexion ($p<0.05$) and left lateral flexion ($p<0.05$) among spectacle users and left neck rotation ($p<0.05$) among non-users with the lordosis angle were found. The neck ROMs and lordosis angle between spectacle users and non-users were found to be non-significant and therefore it can be concluded that neck ROM and lordosis are not affected by spectacle wearing in general. Positive correlations between neck ROM and lordosis angle among both groups were novel findings.

Learning style preferences among second year medical students in Rajarata University of Sri Lanka

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Current medical students are a diverse group in terms of level of preparedness for learning and the learning preferences. It is the responsibility of teachers to address the diversity of learning needs and preferences among students and to take appropriate teaching approaches to improve their education. This study aimed to assess the learning styles of 2nd year medical students of the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (FMAS, RUSL). A descriptive cross-sectional study was conducted among two batches of students in FMAS, RUSL after completion of the pre-clinical course. The Visual, Auditory, Read/Write, Kinesthetic (VARK) questionnaire, which is a validated tool for the assessment of learning preferences was administered to all the students in 2 batches, after obtaining informed written consent. The data were analyzed and presented using descriptive statistics, according to the guidelines in VARK website. The questionnaire was distributed to 347 students, and response rate was 88.2% (n=306). Among the respondents, 57.3% preferred a single mode of information presentation (V, A, R or K); 9.3% preferred visual (learning from graphs, charts, and flow diagrams), 21.7% preferred auditory (learning from speech), 12.1% preferred printed words (learning from reading and writing), and 14.2% preferred using all their senses (kinesthetic: learning from physical involvement- touching, doings, and manipulation). Of the respondents, 42.7% preferred the multiple modes of information presentation. This included 34.7% of bi-modal learners, 7.0% of tri-modal learners and 1.0% of quad-modal learners.

Second year medical students in FMAS, RUSL have a variety of learning styles indicating the preference to different modes of information presentation in their learning. To meet these needs, teaching should be multisensory and should include a variety of teaching learning modalities to enrich the students' learning experience.

Student perception of the educational environment in an evolving medical school in Sri Lanka

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Educational environment is crucial in determining students' learning and the success of an educational program. This study assessed the students' perception regarding the educational environment in the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (FMAS, RUSL). Dundee Ready Education Environment Measure (DREEM) was administered online to all undergraduates of FMAS, RUSL maintaining anonymity. Results were analyzed using SPSS-20, descriptive statistics and Mann-Whitney-U test based on DREEM guidelines. The study was approved by Ethic Review Committee, FMAS, RUSL. Total of 681 students (63.1%) responded [Preclinical 448 (65.8%); Senior (paraclinical and clinical) 233 (34.2%)]. The majority (67.7%) were females. Overall mean score was 139.6±28.5 (69.8%) indicating 'more positive than negative' perception. Mean scores for all sub-domains were one grade below the best. The highest score was related to perception of learning (75.8%) and the lowest scores to atmosphere (66.7%) and social self-perception (66.8%). Items with the highest scores were the knowledge (3.53) and preparedness (3.42) of teachers and the lowest score was related to the ability to memorize (2.07). Academic self-perception was significantly higher among males (Mann-Whitney-U 40974.0, $p<0.001$), and no significant gender difference was observed in other sub-domains. Perception of educational environment was significantly more positive among pre-clinical students ($p<0.05$) in all sub-domains, compared to senior students. Of the 50 items, 37 showed significant difference between pre-clinical and senior students ($P<0.05$), and scores of 35 items were higher among pre-clinical students. Majority of undergraduates of FMAS, RUSL perceived educational environment positively. This study has identified areas requiring improvement for the success of educational programme of FMAS, RUSL.

A study on student perception on a formative 'mini-viva' examination in Anatomy

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Using assessments 'for learning' is achieved by providing opportunities to students to reflect on self, receive feedback and advice on improvement. This study evaluated students' perception on a formative 'mini-viva' examination in Anatomy conducted with this purpose. Mid-semester viva-voce examination was held for first year students of Faculty of Medicine, Rajarata University, at the end of the 'Regional Anatomy of Abdomen' module. The batch was divided into 6 groups, each with 29-30 students. Two examiners conducted the viva for each group, 5 minutes per student in the presence of the whole group of students. Students were assessed using a structured set of questions with a marking scheme. Feedback was obtained in-writing and through an online questionnaire. Of the total 177 students who participated in the viva, 134 (75.7%) students responded to the questionnaire. Majority of students were contented with the organization of viva (98.5%), examiners' support (98.5%), clarity (89.5%) and relevance of questions (96.3%), time allocated (92.5%) and use of relevant material (81.3%). Majority (81.3%) was satisfied with the viva and 93.3% were motivated to study further. Thematic analysis of written feedback from all (177) the students revealed the use of viva to stimulate further learning (6.8%), to improve knowledge (21.4%) and communication skills (11.3%), and for self-evaluation (9.6%). Some students expressed its usefulness in future assessments (15.3%), and to reduce nervousness (5.1%). Viva was reported as stressful (14.7%) and few disapproved its use for summative exams (2.9%). The study demonstrated that, despite viva being a traditional assessment tool, when appropriately used it can be used to enhance learning by promoting internal motivation and providing opportunities for self-evaluation, knowledge and skills enhancement.

Knowledge, awareness and perception regarding physiotherapy services among clinical students in Rajarata University

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Patients rely on doctors for referral to allied healthcare services including physiotherapy. Doctors need to be well-informed about physiotherapy to provide effective patient care in many situations. This study assessed the knowledge, awareness and perceptions regarding physiotherapy among the clinical students in Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (FMAS, RUSL). A descriptive cross sectional study was conducted among clinical students of FMAS, RUSL. Data was collected using a pre-tested, structured, self-administrated questionnaire and analyzed using SPSS-20. Among 84 respondents, all were aware of the presence of physiotherapy profession. Most commonly known areas of physiotherapy specializations were orthopedic (39; 46.4%), neurology (30; 35.7%) and pediatric (23; 27.3%). Majority (92.9%) were familiar with the use of exercises in physiotherapy, however knowledge regarding other physiotherapy modalities were low. Commonly known physiotherapy techniques were joint mobilization (94%), stretching (91.7%), soft tissue mobilization (66.6%) and movement rehabilitation (60.7%). Most of the students knew the application of physiotherapy in spinal cord injury (75%), cerebral palsy (70.2%) and stroke (67.8%) but it was poor in conditions like torticollis (5.9%), scoliosis (27.4%) and burns (26.2%), where physiotherapy is more applicable. Majority of the students agreed that physiotherapy play an important role in hospitalized patients (94%) and community-based rehabilitation (79.8%). Most of the students perceived inadequacy of knowledge regarding physiotherapy (72.6%). The study revealed that the medical students' knowledge and awareness regarding physiotherapy services needs improvement and more effective means of informing medical students about physiotherapy are required.

Estimation of stature from hand and handprint measurements in a sample of Sri Lankan Sinhalese population

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Stature is an essential attribute in creation of a biological profile in forensic anthropometry. Anthropometric approaches of the hand and handprint measurements in forensic investigations have been widely demonstrated. Hence, the present study was undertaken to estimate stature from hand and handprint measurements using sex specific regression models in a group of Sri Lankan Sinhalese. A sample comprising of 51 males and 66 females belonging to the Sinhalese ethnic group in the age range of 20 to 28 years was recruited in the study. Stature and eight measurements of both hands and their corresponding prints were collected and recorded using standard instruments and techniques. Significant positive correlation was shown between all hand and handprint measurements and stature ($p<0.05$-$p<0.001$) in both sexes. Simple linear regression equations showed a stature prediction accuracy ranging from±4.41 to ±5.88 cm and±4.04 to ±5.22cm for hand measurements in males and females respectively. Corresponding figures for handprint measurements were ±4.57 cm to±5.95cm and ±4.36 to ±5.52 cm, respectively while multiple regression models derived from hand measurements showed superior stature prediction accuracy rates (± 3.95 cm in males and ± 3.82 cm in females).The present study demonstrates that stature can be predicted from hand and handprint measurements with higher accuracy rates than those recorded in previous studies for other population groups. Hence, the stature prediction models established in this study will be useful in disaster victim identification from mutilated human remains and in crime scene investigations in Sinhalese population in Sri Lanka.

Anatomical variations of the obturator artery in a group of Sri Lankan subjects: A cadaveric study

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The aberrant obturator artery (AOA) which crosses the superior pubic ramus is at risk of damage in pelvic trauma and surgeries. Therefore, the knowledge of its common variations and incidence are important in clinical practice. The aim of the study was to identify its variations in a group of Sri Lankan subjects. The study was conducted in the Department of Anatomy, Faculty of Medicine, University of Ruhuna using pelvises of the cadavers during educational dissections. The origin, course and variations of the OA were noted. The study included 30 hemipelvises. There were 8 males and 7 females. The normal pattern of the OA originating from the anterior division of internal iliac artery (IIA) was found in 17 (56.6%) hemipelvises. Numerical and morphological variations were seen in 13 (43.3%) hemipelvises. Out of all the hemipelvises 7(23.3%) OAs originated from the inferior epigastric artery (IEA) while 4(13.3%) OAs originated from the posterior division of IIA. Double OA was found in 2 hemipelvises. AOA with or without anastomoses with IIA branch crossing the superior pubic ramus was observed in 8(26.6%) hemipelvises. Most of the variations were present in male subjects [7(53.84%)]. The data of this study shows that AOA is present in nearly 1/3rd of the hemipelvises. The knowledge of their pattern and relations are of academic interest to anatomists and clinically important to surgeons in preventing accidental damage during surgery.

Anatomical variations of Peroneus tertius and Extensor digitorum longus muscles: Cadaveric Study

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Peroneus tertius (PT) and extensor digitorum longus (EDL), are muscles of the anterior compartment of the leg, important for the bipedal gait in humans. They are used as anatomical boundaries during arthroscopy of the ankle joint and widely used for soft tissue replacement surgeries. PT is implicated in the pathophysiology of Jones fracture of the fifth metatarsal bone. It was the objective of this project to study the characteristics of PT and EDL in cadaveric specimens. The PT and EDL muscles of the leg in 25 lower limb specimens were examined for features with regard to muscle length, tendon length, mid circumference, origin and insertion. A single muscle belly replacing PT and EDL was observed in 7 specimens giving rise to 5 tendons which had distal attachments to the lateral 4 digits of the foot and the base of 5th metatarsal. The average circumference of these single muscles was 4.18 cm. In the specimens where PT and EDL were separate, their average circumferences were 3.46 cm and 4.5 cm respectively. Distal insertion of the PT had variable attachments to the base and shaft of the fifth metatarsal bone and the fourth interosseous space. Further, in one cadaver, PT and EHL appeared to be attached closely together by a common fascial layer in between them, which extended to enclose the lower most fibers of the PT. Correct identification of individual anatomy of these muscles has important surgical implications. Susceptibility to Jones fracture may vary with the variability of PT especially with regard to its insertion.

Anatomical variations of the sciatic nerve: A descriptive study

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The sciatic nerve (SN) (L4-S3) usually leaves the pelvis below piriformis and descends, dividing into the tibial nerve (TN) and common peroneal nerve (CPN) at varying levels proximal to the knee. Understanding about these variations is important in clinical medicine and for the knowledge in anatomy. Here we present the variations of high and low divisions of the SN. Twenty seven cadavers (53 limbs) at the Department of Anatomy, Faculty of Medicine University of Peradeniya, Sri Lanka, which were used for routine undergraduate dissections were studied, and the course of the SN with the levels of bifurcation were quantitatively documented from the midpoint of the two femoral condyles to the point of division of the nerve. Of the 53 limb specimens studied, the division was in the normal confinements in 24 (45.28%) limbs. In 14 (26.42%), there was high division of SN, and in another 14 (26.42%), there was low division. In 01 (1.88%) case, the CPN was emerging through the piriformis muscle to begin with, while the TN was emerging below PM. The knowledge on abnormal variations of the SN is known for causing non-discogenic sciatica with sciatic nerve compression, especially piriformis syndrome when it traverses through the piriformis muscle. Even though the lower division of the SN is relatively uncommon, we found it rather in higher percentages, as well as its high division of SN. This knowledge is useful in pure anatomical point of view as well as in clinical medicine.

Anatomical landmarks for the intercostal nerve blocks: A cross sectional cadaveric study

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In ultrasound guided intercostal nerve blocks the nerve is often not observed despite the ability to visualize the neurovascular plane. The objective of this study was to identify the location of the intercostal nerve in relation to the lower border of the corresponding rib at the plane where intercostal nerve block is delivered. In this cross-sectional study, phenoxymethanol fixed cadaveric thoracic walls were frozen and parasagittal sections were made 7cm from the mid-sagittal plane. Images were analysed using ImageJ (version-1.52a).Ninety-one intercostal (including subcostal) spaces of twelve cadaveric hemithoraces [n=6(right), n=6(left)] were studied. The typical neurovascular pattern, i.e. vein(V), artery(A) and nerve(N) lying from above downwards in VAN order was seen only on 37/91 (40.7%) intercostal spaces. This was followed by the patterns NVA (n=27/91, 29.7%) and VNA (n=12/91, 13.2%). A binary logistic regression was conducted to assess the determinants of the neurovascular pattern (dichotomous outcome variable="VAN-pattern" or "other-pattern"). The model with five predictors (sex, numerical value of the intercostal space, waist circumference, length of upper segment and shoulder width) was not statistically significant [$\chi 2(5,n=91)=19.12,p=.059$]. The nerve was located 4.4±1.7mm deep and 0.5±2.6mm inferior to the lower border of the corresponding rib. In conclusion, the typical intercostal neurovascular pattern (VAN) was observed only in 41% of the intercostal spaces, while the presence of this pattern was not predicted by the investigated independent variables. We recommend inserting the anaesthetic needle perpendicular to the skin, immediately below (~0.5 mm) the corresponding rib. Clinical correlations are necessary for validation of these results.

Analysis of foot and footprint measurements to determine the sex of an individual

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Sex determination becomes a major problem to forensic experts especially when only fragments of the body are left following mass disasters. Sex determination is a major aspect in the identification of a person along with the estimation of age, stature and ethnicity. Analysis of skeletal biology is an integral part of this process. Previous studies done on human skeletons have shown population specificity in sexual dimorphism. Similar studies applicable to the Sri Lankan population are scarce. The present study is an attempt to use foot and footprint measurements for sex determination. A total of 116 (51 males and 65 females) Sinhalese individuals participated in the study. Three anthropometric measurements of the foot and seven linear measurements of the footprint were obtained from each individual for the analysis. All foot and foot print dimensions were significantly greater in males than in females ($p<0.001$). Univariate and stepwise discriminant function analyses performed to assess the sex classification potential of foot and footprint variables showed that overall cross-validated per cent correct classifications range from 73.9%-96.6% and 77.4%-94% for the right and left foot, respectively. The most dimorphic single parameter was the foot length providing cross validated accuracy rates of 96.6% and 94.0% for the right and left foot, respectively. The study shows that foot and foot print measurements can be used to classify sex with a high degree of accuracy among the Sinhalese population in Sri Lanka.

Protocol for harvesting adult human hippocampus

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Hippocampus has gained much attention recently as a niche of adult neural stem cells, thus a potential therapeutic target in neurodegenerative diseases. Methods of harvesting hippocampi in rodents are described in detail. Here, we aimed to develop a protocol to harvest adult human hippocampi from post-mortem brain specimens. Brains were fixed en-bloc in 10% paraformaldehyde for 18 hours. Dorsal approach was selected as the best approach after testing ventral and lateral approaches. Five-millimetre-thick axial sections were made starting from the level of corpus callosum to expose the lateral ventricles. A 14-gauge needle was inserted into the temporal horn of the lateral ventricle and the position of the anterior and posterior ends of the temporal horn was marked on the cortex by piercing the temporal lobe with the needle. An incision was made on the temporal lobe joining the above mentioned two points to open the temporal horn. Hippocampus was visualized and separated from the parahippocampal gyrus by "rolling", starting from its posterior end. The amygdala was dissected with the hippocampus at the anterior end to preserve its connections. Macroscopically, the isolated tissue consisted of entire hippocampus and dentate gyrus. The proposed method of harvesting human hippocampus was repeatable. We plan to validate our protocol by visualizing different regions of the harvested hippocampal tissue after immunostaining.

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Location and Number of Nutrient Foramina in Human Long Bones of a Sri Lankan sample

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Nutrient foramen is an opening in the shaft of the bone from which nutrient arteries enter the medullary cavity providing the main blood supply for long bones. Although foramina in long bones are located away from the growing end, the topographical anatomy may vary and can even alter during growth. The objective of the present study was to determine the location and the number of the nutrient foramina in long bones of a Sri Lankan population. In this study 253 adult human long bones of the upper (43 humeri, 43 radii, 48 ulnae) and lower (52 femora, 49 tibiae, 38 fibulae) limbs were used. For each bone the foraminal index indicating the location of the nutrient foramen in relation to its proximal end was calculated (Foraminal index=Distance of the foramen from the proximal end/Total length). In the upper limb, foramina were located on the diaphysis with foraminal index ranging from 5.2 - 73.84% of the overall length of the humerus, 11.11-55.16% for the radius and 9.34-73.64% for the ulna. In the lower limb foramina were located on the diaphysis 3.97-89.74% of the overall length of the femur, 11.76-72.17% for the tibia and 23.42-56.76% for the fibula. In addition, the number and the distribution of the foramina in relation to specific regions/surfaces of the diaphysis were identified. This study provides additional and important information on the location and number of nutrient foramina in the long bones of the upper and lower limbs in a Sri Lankan sample. The knowledge of these variations is important during surgical procedures in order to preserve nutrient arteries.

Association of volume estimation accuracy obtained using USS, CT and volume calculation formulae for different shaped objects

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Ultrasonography (USS) and computer-tomography (CT) are frequently warranted for volume estimation of internal organs. Volume estimation accuracy is said to be influenced by the shape of the object. The scanner utilizes formula to calculate the volume of a specific shaped object. Therefore the volume estimation error described for non-globular objects may be related to the calculation formula used. The study aimed to evaluate the association of volume estimation error among USS, CT and volume estimation formula. Globular (Gb); elongated (IR) and near spherical (NGb) shaped hollow plastic objects were filled with water and subjected to USS and CT to obtain length, width, depth and estimated volume (EV). Manual volume calculation was performed using prolate (PV), ellipsoid (SV) and Lambert (LV) formulae. The actual volume (AV), EV and calculated volume was compared. The AV ranged from 10 to 445ml. Mean EV of USS; CT;SV;PV and LV were 97.9ml ($r=0.98;p<0.001$); 89.1ml ($r=0.97;p<0.001$);97ml ($r=0.99;p<0.001$); 108.5ml ($r=0.97;p<0.001$) and 132.5ml ($r=0.99;p<0.001$) respectively. Volume of all shapes were estimated with highest and lowest accuracy using PV (bias =-0.7) and LV (bias=+23.3) respectively. Regardless of the shape of object, EV of PV and LV has shown the highest (bias=-0.7; precession=19.8) and lowest accuracy (bias=21.8; precession=12.8) respectively. Among all shapes, volume of Gb were estimated with highest accuracy irrespective of the method used: (USS[bias=-0.31]; CT[bias=-0.14]; SV[bias=-1.5]; PV[bias=-0.7]; LV[bias=32.9]); volume of IR were estimated with lowest accuracy:(USS[bias=-17.5]; CT[bias=-32.6]; SV[bias=-18.4]; PV[bias=-0.3]; LV[bias=11.4]).Shape of the object and calculation formula used were shown to have an impact on the volume estimation accuracy.

Anatomical Variations of the Cephalic Vein at its termination in a Group of Sri Lankan Subjects: A Cadaveric study

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The cephalic vein (CV) in the deltopectoral groove is used for central venous access with lower incidence of complications than other sites. However, the presence of variations at this site would cause side effects like bleeding and misplacement of implant devices. We examined CVs at the deltopectoral groove in cadavers during educational dissection in the Department of Anatomy, Faculty of Medicine, University of Ruhuna. Any variation of the CV termination and relation to the clavicle was noted. The study included 64 CVs in 32 cadavers. There were 18 males and 14 females. Two female cadavers had unilateral variations while one male had bilateral variations of the CV. Two different types of variations were identified among these four CVs. The first variation was the presence of a persistent jugulocephalic vein where the CV drains into external jugular vein (EJV) after crossing the clavicle. This variation was seen in the left side of a female cadaver and the right side of the male cadaver. The other variation was the presence of a bifid cephalic arch where CV terminates in the axillary vein and an additional communication with the EJV. This was seen in right side of a female cadaver and the left side of the male cadaver. In these four instances the cephalic vein or the communicating vein crosses the clavicle lateral to the midpoint of the clavicle. Persistent jugulocephalic vein and bifid cephalic arch were the variations noted in this study. Awareness of these variations and their incidence are important for radiologists, vascular surgeons and interventional cardiologists to avoid complications during vascular interventional procedures.

Diaphragmatic hernia presenting in adult life

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Diaphragmatic hernia (DH) usually presents in the newborn and rarely present as undetected cases in adult life. Defects in the diaphragm can cause abdominal organs to herniate into the thoracic cavity resulting in respiratory distress or strangulation of abdominal organs. Reported here are 3 cases of congenital DH presenting in adult life. A 59-year-old male presenting with epigastric pain, bilious vomiting and constipation demonstrated an air-fluid level in the anterior mediastinum on chest radiograph. Surgery revealed an anterior Morgagni defect with a loop of terminal ileum herniated and strangulated into the pericardial sac. A 13-year-old girl presenting with abdominal pain and pyrexia for 2 days, on CT chest and abdomen revealed right lobe of liver and transverse colon in the right hemithorax with the pericardium pushed to the opposite side. Surgery revealed a large right sided central tendon defect. A strangulated gangrenous transverse colon was removed from within the thoracic cavity. A 73-year-old woman presented respiratory distress and was found to have left sided pleural effusion and bowel shadows in the chest cavity on radiography. CT revealed the stomach and several small intestinal loops herniating through a posterior defect in the left hemi-diaphragm. In these cases the body had adapted by maintaining total lung volume by compensatory hyperplasia of the contralateral lung. It was fascinating to find the liver moulded to the shape of the thoracic cavity in case 2. It appeared that the herniation of bowel loops had occurred as an acute event giving rise to symptoms. Even though rare, the possibility of congenital DH presenting as acute abdomen or respiratory distress is important to remember.

Morphological variations of human adult spleens: A cadaveric study

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Spleen is an organ enriched in vascular and lymphoid tissue located in left hypochondrium. It has morphological variations which may be misinterpreted as a disease condition involving the spleen. Therefore, knowledge on such morphological differences in a population is vital to distinguish normal variations from the disease conditions. The objective of this study was to identify the morphological variations such as notches, fissures and lobulations in cadavers. Ten per cent formalin fixed, self-donated cadaveric human spleens (n=13) in the Department of Anatomy, Faculty of Medicine, University of Colombo were included in the present study. In the study population, male to female ratio was 10: 3 with the age ranging from 26 to 95 years. The morphological features such as shape, notching of borders, fissures and lobulations were photographed, dissected and analyzed according to Michels NA classification. The morphological types of spleens were categorized by measuring hilar lengths. Notches were present in superior (n=8), inferior (n= 1) or intermediate (n=2) borders in ten spleens, while notches were absent in three. Five spleens had supernumerary notches. Six spleens contained fissures in both visceral and diaphragmatic surfaces. Four spleens had more than three lobules, while three had more than one hilum. The splenic types included compact, intermediate and distributed, which accounted for four, three and six respectively. Inconstant location of notches, presence of supernumerary notches, fissures and lobules were a common morphological variation observed.

A study of variations in the origin of Profunda femoris artery

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Profunda femoris artery, the main artery supplying the thigh, is a branch arising from the femoral artery, 3-4 cm below the inguinal ligament. Knowledge on common variations is important to make procedures like femoral artery catheterization, embolectomy and harvesting myocutaneous flaps safe and easy. Twenty seven formalin fixed cadavers from the Department of Anatomy, Faculty of Medicine, University of Peradeniya, were used and routine dissections were carried out to study the relationship of Profunda femoris artery to the femoral artery. The distance from inguinal ligament, immediately above the common femoral artery, to the origin of Profunda femoris artery was measured using a tape and a string. The Profunda femoris artery originated from the posterolateral aspect of the common femoral artery in 66% of limbs. The next common origins were from the posterior (15%) and posteromedial (11.3%) aspects. There were few from the lateral aspect (5.6%) and one from the medial aspect(1.9%).The mean distance of origin of Profunda femoris artery was 3.2 cm. In 30.1% it was < 3 cm and in 2 limbs it was less than 1cm. Considerable variations in the origin of the Profunda Femoris artery were noted in our study. In contrast to standard anatomical texts, 33% of the limbs had arteries originating from sides other than posterolateral and 30.1% of arteries originated within 3cm from the inguinal ligament. Knowledge of these variations is important to avoid inadvertent injury to the vasculature during clinical procedures.

Variations of the Subclavius Muscle: a preliminary study

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Variations of the Subclavius muscle have been identified as a cause for Thoracic outlet syndrome (TOS). Knowledge about these variations is particularly important for surgeons as well as radiologists. Twenty eight shoulder pectoral specimens of 14 cadavers were studied in the Department of Anatomy, Faculty of Medicine, University of Peradeniya, Sri Lanka. The skin and the subcutaneous tissues were dissected. The clavicle was lifted up from the medial side. The origins and insertions of the subclavius were documented. Twenty (71.4 %) male and 8 (28.5%) female specimens were studied. In 2 (7.14%) specimens, the subclavius muscle was absent. Twenty six cases showed normal site of origin, that is from the first costochondral junction.Twenty four cases (85.7%) had attachments to the clavicle. In 1 case (3.6%) an aberrant muscle (subclavius posticus) had gone posterolaterally crossing the subclavian vessels and brachial plexus towards the scapula, without any attachment to the clavicle and inserted in to the transverse scapular ligament, lateral to the inferior belly of omohyoid. In 1 specimen (3.6%) subclavius coexisted with subclavius posticus where subclavius attached distally to both clavicle and to the coracoid process. This study revealed aberrant attachment of subclavius and aberrant muscle known as subclavius posticus. These can cause TOS due to either compression of the neurovascular structures, narrowing the thoracic outlet and cervico axillary canal. Therefore, this knowledge is important for diagnostic and therapeutic purposes by both surgeons and radiologists.

Anatomical variations of terminal branching pattern of the brachial artery

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The arterial distribution of the upper limb shows numerous anatomical variations (9% -18.5%). Knowledge about these variations is important during surgeries, intra-arterial injections, venipuncture and imaging of the upper limb. With this background a study was conducted on 54 upper limb specimens of 27 cadavers during routine dissection at the Department of Anatomy, Faculty of Medicine, Peradeniya. The anterior compartment of the arm, cubital fossa and the forearm were dissected accordingly, and the pathway and the distribution of the brachial artery and its branches were documented. Variations of the brachial artery anatomy were noted in 3 specimens (5%). Unilateral high bifurcation of brachial artery was noted in two specimens on the left side. In one specimen, the bifurcation into its terminal branches, ulnar and radial, was directly from the axillary artery at the axilla just proximal to the formation of the median nerve. In the other specimen, the brachial artery bifurcation into ulnar and radial branches was found at the level of the mid humerus. Unilateral trifurcation of the brachial artery was noted in the third specimen. The brachial artery was seen to trifurcate into radial, ulnar and common interosseus arteries at the level of the neck of the radius. High brachial artery bifurcation and trifurcation of the brachial artery in the upper arm were noted in this study. Reporting of such variations in different populations is important for the practicing clinician during diagnostic and surgical procedures.

Anatomical variations of the Ulnar Artery

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The arterial distribution of the upper limb shows a large number of anatomical variations (9% - 18.5%). Knowledge about these variations is important during surgeries, intra-arterial injections, venipuncture and imaging of the upper limb. With this background a study was conducted on 54 upper limb specimens of 27 cadavers during routine dissection at the Department of Anatomy, Faculty of Medicine, Peradeniya. The anterior compartment of the arm, cubital fossa and the forearm were dissected accordingly, and the pathway and the distribution of the ulnar artery were documented. Variations of the ulnar artery anatomy were noted in 2 specimens (3.7%). Unilateral superficial ulnar artery was noted in one of them. The ulnar artery commenced at the level of the neck of the radius and passed superficial to the pronator teres, fibrous arch of the flexor digitorum superficialis, flexor carpi ulnaris and continued lateral to flexor carpi ulnaris tendon at the distal forearm. In the other specimen, the brachial artery trifurcated into the ulnar, radial and common interosseous arteries at the level of the neck of the radius. The ulnar artery continued superficial to the flexor digitorum superficialis, flexor carpi ulnaris up to mid forearm (7cm from trifurcation) and then ran deep into the plane between flexor carpi ulnaris and flexor digitorum profundus.The superficial course of the ulnar artery was noted in two specimens. It increases the risk of arterial injury especially during venipuncture. Reporting of such variations in different populations is important for the practicing clinicians during diagnostic and surgical procedures.

Variations of the human retinal vascular patterns: A descriptive cross-sectional study in Sri Lanka

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The retina is the commonest site used to observe the vascular architecture in-vivo. Changes in retinal vasculature have been described in numerous medical conditions; detection of which can aid diagnosis. However, variations of the normal vascular pattern have not been studied extensively. Fundoscopic images (n=150) of normal individuals were manually evaluated qualitatively for distribution patterns of main vessels. Images with retinal, vascular pathologies were excluded by a consultant ophthalmologist. Of the 104 images 67.3 % (n=70) were of females and 49 % (n=51) were from the left eye. Standard pattern of 2 nasal and 2 temporal were seen in 89.4 %(n=93). Six other different vascular patterns were seen. Distribution of the temporal vessels was constant. Three eyes (2.9%) from 2 individuals had only one nasal branch (transverse). Both eyes from one person (1.9%) had a transverse nasal branch in addition to two upper and lower nasal branches. Four eyes (3.8%) of three people had two upper nasal branches of which three eyes (2 from the same person) had one lower nasal branch and the other eye had 2 lower nasal branches. There was one (0.9%) eye with three upper nasal branches and one (0.9%) lower nasal branch and one eye with one upper nasal branch and two lower nasal branches. Although standard pattern of vascular distribution was seen in majority, variations were seen in the nasal branches. These findings can be used for development of digital technology in detecting retinal and vascular diseases involving angiogenesis.

A variation in the course of sciatic nerve in relation to the piriformis muscle: A case report

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Non discogenic sciatica is a disease, where the sciatic nerve (SN) or its branches, the Common Peroneal Nerve (CPN) and the Tibial Nerve (TN) compressing against the piriformis muscle (PM) because of its anatomical relation to the PM. Understanding about these variations is important in early diagnosis of Piriformis syndrome (PS) in clinical medicine. Twenty seven cadavers (53 lower limbs) used for routine undergraduate dissections at the Department of Anatomy, Faculty of Medicine, University of Peradeniya, Sri Lanka, were studied and the course of the SN as it travels in relation to the PM was observed and classified according to the Beaton's and Anson's classification. Of the 53 lower limb specimens observed, there was 01 (1.88%) case, where the CPN was emerging through the piriformis muscle to begin with, while the TN passes below the PM (Type II of Beaton's and Anson's classification). It was a right limb, and the left lower limb was having normal anatomy (Type I). Other specimens (98.22%) were showing the normal anatomical variation (Type I). The Type III and type IV of aforementioned classification was not observed. The normal course of SN is that it emerges below the PM (Type I). In the present study a variation of SN which can lead to PS with type II variation was observed. Therefore, knowledge on anatomical variations of the SN is important, in diagnosing the syndrome. Having high clinical suspicion will make the treatments much easier, and it will be helpful for the radiologists as well.

A cadaveric study of the variations in pulmonary fissures

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The right lung consists of three lobes separated by oblique and horizontal fissures and left lung consists of two lobes separated by a single oblique fissure. The knowledge on possible variations of pulmonary fissures is important in interpretation of radiological images and in pulmonary lobectomies or segmental resection. This study was carried out at the Department of Anatomy, Faculty of Medicine, University of Peradeniya, using 21 formalin fixed cadavers where routine dissections of the thorax was done by the medical undergraduates. Both lungs of each cadaver were removed and positioning of the pulmonary fissures and lobes were observed. Out of 21 pairs of right and left lungs, thirty three per cent of the right lungs and ninety per cent of the left lungs showed a typical presentation. Horizontal fissure was absent in twenty nine per cent of the right lungs and in thirty three per cent it was incomplete where it extended from the oblique fissure ending midway without extending up to the anterior border. The horizontal fissure was running low in one right lung enclosing a very small middle lobe. Small accessory fissures were present in two of the right and two of the left lungs. No sexual dimorphism noted in overall variations. The Present study revealed several variations where sixty six % of the right lungs had either an incomplete or absent horizontal fissure where the typical fissure pattern was seen less. Ten % of left lungs had accessory fissures. Such knowledge on variations is important during radiological interpretations and lung surgeries.

A preliminary study of morphological and morphometric variations of internal iliac artery in a group of Sri Lankan subjects: A cadaveric study

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Internal Iliac Artery (IIA) is the principal blood supply to the pelvic viscera, gluteal region, medial compartment of the thigh and perineum. Ligation of the IIA is carried out for controlling pelvic bleeding in trauma, pelvic surgeries and post-partum haemorrhage. Therefore, it is very important to have a thorough knowledge of its anatomical relations and common variations. We examined IIA of both sides of pelvises of cadavers during educational dissection in the Department of Anatomy, Faculty of Medicine, University of Ruhuna. Branching pattern of the IIA, lengths of the common iliac artery (CIA) and IIA, and the diameters of main divisions of IIA were measured using a vernier caliper. Relation of the IIA to the external iliac artery (EIA) and the internal iliac vein (IIV) were noted. Independent t-test at 0.05 significant levels was used to compare sex differences in mean lengths of CIA and IIA and the mean diameters of main divisions of the IIA. The study included 30 IIAs in 15 cadavers. There were 8 males and 7 females. Four types of branching patterns of arteries were identified according to the Adachi's classification and most of them were in Adachi's type I (53.33%). Majority of IIAs (63.33%) coursed posteromedial to the EIA and anteromedial to the IIV (46.66%). There were no sex differences in mean lengths of CIA ($p=0.47$), IIA ($p=0.67$) and mean diameters of main divisions of IIA ($p=0.06$ each). Awareness of these variations is important for surgeons, obstetricians and radiologists to prevent inadvertent damage to neighbouring structures during surgeries and interventional procedures.

Development and implementation of a tool to assess anatomy knowledge retention

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Anatomy knowledge is fundamental in practicing medicine. Knowledge acquired during preclinical years may be lost in clinical years. To date, a universally acceptable tool to assess anatomy knowledge retention (AKR) is not available. The objective of this study was to develop and validate AKR tool. Semi-structured interviews were held with clinicians from different disciplines to establish essential anatomy knowledge. Fifty single-best-response questions were developed based on this, and discussed with anatomists and clinicians for content validity. Best questions were administered to 30 clinical year students. Point bi-serial index, difficulty-index and KR-20 were calculated. Low point bi-serial was defined as <0.15 and response rate <35% answering correctly. Questions were graded according to these two parameters into poor, marginal, good and excellent. Marginal questions were edited, poor ones were removed. Developed AKR tool was administered to 15 clinical-year-students, 15junior doctors and10 senior doctors.The point bi-serial ranged from (-0.02)-0.97.Difficulty index ranged from 29%-94%. Out of 50 questions, 25 were excellent, 10 good, 11 marginal and 4 poor. Final tool consisted of 46 questions. KR-20 was 0.7 (high reliability). Table shows the marks achieved.

	Limbs%	Thorax abdomen	Pelvis%	Neuro%	Total%
Students	66.04	82.6	75.13	78.7	75.46%
Junior doctor	59.2	73.3	72	62.5	66.75%
Senior doctor	48.3	52.3	49.2	44	48.45%

Students scored highest marks in all regions. Thorax and abdomen region scores were better than that of other regions. Limbs section show lowest marks in students and junior doctors, while the senior doctors had scored lowest in neuroanatomy. It appears that clinical year students have a good anatomy knowledge retention which seems to be continuing as they become doctors but diminishing as they become more experienced.

Psychological effects of Anatomy dissections among pre-clinical medical students in Rajarata University of Sri Lanka

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Anatomy teaching/learning in medical schools is frequently based around the use of human cadavers. Dissections are associated with multiple stresses including psychological effects. This study assessed the psychological impact of anatomy dissections among pre-clinical students of Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (FMAS, RUSL). A descriptive cross-sectional study was conducted among preclinical students in the first and second-year in FMAS, RUSL. The data was collected using a pilot-tested, self-administered questionnaire, and was analyzed using descriptive statistics and Chi-square test. The study was approved by Ethics Review Committee, FMAS, RUSL. Total of 305 of preclinical students [158 (51.8%) first-year and 147 (48.2%) second-year students] participated. Majority (68.9%) were females. Factors that made students uncomfortable most during dissections were formaldehyde smell (47.2%) and infrastructure of the dissection lab (16.4%). Psychological effects of dissections included sleeping problems (2%), learning difficulties (5.9%), nightmares (3.9%) and stress (6.9%). Learning difficulties following exposure to cadavers were significantly more common among first-year students (10.1% vs. 1.4%; $p<0.001$). No significant sex difference was observed. The main complains following dissection were reduced energy, diminished appetite and loss of interest and enjoyment (24.0% vs. 11.6%, 14.6% vs. 17.0%, 14.6% vs. 10.9% respectively among first-year and second-year students). Depressed mood was reported by 15.8% of first-year students and 8.8% of second-year students. Multiple psychological effects are shown to be associated with anatomy dissections. Identification and appropriate management of these stresses are important for enhancement of students' learning experience in Anatomy laboratory.

A survey on the attitudes of the Ayurveda medical students towards cadaver dissection

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Cadaveric dissection is a fundamental tool in teaching anatomy. Although this facilitates students to understand the theories and the concepts of the discipline, presently cadaveric dissections seems to be reduced due to negative attitudes such as exposure to hazardous chemicals, and time consumption among other things. In this study, the attitudes of Ayurveda medical students towards the cadaveric dissection were analyzed during their BAMS (Bachelor of Ayurveda Medicine and Surgery) course.This was a cross-sectional study. Data were collected from students of Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya who have completed Anatomy course in Preclinical phase. A self-administered questionnaire in five point Likert scale was given to 110 students. This study revealed that 67% of the respondents found their first visit to the dissection hall exciting and 90% were shocked to see the cadaver for the first time. Between 60% and 94% of students held positive attitudes towards the dissection. Ninety-four per cent of the students were determined, interested, and ready to study anatomy with cadaveric dissection. Eighty-five per cent students reported that the undergraduate curriculum should include the dissection method for teaching modality of Anatomy. Hundred per cent of students have showed a sense of gratitude to the people and/or the relatives who had donated their bodies. Although dissections have given an unpleasant experience for majority of BAMS graduates at the beginning they conclude that cadaveric dissections are an essential part of teaching Anatomy in BAMS degree program.

Optimizing a protocol for isolating fibroblasts from human oral cavity

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Fibroblasts are one of the most abundant cell types in the connective tissue. In patients diagnosed with oral squamous cell carcinoma (SCC), who undergo radiation therapy, fibroblasts act as initiating agents for many different post-radiation therapy side effects. Fibroblasts have become the subject of study in research into radiation-induced side effects of oral SCC. The aim of this study was to isolate and culture expand fibroblasts obtained from human buccal mucosa for future in-vitro experiments related to oral SCC. Adhering to Helsinki Declaration principals and following the approval of University of Colombo Ethics Review Committee (No. EC-17-158) the study was conducted at the Human Genetics Unit. Tissue samples were obtained from biopsies of two oral SCC patients and cultured consecutively; modifying the culture conditions. A previously described explant method was used and modifications done with regards to tissue digesting time, reagents and their concentrations. Cell growth was observed under inverted microscope until 80% confluence was achieved. Adherent population of monomorphic cells displaying elongated disk like shapes, characteristic of fibroblasts, were identified in the cultures of the second patient. We were able to successfully develop and optimize a protocol to isolate and expand fibroblast cell cultures in vitro that is most appropriate for our laboratory.

A case report of a high division of Extensor digitorum longus

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Extensor digitorum longus (EDL) is a muscle of the extensor compartment of the leg. It arises from inferior surface of the lateral condyle of tibia, upper three quarter of extensor surface of fibula, anterior surface of interosseus membrane and deep surface of deep fascia, becomes tendinous in the leg, then passes deep to both superior and inferior extensor retinacula. In the dorsum of the foot EDL tendon usually divides into 4 slips which passes forward to 2nd, 3rd, 4th, 5th toes to form extensor expansions. It is an extensor of the toes as well as the ankle joint. While it is essential for the gait, it has an importance in tendon reconstruction surgeries. During routine dissections a variation of EDL muscle was noted. It originated from lateral tibial condyle, deep fascia, fibula, and interosseus membrane. The muscle belly divided into 2 as it became tendinous in the proximal leg. The 2 tendons passed under both superior and inferior extensor retinacula. The medial tendon gave slips to 2nd and 3rd toes while the lateral tendon gave slips to 4th and 5th toes. The lateral tendon additionally had an inter-tendinous connection with the tendon of Peroneus tertius. Variations of EDL are not commonly reported. This high bifurcation of EDL may have implications in surgery, especially during reconstruction following trauma.