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Original Article

To assess and compare supra-clavicular and infra-clavicular approaches to obtain blood samples from the subclavian vein in cadavers at autopsy

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ABSTRACT

Objectives: The study of microbes in the body of a person after death can be perhaps termed appropriately as Post-mortem Human Microbiology. Microbes can indicate the state of bacteremia at the time of death if the blood samples collected at autopsy are taken in an aseptic way from an appropriate sampling site. The aim of the study was to compare the sampling efficacy of the supraclavicular and infraclavicular approaches for both the left and right subclavian veins and determine the most effective approach among the four options.

Material and Methods: In this study, post-mortem blood samples were collected from both subclavian veins of 30 dead bodies to evaluate the effectiveness of approaches to subclavian veins. To achieve this, blood was collected from one side of the dead body through the supraclavicular approach and the other side through the infraclavicular approach, noting down the number of attempts which were required to successfully obtain at least 10 mL blood sample which is adequate for post-mortem microbiological examination.

Results: The study revealed that the supraclavicular approach is superior to the infraclavicular method. The left supraclavicular approach yielded maximum successful sample collection (100%) and also showed maximum successful single attempt sample collection (53%) compared to the other three approaches.

Conclusion: Following recommended aseptic procedures, using the procedure mentioned and obtaining blood samples from the left supraclavicular approach can improve the chances of getting a non-contaminated, adequate sample for Post-mortem Microbiology (PMM).

Keywords: forensic pathology, post-mortem microbiology, subclavian vein, post-mortem human microbiology, microbes

INTRODUCTION

Post-mortem Microbiology (PMM) is the study of microbes in the body of a person after death when perhaps the term should most appropriately be Post-mortem Human Microbiology. It can reflect the ante-mortem microbial state in the person when studied using aseptic measures at autopsy, within a certain range of terms and conditions. Definitions and perspectives related to PMM abound in literature from the mid-19th century to recent times.[1] The definition of PMM with limited meaning still finds its place in scientific literature as the study of germs that settle on or infect a dead body after death. The presence of microbes can indicate the state of bacteremia at the time of death if the blood samples collected at autopsy are taken in an aseptic way in accordance with recommendations formulated after rigorous scientific thought. Bacterial growth occurs

after death and the bacteria can spread by a process known as translocation. Studies have been conducted to study the bacterial flora on the skin, the blood, and in the gut, and the factors that govern the translocation of bacteria after death. Interpretation of the autopsy cultures and the applicability of the information is being discussed on a collaborative platform between microbiologists and autopsy pathologists. A culture report may reflect ante-mortem bacteremia of the deceased or postmortem translocation of bacteria or contamination during blood sampling, which requires evaluation. European Society of Clinical Microbiology and Infectious Diseases-ESCMID Study Group of Forensic and Post-mortem Microbiology (ESCMID-ESGFOR) has recently put forward recommendations for sample collection for PMM at autopsy. This includes collecting blood samples from the subclavian vein before conducting the autopsy.

Post-mortem microbiology can be used to ascertain the deceased person's time since death, the cause of death in some cases, and where the dead body has been by looking at the microbial communities on the dead body. Investigating criminal cases, locating those hurt in natural catastrophes, and monitoring the spread of infectious diseases can all benefit from this information.

Post-mortem microbiology is a promising field of study with a wide range of uses in forensics, public health, and environmental monitoring. This information can be valuable in solving criminal cases, identifying victims of natural disasters, and tracking the spread of infectious diseases.

In post-mortem microbiology, it is imperative to ensure the blood or tissue samples obtained are sterile and devoid of foreign microbes. Several microbiological studies have identified that one of the best sites to collect the blood sample is the subclavian vein for post-mortem microbiology blood sample collection.[2–4] The subclavian vein can be considered a peripheral vein rather than a central vein in terms of the redistribution of microbes and metabolites after death. The blood sample from the peripheral vein is considered superior to the cardiac sample when the blood culture for microbial activity is questioned.[5] This has now been incorporated in the International Guidelines (2019) by ESCMID (European Society for Clinical Microbiology and Infectious Diseases) for Minimally Invasive Autopsy (MIA).[6,7] Collecting blood samples from the subclavian vein has challenges for the living and the dead. This difficulty in accessing the subclavian vein has been mentioned in studies undertaken by anesthetists to establish the best approach for cannulation. Dead body studies by anesthetists using knowledge of clinical anatomy have focused on access to a subclavian vein using the deltoid tubercle. Anatomical or dead body studies are likely to correlate better with autopsy sample collection methods. At autopsy, the stiffness of muscles (Rigor mortis) in the body, the flaccidity of walls of veins and collapsed position of veins, and the availability of limited blood in the subclavian vein are factors distinct from both the clinical approach of anesthetists and anatomical system in embalmed dead body. Traditionally, blood samples at autopsy have been collected from the femoral vein or the heart for toxicological or chemical analysis.[8–10]

The subclavian vein is available on both sides of the human body. Each subclavian vein is a direct continuation of the axillary vein and is renamed as the subclavian vein once it passes the lateral border of the first rib. So, the area of interest is very narrow, surrounded by other vessels, and thus, the possibility of pricking other vessels is greater. Hence, proper technique must be followed for subclavian vein blood collection.

The subclavian vein can be approached through either supraclavicular or infraclavicular approach.[11,12] Thus, there are four options for collecting blood from the subclavian vein, which need to be evaluated further to determine the best among the four. In this study, the workers have tried to focus on approaching the subclavian vein through the four options and to classify each based on accuracy and number of attempts required to collect the sample.

MATERIAL AND METHODS

The objective of this study was to assess which site out of the supraclavicular or infraclavicular approach of the left and right subclavian vein offered more definite sampling and which among the four was the best.

All dead bodies brought for autopsy at the Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, Bhopal, were included in the study except where informed consent from next of kin was not obtained, bodies in moderate to advanced stage of decomposition where the chance of blood retrieval is scarce, in cases with cervicothoracic injuries leading to a deformed collection site, or in dead bodies with injuries leading to severe blood loss. It is a cross-sectional clinical trial on dead bodies by clustered sampling in singularity.

In this study, 30 fresh dead bodies were included and divided into groups of 15 each as Group A and Group B.

In Group A, blood was collected from the left supraclavicular and right infraclavicular approaches, whereas in Group B, blood was collected from the left infraclavicular and right supraclavicular.

A blood sample was collected by qualified autopsy surgeons (right-handed) who were practically trained to collect subclavian blood samples using a Quincke needle (20 g).

The cadaver was placed supine initially, and following the standard aseptic procedure, a 20-gauge Quincke needle was inserted blindly with a guarded and slow needle advancement technique with constant negative pressure in the syringe. If blood was not aspirated, then, with the needle in situ, the dead body was elevated to the Trendelenburg position to help the blood to gravitate toward the neck.

Aspiration of 10 mL of blood into the needle's hub was considered a successful attempt when achieved with a maximum of 10 individual attempts. If blood could not be aspirated even after ten attempts, the procedure was labeled "failure". The successful attempts were confirmed by visualizing prick marks on subclavian veins during routine autopsy procedures while reflecting the skin of the neck and chest area.

The following uniform approach was followed to retrieve blood samples in all cases.

In the supraclavicular approach, the patient's head was turned slightly to the contralateral side, and the same side arm was kept to the sides (adducted). The needle was inserted in the claviculo-sternocleidomastoid angle at the base of the neck, the needle directed downwards to the contralateral side and slightly backward, in other words, towards the contralateral nipple reaching just inferior to the clavicle[13] [Figure 1].

In the infraclavicular approach, the patient's head was turned slightly to the contralateral side, and the same side arm was kept in abduction. Place the thumb of one hand on the middle part of the clavicle and the index finger on the jugular notch. The needle tip should enter the skin inferior to the thumb, 1 cm lateral to the curvature of the middle third of the clavicle, and the hand is advanced medially towards the index finger[13] [Figure 2].

Figure 1: Showing the use of a syringe with a Quincke needle attached for collecting blood samples from the left side subclavian vein through the supraclavicular approach.

Figure 2: Showing the use of a syringe with a Quincke needle attached for collecting blood samples from the right side subclavian vein through the infraclavicular approach.

The relevant data were collected in an Excel spreadsheet, and statistical analysis such as descriptive analysis, regression analysis, and Test of sums was performed using SPSS software.

RESULTS

On statistical descriptive analysis of collected data of 30 cases, 22 were male (73.3%) and eight were female (26.7%). All individuals in the study were between the ages of 15 to 90 years. The mean age of all the subjects was 38.9 ± 20.95. And 75% of subjects were inside the age group of 18 years to 66 years.

The height of all subjects who participated in the study ranged between 142 cm and 182 cm, with a mean of 163.9 cm.

Out of 60 subclavian vein blood samples to be collected, 52 were successful when both single attempts and multiple attempts up to 10 attempts per site were included. The rate of success was 86.7%. The sample collection from the left supraclavicular site was achieved 100% with a mean shot of 1.733 ± 1.793. The right infraclavicular approach showed a minimum success rate of 73.33% with a mean shot of 2.272 ± 1.135. The right supraclavicular approach yielded 93.33% success with a mean attempt of 1.429± 0.433, and the left infraclavicular approach got a success rate of 80% with a mean shot of 2.667 ± 1.899. When all four methods were considered together, in the left supraclavicular approach, the subclavian vein was punctured successfully 100% of the time, whereas the right infraclavicular process showed the lowest success rate of 73%. The right supraclavicular process showed the second best success rate (93%), and the left infraclavicular process was third (80%) [Table 1].

When comparing the supraclavicular and infraclavicular approaches using regression analysis, it is observed that the

Table 1: Showing successful sample collection in all four approaches.

Supraclavicular	Successful	Unsuccessful	Success rate
Right	14	1	93%
Left	15	0	100%
Infraclavicular	**Successful**	**Unsuccessful**	**Success rate**
Right	11	4	73%
Left	12	3	80%

Table 2: Showing successful sample collection comparing sides and approach types.

Approach	Successful	Unsuccessful	Success rate
Supraclavicular	29	1	96%
Infraclavicular	23	7	76%
Approach	**Successful**	**Unsuccessful**	**Success rate**
Right	25	5	83%
Left	27	3	90%

success rate is higher in the supraclavicular approach, with 29 successful attempts (out of a possible 30) as compared to the success rate of 23 with the infraclavicular system (out of possible 30). It is also observed that the success rate is marginally higher in the left-sided approach, with 27 successful attempts (90%), compared to the right-sided approach, with 26 successful attempts [Table 2].

When all four approaches are considered together using regression analysis, in the left supraclavicular approach, the subclavian vein was punctured successfully 100% of the time, whereas the right infraclavicular process showed the least success rate of 73%. The right supraclavicular process showed the second best success rate (93%), and the left infraclavicular approach was third (80%).

The supraclavicular approach gave a maximum single attempt successful puncture of the subclavian vein (73%), whereas the infraclavicular approach had only 26% first attempt successes. When sides are considered, the first attempt success rate is distributed almost equally, with 46% on the right side and 53% on the left side [Table 3].

The depth at which the needle has pierced the subclavian vein was also calculated in this study. The needle depth ranged between 1.2 cm to 9 cm when considering all 52 successful punctures. In the left supraclavicular approach, the range was between 2 cm to 4.2 cm, with a mean depth of 2.94 cm. In the right supraclavicular process, the range was 1.2 cm to 5.8 cm with a mean of 3.29 cm. In the left infraclavicular approach, the field was 2.5 cm to 5.7 cm, with a mean attempt depth of 3.99 cm. The right infraclavicular process showed a maximum capacity of 1.4 cm to 9 cm with a mean depth of 4.03 cm [Table 4].

Table 3: Showing the rate of successful first attempts in all four approaches.

Approach	Success in the first attempt	Rate
Supraclavicular (n = 30)	22	73%
Infraclavicular (n = 30)	8	26%
Approach	**Success in the first attempt**	**Rate**
Right (n = 30)	14	46%
Left (n = 30)	16	53%

Table 4: Showing the difference in depth at which the needle has pierced the subclavian vein in all four approaches.

Site	Mean	Median	Range
Supraclavicular, Left (n = 15)	2.94	2.9	2–4.2
Supraclavicular, Right (n = 14)	3.29	3.65	1.2–5.8
Infraclavicular, Left (n = 12)	3.99	3.7	2.5–5.7
Infraclavicular, Right (n = 11)	4.03	3.5	1.4–9

Table 5: Showing the difference in distance from the midline to the puncture site at which the needle has pierced the subclavian vein in all four approaches.

Site	Mean	Median	Range
Supraclavicular, Left (n = 15)	6.30	6	5–8
Supraclavicular, Right (n = 14)	6.65	7	4.5–9.5
Infraclavicular, Left (n = 12)	7.43	7.75	5–11
Infraclavicular, Right (n = 11)	7.27	8	6–9

The distance from the midline to the puncture site was measured in this study and was found to be 4.5 cm to 11 cm. The left supraclavicular approach had a distance range of 5 cm to 8 cm from midline with a mean of 6.3 cm. The right supraclavicular approach ranged from 4.5 cm to 9.5 cm with a mean distance of 6.65 cm from the midline. The left infraclavicular approach had a maximum capacity of 5 cm to 11 cm with a mean of 7.43 cm, and the right infraclavicular direction had a range of 6 cm to 9 cm with a mean distance of 7.27 cm [Table 5].

On using the Test of ranks of sum for predicting the difference in the number of attempts of piercing, there was a significant difference in the mean number of piercing on the left side between infra- and supra-clavicular approaches, p=0.034; however, the difference between the mean number of piercings in the right side between both approaches was not found statistically significant (p-value=0.063). The mean number of piercings required on the infraclavicular approach was 2.66, and for the supraclavicular approach was 1.73; thus, the supraclavicular approach required a lesser number of piercing attempts in our study, which was significantly less

than the number of piercings required in infraclavicular approach.

DISCUSSION

The present study focused primarily on ascertaining the best approach to get an optimum blood sample from the subclavian vein for PMM. Access to a subclavian vein in the living has been described by anesthetists and in the dead by anatomists. Collecting blood samples from the dead body at autopsy has yet to be discussed in the literature. Autopsy pathologists, more so Forensic Pathologists, are not trained in managing blood samples from subclavian veins as a blind procedure. Maxwell Hove *et al*. reported that out of different sites for obtaining a post-mortem blood sample for microbial culture, the rate of false positivity from subclavian venous samples was significantly lower than from other areas.[14] In this study, the male participants were in higher numbers than female participants, mainly due to the increased number of male medicolegal deaths in the area of study and the variation in obtaining informed consent from relatives or next of kin. Their study supports the ESCMID-ESGFOR recommendations of collecting blood from the subclavian vein for PMM. The recommendations neither mention the sample collection process nor the site as the right nor left subclavian vein or the approach (supraclavicular or infraclavicular) to the subclavian vein.

In the present study, a blood sample was obtained from four sites to assess the ease with which an adequate blood sample can be obtained for PMM. The supraclavicular approach on the left gave the best success rate, the terms success and failure being defined for the present study. A maximum number of samples were obtained in a single attempt also from the left supraclavicular site. Considering a study by Amparo Fernandez Rodriguez *et al*. which implies a more significant number of attempts will lead to higher chances of samples obtained for post-mortem microbiology being contaminated,[7] it is essential to consider where the possibility of getting a model with a single attempt is maximum. The study is small, yet it indicates that the left supraclavicular site sample should be preferred to avoid contamination as the possibility of getting the piece in one attempt is maximum with the left supraclavicular site. The study findings of Tomasz Czarnik *et al*. in live clinical patients also state that the supraclavicular approach yields better first-attempt catheterization of the vein and causes minimal complication in live patients.[15]

On comparing the combined success rate between supraclavicular and infraclavicular approaches, the supraclavicular approach has a higher success rate (96%) in the present study. Our study revealed the supraclavicular strategy through the left side as slightly better than the right supraclavicular approach. However, other studies find the supraclavicular path on the right side to be better. This difference is slight and can be explained by the difference in skill and handedness of the persons. A study with a bigger sample size in the future could better address this issue. Practically, this difference could mean that both left and right supraclavicular sites may be considered for successful catheterization of the subclavian vein and for obtaining blood samples for post-mortem microbiology. The supraclavicular approach has provided better results than the infraclavicular approach, which is corroborated by the study finding of 29 out of 30 successful attempts in the supraclavicular approach as compared to 23 out of 30 successful attempts in the infraclavicular approach. The single-attempt success rate in the supraclavicular approach is also higher (22 out of 30) compared to the infraclavicular single-attempt success rate (8 out of 30).

The study also considered the variability of subclavian vein position by obtaining measurements of the depth of the needle from the puncture site and the distance from the midline of the successful puncture site. Considering all four approaches, the mean depth of the puncture site varied between 2.94 cm to 4.03 cm. The minimum difference may be because the subclavian vein is found just below the clavicle, a superficial horizontal bone of the upper chest.

The mean distance from the midline of the anterior plane was between 6.30 cm to 7.43 cm, considering all four approaches together. This also implies the minimal variation of the position of the subclavian vein in different individuals irrespective of their height and build. Even though the subclavian veins are asymmetrical on both sides, the main difference in distance from the midline was perceived in the supraclavicular approach vs the infraclavicular approach. In the supraclavicular approach, the mean distance varied between 6.30 cm to 6.65 cm; in the infraclavicular approach, the mean varied between 7.27 cm to 7.43 cm. This shows that despite the anatomical variation of the subclavian vein on both sides, the distance between the subclavian vein and midline is comparable. In the present study, documenting the angle at which the needle pierced the surface of the skin to reach the subclavian vein could have given valuable insight into the overall anatomical variation and its correlation with sampling approaches.

Subclavian blood sample collection has been done in previous studies to obtain qualitative information on post-mortem microbes present in the dead body and has helped to find many pathogens, including viruses, bacteria, and fungi. Such studies mainly focused on obtaining blood samples in Minimal Invasive Autopsy, especially in suspected highly infective dead bodies.[16]

The discoveries of this study could have enormous ramifications for posthumous microbial science. After death, microbial science includes the recognizable proof and examination of microorganisms in dead bodies, which can give significant data to forensic examinations and clinical exploration. Blood culture analysis is one of the most ordinarily involved examples for posthumous microbial science investigation, as it can give data on the presence of pathogens, sepsis, and/or any other ailments.

CONCLUSION

This study of 30 sample sizes is expected to analyze the adequacy of four approaches to obtain blood samples from subclavian veins on the two sides of the body. The left supraclavicular approach was the better strategy, outperforming the left and right infraclavicular and suitable supraclavicular approaches.

Following recommended aseptic procedures, using the method mentioned and obtaining a blood sample from the left supraclavicular approach can improve the chances of getting a non-contaminated, adequate sample for PMM. Based on this study, the skill enhancement of autopsy surgeons helps in conducting Minimally Invasive Autopsies, having evidence for stating bacteremia in a particular case, and helping in identifying microbes better in a future pandemic.

Generally, this study gives essential knowledge into the adequacy of various methodologies for acquiring blood samples from subclavian veins and features the expected ramifications for posthumous microbial science. Future research could explore the pertinence of this method in a more extensive range of cases and investigate the factors that contribute to its high success rate, such as the depth of insertion, angle of the needle, and correlation between heights and build of the person with change in success rate.

Ethical approval

The research/study was approved by the Institute Human Ethics Committee of AIIMS Bhopal, number IHEC-LOP/2022/IL031, dated 16-09-2022.

Declaration of patient consent

Patient consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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